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

NTP Technical Report on the Toxicology and Carcinogenesis Studies of Pyrogallol (CAS No. 87-66-1) in F344/N Rats and B6C3F1/N Mice (Dermal Studies)

NTP TR 574

FEBRUARY 2013

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF PYROGALLOL
(CAS NO. 87-66-1)

IN F344/N RATS AND B6C3F1/N MICE
(DERMAL STUDIES)

NATIONAL TOXICOLOGY PROGRAM
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FOREWORD

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

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

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SUMMARY

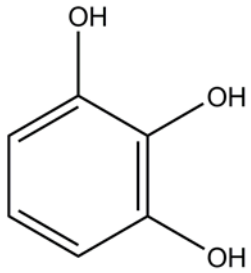
Background
Pyrogallol is used to produce pharmaceuticals and pesticides and formerly was used in hair dyes. We studied the effects of pyrogallol on male and female rats and mice to identify potential toxic or cancer-related hazards.

Methods
We applied solutions containing pyrogallol in ethanol on the backs of male and female rats and mice. Groups of 50 male and female rats and mice received 5, 20, or 75 milligrams of pyrogallol per kilogram of body weight five days per week for two years. Groups of animals receiving ethanol alone served as controls. At the end of the study tissues from more than 40 sites were examined for every animal.

Results
The only effects observed were in the skin at the site where the chemical was applied. Occurrences of epidermal hyperplasia and hyperkeratosis were greatly increased in all rat groups receiving 20 mg/kg or more of pyrogallol and in all groups of mice receiving pyrogallol. Two squamous cell papillomas of the skin occurred in male mice and four squamous cell carcinomas of the skin occurred in female mice at the site where pyrogallol was applied.

Conclusions
We conclude that exposure to methyl *trans*-styryl ketone caused skin lesions including hyperplasia, hyperkeratosis, and inflammation at the site of application. Pyrogallol caused cancer of the skin in female mice and possibly also in male mice.

ABSTRACT



PYROGALLOL

CAS No. 87-66-1

Chemical Formula: $C_6H_6O_3$ Molecular Weight: 126.11

Synonyms: 1,2,3-Benzenetriol; 2,3-dihydroxyphenol; gallamine; pyrogallic acid; 1,2,3-trihydroxybenzene
Trade names: C.I. Oxidation Base 32, C.I. 76515, C.I. 76551, Fouramine Base AP, Fouramine Brown AP, Fourrine PG, Fourrine 85, Piral, Pyro

The current main commercial use of pyrogallol is the production of pharmaceuticals and pesticides. In analytical chemistry, pyrogallol is used as a complexing agent, reducing agent, and, in alkaline solution, as an indicator of gaseous oxygen. Pyrogallol was nominated for testing by private individuals based on its frequent occurrence in natural and manufactured products, including hair dyes, and the apparent lack of carcinogenicity data. Male and female F344/N rats and B6C3F1/N mice were administered pyrogallol (99% pure) dermally for 3 months or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, mouse bone marrow cells, and mouse peripheral blood erythrocytes.

3-MONTH STUDY IN RATS

Groups of 10 male and 10 female rats received dermal applications of pyrogallol in 95% ethanol at doses of 0, 9.5, 18.75, 37.5, 75, or 150 mg pyrogallol/kg body weight, 5 days per week for 14 weeks. Additional groups of 10 male and 10 female special study rats were administered the same doses, 5 days per week for 23 days. All rats survived until the end of the study except for one vehicle control female. Mean body weights of dosed groups of males and females were generally similar to those of the vehicle controls. Chemical-related clinical findings included brown staining and irritation of the skin at the site of application. There were no changes in the hematology, serum clinical chemistry, thyroid hormone values, or organ weights attributable to the dermal administration of pyrogallol. The incidences of squamous hyperplasia, hyperkeratosis, and chronic active inflammation of the skin at the site of application were significantly increased in all dosed groups of males and females.

3-MONTH STUDY IN MICE

Groups of 10 male and 10 female mice received dermal applications of pyrogallol in 95% ethanol at doses of 0, 38, 75, 150, 300, or 600 mg pyrogallol/kg body weight, 5 days per week for 14 weeks. All mice survived until the end of the study. Mean body weights of dosed groups of males and females were similar to those of the vehicle controls. Chemical-related clinical findings included brown staining and irritation at the site of application. There were no changes in the hematology values or organ weights attributable to the dermal administration of pyrogallol. The incidences of squamous hyperplasia, hyperkeratosis, and chronic active inflammation of the skin at the site of application were significantly increased

in all dosed groups of males and females. The incidence of hematopoietic cell proliferation of the spleen in 600 mg/kg males was significantly greater than that in the vehicle control group.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female rats received dermal applications of pyrogallol in 95% ethanol at doses of 0, 5, 20, or 75 mg pyrogallol/kg body weight, 5 days per week for up to 104 weeks. Survival of dosed groups of male and female rats was similar to that of the vehicle control groups. Mean body weights of dosed male and female rats were similar to those of the vehicle control groups throughout the study. Irritation of the skin at the site of application was the only chemical-related clinical finding and occurred in the 20 and 75 mg/kg groups.

In the skin at the site of application, there were significant increases in the incidences of hyperplasia in all dosed groups of males and females, hyperkeratosis in 20 and 75 mg/kg males and all dosed groups of females, inflammation in 75 mg/kg males and 20 and 75 mg/kg females, and sebaceous gland hyperplasia in 20 and 75 mg/kg males and females.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female mice received dermal applications of pyrogallol in 95% ethanol at doses of 0, 5, 20, or 75 mg pyrogallol/kg body weight, 5 days per week for up to 105 weeks. Survival of dosed groups of male mice was similar to that of the vehicle control group. Survival was significantly decreased in 75 mg/kg females; most early deaths in this group were due to ulcers at or adjacent to the site of application. The mean body weights of 75 mg/kg female mice were generally over 10% less than those of the vehicle controls during year 2 of the study. Irritation and/or ulceration of the skin at the site of application were the only chemical-related clinical findings and occurred predominantly in the 20 and 75 mg/kg groups.

In the skin at the site of application, the incidence of squamous cell carcinoma in 75 mg/kg females was significantly greater than that in the vehicle control group. Two 75 mg/kg males had squamous cell papillomas; squamous cell papillomas have not been observed in historical control male mice in four ethanol dermal studies.

Increased incidences of nonneoplastic lesions at the site of application included hyperplasia and hyperkeratosis in all dosed groups; inflammation, fibrosis, and pigmentation in the 20 and 75 mg/kg groups; and sebaceous gland hyperplasia and ulcer in the 75 mg/kg groups. Similar lesions in the skin of the neck and back immediately adjacent to the site of application were observed; the incidences of hyperplasia, hyperkeratosis, ulcer, inflammation, and fibrosis at these sites were significantly increased in 75 mg/kg male and female mice, and the incidence of sebaceous gland hyperplasia was significantly increased in 75 mg/kg female mice.

Dermal application of pyrogallol also resulted in significant increases in the incidences of bone marrow hyperplasia in males and females and lymphoid hyperplasia of the axillary, inguinal, and mandibular lymph nodes; adrenal cortical hematopoietic cell proliferation; and mammary gland hyperplasia in females.

GENETIC TOXICOLOGY

Pyrogallol was tested in two independent bacterial mutation studies; both studies gave positive results in one or more strains of *S. typhimurium* or *E. coli*. In the first study, positive results were seen in *S. typhimurium* strain TA100 with and without S9 exogenous metabolic activation, and negative results were obtained in strain TA98. In the second study, which was conducted with the same lot of pyrogallol that was used in the 3-month and 2-year studies, positive results were obtained in *S. typhimurium* strains TA98, TA100, and in *E. coli* strain WP2 *uvrA*/pKM101 in the absence of S9. With S9, this sample of pyrogallol was mutagenic in the *E. coli* strain but gave equivocal responses in *S. typhimurium* strains TA98 and TA100.

In vivo, a micronucleus test that measured frequency of micronucleated polychromatic erythrocytes in bone marrow of male B6C3F1/N mice following three intraperitoneal injections of pyrogallol, gave negative results. In a second *in vivo* test, no increase in the frequency of micronucleated erythrocytes was observed in the peripheral blood of female B6C3F1/N mice treated with pyrogallol via dermal application for 3 months; in male mice, however, results were equivocal, based on a significant increase in micronucleated erythrocytes observed at a single dose level at the end of the 3-month study.

CONCLUSIONS

Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenic activity** of pyrogallol in male or female F344/N rats administered 5, 20, or 75 mg/kg. There was *equivocal evidence of carcinogenic activity* of pyrogallol in male B6C3F1/N mice based on increased incidences of squamous cell papilloma of the skin at the site of application. There was *some evidence of carcinogenic activity* of pyrogallol in female B6C3F1/N mice based on increased incidences of squamous cell carcinoma of the skin at the site of application.

Dermal administration of pyrogallol caused increased incidences of nonneoplastic lesions of the skin at the site of application in male and female rats and mice, skin adjacent to the site of application in male and female mice, and mammary gland in female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Peer Review Panel's comments and the public discussion on this Technical Report appears on page 13.

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

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Doses in ethanol by dermal application	0, 5, 20, or 75 mg/kg	0, 5, 20, or 75 mg/kg	0, 5, 20, or 75 mg/kg	0, 5, 20, or 75 mg/kg
Body weights	Dosed groups similar to the vehicle control group	Dosed groups similar to the vehicle control group	Dosed groups similar to the vehicle control group	75 mg/kg group generally 10% less than the vehicle control group after week 52
Survival rates	23/50, 28/50, 28/50, 28/50	29/50, 33/50, 26/50, 31/50	37/50, 36/50, 34/50, 31/50	33/50, 30/50, 36/50, 17/50
Nonneoplastic effects	Skin (site of application): hyperplasia (0/50, 6/50, 20/50, 50/50); hyperkeratosis (0/50, 2/50, 21/50, 48/50); inflammation (0/50, 0/50, 0/50, 46/50); sebaceous gland, hyperplasia (0/50, 0/50, 12/50, 48/50)	Skin (site of application): hyperplasia (0/50, 9/50, 11/50, 49/50); hyperkeratosis (0/50, 6/50, 23/50, 49/50); inflammation (0/50, 3/50, 6/50, 49/50); sebaceous gland, hyperplasia (0/50, 0/50, 5/50, 41/50)	Skin (site of application): hyperplasia (8/50, 24/50, 47/50, 50/50); hyperkeratosis (11/50, 43/50, 50/50, 50/50); inflammation (2/50, 6/50, 37/50, 44/50); fibrosis (3/50, 6/50, 28/50, 47/50); pigmentation (0/50, 0/50, 9/50, 39/50); sebaceous gland, hyperplasia (1/50, 6/50, 4/50, 24/50); ulcer (1/50, 1/50, 2/50, 23/50) Skin: hyperplasia (1/50, 1/50, 3/50, 10/50); hyperkeratosis (1/50, 1/50, 3/50, 10/50); ulcer (0/50, 1/50, 3/50, 10/50); inflammation (1/50, 1/50, 3/50, 10/50); fibrosis (1/50, 1/50, 3/50, 10/50)	Skin (site of application): hyperplasia (20/50, 31/50, 49/50, 49/50); hyperkeratosis (24/50, 38/50, 49/50, 49/50); inflammation (12/50, 14/50, 42/50, 48/50); fibrosis (5/50, 6/50, 31/50, 49/50); pigmentation (0/50, 0/50, 35/50, 40/50); sebaceous gland, hyperplasia (1/50, 2/50, 6/50, 34/50); ulcer (2/50, 0/50, 3/50, 33/50) Skin: hyperplasia (1/50, 2/50, 1/50, 9/50); hyperkeratosis (1/50, 2/50, 1/50, 9/50); ulcer (1/50, 1/50, 1/50, 9/50); inflammation (1/50, 0/50, 0/50, 9/50); fibrosis (1/50, 2/50, 1/50, 9/50); sebaceous gland, hyperplasia (1/50, 0/50, 1/50, 7/50) Mammary gland: hyperplasia (5/50, 9/50, 3/50, 16/50)
Neoplastic effects	None	None	None	Skin (site of application): squamous cell carcinoma (0/50, 0/50, 0/50, 4/50)
Equivocal findings	None	None	Skin (site of application): squamous cell papilloma (0/50, 0/50, 0/50, 2/50)	None
Level of evidence of carcinogenic activity	No evidence	No evidence	Equivocal evidence	Some evidence
Genetic toxicology				
Bacterial gene mutations:		Positive in one study in *S. typhimurium* strain TA100 with and without S9 and negative in TA98 under the same conditions; positive in a second study in TA98, TA100, and in *E. coli* WP2 *uvrA*/pKM101 without S9; equivocal in TA98 and TA100 and positive in *E. coli* with S9		
Micronucleated erythrocytes				
Mouse bone marrow *in vivo*:		Negative in males		
Mouse peripheral blood *in vivo*:		Equivocal in males and negative in females		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft NTP Technical Report on pyrogallol on February 8, 2012, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing the NTP studies:

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

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SUMMARY OF PEER REVIEW PANEL COMMENTS

On February 8, 2012, the draft Technical Report on the toxicology and carcinogenesis studies of pyrogallol received public review by the National Toxicology Program's Technical Report Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. M. Mercado-Feliciano, NIEHS, introduced the toxicology and carcinogenesis studies of pyrogallol by describing its occurrence as a natural decomposition by-product of plant tannins and its use in a variety of manufacturing processes and consumer products, its positive response in genetic toxicity tests and short-term contact hypersensitivity tests, and the nonneoplastic lesions observed in short-term studies and the neoplasms observed at the site of application in the long-term rodent studies. The proposed conclusions were *no evidence of carcinogenic activity* of pyrogallol in male or female F344/N rats, *equivocal evidence of carcinogenic activity* of pyrogallol in male B6C3F1/N mice, and *some evidence of carcinogenic activity* of pyrogallol in female B6C3F1/N mice.

Dr. Cattley, the first primary reviewer, said the study report was clear and justified the conclusions. He noted that discussion in the report had mentioned determination of a no-observable-adverse-effect level (NOAEL), which he was unaccustomed to seeing in an NTP report, and asked if it should be addressed in the conclusions. He thought the dose selection was reasonable but wanted more rationale about the choice of the top dose. He asked about the criteria for removing animals from the study. He noted that all of the findings in the 2-year study were at the site of application except the mammary gland hyperplasia, and asked if there was some explanation for those.

Dr. Alcorn, the second primary reviewer, asked for clarification regarding the lower body weight of the 75 mg/kg female mice compared to vehicle controls in the 2-year study. She asked if transference from the site of application may have taken place, perhaps as a result of grooming behavior, that might explain the squamous cell papillomas found on the ear and nose of the three rats.

Dr. Soper, the third primary reviewer, thought the study was well designed and that the conclusions were well justified. He was interested in the skin papillomas in the male rats but did not think they rose to the level of *equivocal evidence*.

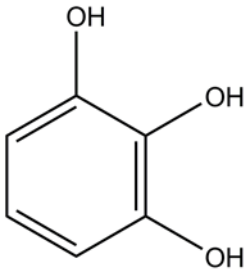
Dr. Mercado-Feliciano said that 75 mg/kg had been chosen as the top dose because that appeared to be the minimum concentration that would give the maximum response, as had been seen in the 3-month study. Dr. R.A. Herbert, NIEHS, said that NTP specifications were followed for removal of animals, although the attending veterinarian is allowed latitude, particularly where the welfare of the animals is concerned. He said that there did not seem to be any qualitative difference between the mammary gland hyperplasia in the vehicle control animals and that in the dosed animals. Dr. Mercado-Feliciano said that feed consumption is not routinely monitored in dermal application studies, so there was no ready explanation for the lower body weights of the 75 mg/kg female mice. She said the three squamous cell papillomas not at the site of application in the rats were not considered to be treatment related.

Dr. Cattley found the top dose choice to be reasonable and suggested that instead of referring to it as a NOAEL, it should be expressed as highest dose tolerated with no effect on survival. Dr. Alcorn suggested that monitoring of feed consumption be included as part of a study's humane intervention checklist. Dr. Soper spoke in support of the 75 mg/kg dose as the top dose, actually extrapolating from human to rodent, citing a human male who had dosed himself at approximately 143 mg/kg and died acutely.

Dr. Pino suggested the abstract should include a statement that the skin papillomas were not considered treatment-related; otherwise reference to the lesions should be removed from the abstract. Dr. Elwell thought 75 mg/kg may have been excessive once the dosing of female mice was increased based on their doubling in weight. Dr. Mirsalis suggested that the micronucleus data be clarified.

Dr. Soper moved to accept the proposed conclusions as written. Dr. Cattley seconded the motion, which was accepted unanimously with 10 votes.

INTRODUCTION



PYROGALLOL

CAS No. 87-66-1

Chemical Formula: $C_6H_6O_3$ Molecular Weight: 126.11

Synonyms: 1,2,3-Benzenetriol; 2,3-dihydroxyphenol; gallamine; pyrogallic acid; 1,2,3-trihydroxybenzene
Trade names: C.I. Oxidation Base 32, C.I. 76515, C.I. 76551, Fouramine Base AP, Fouramine Brown AP, Fourrine PG, Fourrine 85, Piral, Pyro

CHEMICAL AND PHYSICAL PROPERTIES

Pyrogallol is a hydrophilic compound (626 g/L H_2O; 1.463 specific gravity) and exists at room temperature as white or colorless crystal leaflets or needles (131° to 133° C melting point) that become grayish or beige on exposure to air or light (Leston, 2000; *Merck*, 2006; *CRC Handbook*, 2011).

Pyrogallol is oxidized in air or in the presence of ammonia or alkali, becoming first brown then a black lustrous powder, a process that is faster in moist air or aqueous solution (Allen, 1907; Leston, 2000). The oxidized (black) product is insoluble in water, alcohol or ether, but soluble in basic solution. The red-brown to black mordant dye purpurogallin (CAS No. 569-77-7) forms from mild oxidations of pyrogallol, but other unknown oligomeric species may be formed by self-condensation of a hydroxyquinone intermediate (Haslam, 2003).

The autoxidation of pyrogallol in aqueous solution is a well known process with applications in analytical chemistry and biochemistry, including, for example, the determination of dissolved oxygen (Scholander *et al.*, 1955) and superoxide dismutase activity (Marklund and Marklund, 1974). The fact that superoxide dismutase inhibits pyrogallol autoxidation suggests that the formation of the superoxide radical $^{\bullet}O_2^-$ is required (Marklund and Marklund, 1974). Pyrogallol also produces H_2O_2 *in vitro* more than other polyphenols (pyrogallol > 1,2,4-benzenetriol > hydroquinone > catechol) and is one of only two polyphenols able to produce $^{\bullet}OH$ radicals *in vitro* without the need of a metal ion catalyst (Lin and Lee, 1992; Lee and Lin, 1994).

As a weak acid (pK_{a1}=9.01, pK_{a2}=11.64; Kortüm *et al.*, 1961), pyrogallol forms unstable salts in the presence of bases. Pyrogallol also forms metallic salts or chelates with many metals (Leston, 2000).

Commercially available pyrogallol is up to 99% pure and minor impurities may include a number of inorganic ions like chloride, sodium, potassium and sulfate, as well as aluminum, iron, and other heavy metals (Cerilliant, 2010; Sigma-Aldrich, 2011).

PRODUCTION, USE, AND HUMAN EXPOSURE

Pyrogallol is a benzenetriol produced when carbon dioxide is split from gallic acid by heat (Allen, 1907). Derivatives of pyrogallol occur widely in plants, mostly as

flavones, alkaloids, and tannins; pyrogallol itself is found in nature as a product of the decomposition of plant tannins (Leston, 2000; Tan, 2003).

For commercial purposes, pyrogallol is usually prepared by heating crude gallic acid, a procedure first published by Scheele in 1786 (*Merck*, 2006). Crude gallic acid is extracted from nutgalls (nutlike swellings produced by trees around parasitic wasps) or the seeds of the tara shrub (*Caesalpinia spinosa*), a thorny shrub native to South America. Other chemical synthesis methods can be used including the chlorination of cyclohexanol to form tetrachlorocyclohexanone, followed by hydrolysis (Allen, 1907; Leston, 2000).

As of 2011, pyrogallol was available from at least 32 vendors worldwide (CSI, 2011), in different purity grades and at quantities between 5 g and 4 kg (NCBI, 2011).

Major historical uses of pyrogallol include hair dyeing, leather and wool staining, and photographic developing. The current main commercial application of pyrogallol is the production of pharmaceuticals and pesticides (Leston, 2000). While large scale production of pyrogallol by chemical synthesis is a necessary step in the production of the carbamate insecticide Bendiocarb (2,2-dimethyl-1,3-benzodioxol-4-yl N-methylcarbamate, also known as Ficam; CAS No. 22781-23-3) (Leston, 2000), use of this pesticide was phased out in the United States in 2000 to 2001 (USEPA, 1999); the EPA cancelled its registration and revoked residue tolerance in 2004 (*Fed. Regist.*, 2004). In analytical chemistry, pyrogallol is used as a complexing agent, reducing agent, and, in alkaline solution, as an indicator of gaseous oxygen (*Merck*, 2006).

While pyrogallol seems to be the first synthetic organic dye used on human hair (Merck, 1983; Winter, 2009) and, as recently as the early 1990s, used in the United States as a modifier in oxidation dyes, the most recent chemical manuals make little or no mention of its usage as a hair dye (Leston, 2000; *Merck*, 2006). In the United States, typical pyrogallol concentrations in hair dyes ranged from 0.1% to 5.0% by weight (*Patty's*, 1981), and it was present in at least 42 hair dyes and colors available to consumers during the late 1980s and early 1990s (CIR, 1991). The last known United States consumer product containing pyrogallol as an ingredient was registered with the FDA's Voluntary Cosmetic Registration Program in 1993 (R.L. Bronaugh, FDA, personal communication, 2010). Manufacture of hair products containing pyrogallol in South America has been reported as recently as 2005 (Mazzei *et al.*, 2007).

Pyrogallol is also one of the oldest photographic developers still in use (Leston, 2000), although current use seems to be confined mostly to fine arts photography as the major component of the PMK liquid developer (also known as Gordon Hutchings formula) (Bergger, 2011). In the United States, PMK developer is distributed by at least two companies: Bergger Products, Inc. (Rockford, IL) and Photographers' Formulary Inc. (Condon, MT).

A review of current and recent patents reveals many other possible applications of pyrogallol, including formulation of polymeric coatings (Hasegawa *et al.*, 2011; Russell and Schultz, 2011) and as an antioxidant or corrosion-inhibiting agent to protect a variety of materials during processing or cleaning (Creelman *et al.*, 2010; Reuber *et al.*, 2010; Rovito *et al.*, 2010; Suzuki and Otake, 2010; 21 CFR § 101.108). Additionally, pyrogallol is used as "an oxygen scrubbing solution" for producing high purity nitrogen used in the Monier-Williams Procedure for determining sulfites in food.

Medically, pyrogallol has been used in the past as a topical antipsoriatic, typically applied in an ointment containing 2% to 10% pyrogallol (Pewny, 1925; *Merck*, 1968; Willsteed and Regan, 1985). In Europe in the 1970s, pyrogallol was used in conjunction with ultraviolet B for the treatment of resistant psoriasis (Willsteed and Regan, 1985).

At the time of the National Occupational Exposure Survey (1981-1983), 38,038 U.S. workers were potentially exposed to pyrogallol, 65.5% of them female (NIOSH, 1990). Most of the workers were hairdressers or cosmetologists (49.8%), nurses or health aides (24.8%), or aircraft engine mechanics (17.6%). All aircraft mechanics and 34.3% of hairdressers or cosmetologists were subjected to uncontrolled potential exposure, that is, no personal protective equipment, additional ventilation or other exposure control methods were used.

Humans may also be exposed to pyrogallol in foods. Pyrogallol has been identified in the mg/kg range in brewed coffee, roasted malt, bread crust, and cocoa powder and in the µg/kg range in beer and potato crisps (Lang *et al.*, 2006). It may also be present in smoked foods and smoke condensate used to prepare foods, although chemical identification has not been conclusive (Knowles *et al.*, 1975; Ohshima *et al.*, 1989). Metabolism by human intestinal bacteria seems to convert gallic acid and other polyphenols found in foods, especially tea, to pyrogallol (Meselhy *et al.*, 1997; Daykin *et al.*, 2005; Schantz *et al.*, 2010). Pyrogallol, pyrogallol conjugates,

and 2-*O*-methylpyrogallol were detected in the urine of rats fed a diet containing gallic acid (Booth *et al.*, 1959; Scheline, 1966).

REGULATORY STATUS

Although the Cosmetic Ingredient Review Expert Panel of the United States Cosmetics, Toiletry and Fragrance Association found pyrogallol safe as a cosmetic ingredient at the prevalent concentration and usage in 1991 (CIR, 1991), pyrogallol is not included in the current list of "Color Additives Approved for Use in Cosmetics" (21 CFR Parts 73 and 74). However, pyrogallol is approved in the United States in combination with ferric ammonium citrate for coloring plain and chromic catgut sutures for use in general and ophthalmic surgery (not to exceed 3% ferric ammonium citrate-pyrogallol complex) (21 CFR § 73.1375).

The use of pyrogallol in cosmetics was forbidden by the European Union in 1992 (SCCNFP, 2003) and in Japan by 2001 (MHW, 2000); however, it is not included in the list of cosmetic ingredients regulated by the Mercado Común de Sur (a free trade agreement between Argentina, Brazil, Paraguay, and Uruguay) (MERCOSUR, 2005).

No Threshold Limit Value, Recommended Exposure Limit, or Permissible Exposure Limit has been established for pyrogallol.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

There is limited literature on the metabolism and disposition of pyrogallol. *In vitro*, pyrogallol is metabolized to 2-*O*-methylpyrogallol after incubation with rat catechol-*O*-methyl transferase (COMT) (Masri *et al.*, 1964). Following gavage or intraperitoneal administration of 100 mg/kg pyrogallol in albino rats (sex not specified), pyrogallol, 2-*O*-methylpyrogallol, and traces of resorcinol were detected in the urine 24 hours after dosing (Scheline, 1966). In another investigation, following gavage administration of 100 mg/kg pyrogallol in male albino rats, 6.2% of the dose was detected in the urine 48 hours after dosing as 2-*O*-methyl pyrogallol (Bakke, 1970). In male mice (strain not provided), the maximum concentration of pyrogallol in the brain was detected 10 minutes after intraperitoneal administration of 120 mg/kg (Rogers *et al.*, 1968). These results were confirmed in another intraperitoneal study using 60 mg/kg in female mice (strain not specified); within 15 minutes after administration, the brain concentration of pyrogallol was undetectable (Angel *et al.*, 1969). Following an intraventricular dose of 2 mg of pyrogallol in male Wistar rats, formation of three unidentified esters of pyrogallol was reported in the brain (Eccleston and Ritchie, 1973). Pyrogallol was metabolized to resorcinol by albino rat fecal extract *in vitro*. This conversion was inhibited by a number of antibiotics, suggesting a role of intestinal microflora in the conversion (Scheline 1966, 1968). Pyrogallol was unstable in rodent blood *in vitro*; however, in the presence of ascorbic acid and at pH 3, biological samples were stable up to 3 weeks when stored frozen (Algaier *et al.*, 2003).

Humans

No studies of the metabolism or disposition of pyrogallol in humans *in vivo* were available in the literature. However, pyrogallol was detected in the urine and feces as a metabolite of tea polyphenols (Meselhy *et al.*, 1997; Daykin *et al.*, 2005; Schantz *et al.*, 2010). Also, the conversion of pyrogallol to purpurogallin *in vitro* by human hemoglobin was reported (Miyazaki *et al.*, 2004).

TOXICITY

Experimental Animals

Ingestion of oak and tree legume species high in hydrolyzable tannins is toxic to ruminant animals, and the effect seems to be due in part to metabolism of tannins to pyrogallol (Reed, 1995). Major lesions associated with tannin poisoning in ruminants are hemorrhagic gastroenteritis, necrosis of the liver, and kidney damage with proximal tubular necrosis. Acute intoxication results in high mortality and morbidity in cattle and sheep. In a field study, 6 to 13 ng/g of pyrogallol were found in the rumen of cows that fell ill or died after repeated intake of green acorns and oak leaves (Meiser *et al.*, 2000).

Pyrogallol is a substrate of COMT and a potent COMT inhibitor *in vitro* (Lautala *et al.*, 2001), and several studies suggest that pyrogallol treatment changed catecholamine levels in various regions of the brain in mice, rats, and rabbits (Guldberg and Marsden, 1975). However, since not all studies found changes in catecholamine levels *in vivo*, it has been suggested that a transient rise followed by a fall in brain catecholamines after pyrogallol treatment may be caused by an initial inhibition of COMT followed by a feedback inhibition of catecholamine synthesis. Convulsions observed in 50% of mice after exposure to 720 mg/kg intraperitoneal pyrogallol were concurrent with distinct cyanosis, therefore anoxia could not be ruled out as the cause (Angel and Rogers, 1968).

Pyrogallol is also a potent inhibitor of thyroid peroxidase and seems to inhibit iodine uptake and incorporation into tyrosine. In rats dosed with 12.6 or 31.5 mg subcutaneously, uptake of radioactive iodine was deceased to about 30% and 65% of control, respectively, when measured 2.3 hours after treatment (Arnott and Doniach, 1952). Cooksey *et al.* (1985) showed that pyrogallol is a 1.9-fold more potent inhibitor of swine thyroid peroxidase than 6-propylthiouracil *in vitro*. Pyrogallol oxidation products administered at a concentration of 0.1% in drinking water to male and female rats for 8 to 14 weeks produced changes in the thyroid gland consistent with a pre-goiter condition, including increased height of follicular epithelial cells and new formation of small follicles (Seffner *et al.*, 1995). While pyrogallol has not been associated with thyroid gland effects in humans, exposure to the structurally similar compound resorcinol has been associated with endemic goiter in geographical regions with high resorcinol content in the soil (Gaitan, 1983) and thyroid gland side effects in patients treated with resorcinol-containing ointments (Lynch *et al.*, 2002).

Sprague-Dawley rat oral LD_{50} values for pyrogallol range from 800 mg/kg in females to 1,800 mg/kg in males (CIR, 1991). In one study, the oral LD_{50} in rabbits was 1.6 g/kg, and single gavage doses of 750 to 2,000 mg/kg given to rabbits produced congestion of the lung, liver, kidneys, and spleen as well as gastritis with hemorrhages and/or ulcers in some animals (Dollahite *et al.*, 1962). One study found that the maximum tolerable intraperitoneal dose of pyrogallol for Sprague-Dawley rats was 100 mg/kg per day, after treating for 7 days and monitoring mortality for 30 days (Joharapurkar *et al.*, 2004). No LD_{50} could be found in the literature for the dermal route. Dermal exposure of rabbits to 5% to 50% pyrogallol applied to the interior left ear twice a week during their lifetime did not affect survival (Stenbäck, 1977). Twenty-five mg of pyrogallol applied to the backs of guinea pigs for 24 hours using occlusive dermal patches did not affect survival but produced slight irritation to the skin (CIR, 1991).

Lifetime exposure to up to 0.01 mg pyrogallol in acetone applied to the skin of mice twice per week did not induce significant changes in the skin or shorten lifespan (Stenbäck and Shubik, 1974). Similarly, application of an oxidative hair dye containing 0.49% pyrogallol (mixed with 6% peroxide solution before application) to the shaven skin of mice once per week for 9 or 20 months did not affect average body weight gain or survival (Jacobs *et al.*, 1984). However, a calculated dose of 8 mg/kg per day applied to the shaved skin of rats (exposure duration not specified, but probably less than 21 days) led to skin irritation (Burnett *et al.*, 1976).

Humans

Pyrogallol ingestion or excessive skin application can cause severe poisoning and ultimately death in humans (Gosselin *et al.*, 1984). A psoriatic patient who covered two-thirds of his body with an ointment containing pyrogallol collapsed within 5 minutes and died in a coma 24 hours later (Pewny, 1925). The man absorbed an estimated 10 g of pyrogallol, which corresponds to a 143 mg/kg dose based on a 70-kg body weight. Another psoriasis patient that had applied an aqueous cream containing 10% (793 mM) pyrogallol to his hands nearly every morning for 40 years, and wore white gloves for the remainder of the day, developed ulcerated lesions on the hand's dorsa that could not be explained by exposure to other substances or UV radiation (Willsteed and Regan, 1985). Poisoning through skin absorption or ingestion has been reported to produce a range of symptoms and outcomes, including local pain/edema, malaise, nausea, vomiting, diarrhea, organ congestion, hemorrhage, parenchymatous degeneration of the liver, or nephritis, somnolence, coma, convulsions, cardiac failure, and death (reviewed by von Oettingen, 1949).

IMMUNE SYSTEM TOXICITY

Experimental Animals

Pyrogallol has been shown to be a weak skin sensitizer in experimental animal models. Although positive in the murine local lymph node assay (LLNA), pyrogallol was negative in a mouse ear swelling test (NTP, 2006). In the same study, pyrogallol was positive in the murine irritancy assay, suggesting that at least some of the proliferative response observed in the LLNA was due to the compound's irritant effect. Skin sensitization studies in guinea pigs following pyrogallol exposure have demonstrated conflicting results. In a study using female Hartley guinea pigs more than 50% of the animals became sensitized to pyrogallol following repeated dosing at concentrations as low as 0.1 M (CIR, 1991). However, a similar study showed no evidence of sensitization in guinea pigs after pyrogallol exposure (CIR, 1991).

In Sprague-Dawley rats, treatment with 25 mg/kg pyrogallol per day for 7 days suppressed humoral immunity when measured as inhibition of the antigen-specific antibody response against sheep red blood cells (Joharapurkar *et al.*, 2004). Similarly, inhibition of the production of antibodies specific to sheep red blood cells was observed when murine lymphocytes were cultured *in vitro* in the presence of 5 µg pyrogallol (Archer *et al.*, 1978). Higher doses of pyrogallol (50 and 100 mg/kg) suppressed cell-mediated immunity, phagocytosis, and nonspecific measures of inflammation in Sprague-Dawley

rats (Joharapurkar *et al.*, 2004). This is consistent with studies that showed suppression of cell-mediated immunity in rats following intraperitoneal administration of 25 mg/kg pyrogallol (Bhalla *et al.*, 1970).

Humans

Pyrogallol has been demonstrated to be a contact sensitizer in a number of human studies, particularly those targeting individuals exposed to hairdressing chemicals. A study by Keil (1962) tested eight individuals known to be sensitive to resorcinol; five of those individuals were mildly sensitized by treatment with 2% (159 mM) pyrogallol in alcohol by occlusive patch (exposure length not provided). Three studies investigated the ability of pyrogallol to induce skin sensitization in hairdressers and their customers who had contact dermatitis. The volunteers were administered 1% (79 mM) pyrogallol in petrolatum by occlusive patch for 2 to 3 days. In one study of 302 hairdressers, 1.3% (four individuals) had positive skin sensitization to the treatment (Guerra *et al.*, 1992a). In another study of 261 customers of hairdressers, 2.3% (six individuals) experienced sensitization reactions (Guerra *et al.*, 1992b). In a third study of 781 hairdressers, 0.76% (six individuals) experienced positive sensitization reactions (Frosch *et al.*, 1993). Two more studies (one each in the United States and Germany) compiled medical record data from patients undergoing patch testing (1% pyrogallol) for suspected allergies to hairdressing chemicals. The United States study found that out of 209 patients tested, 9.1% developed a positive reaction; no patients developed an irritant reaction (Wang *et al.*, 2011). The German study found that out of 628 patients tested, 5.4% had a positive reaction (Hillen *et al.*, 2007).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

After pyrogallol was administered by gavage (100, 200, or 300 mg/kg) to Sprague-Dawley rats on days 6 through 15 of gestation, a significant reduction in mean maternal weight gain, increase in resorption, and decreased fetal body weight were observed only at the highest dose; no statistically significant differences in gross, visceral, or skeletal anomalies or variations were found when compared to controls (Picciano *et al.*, 1983). In two dermal studies, hair dye formulations containing 0.4% pyrogallol were applied to the skin of pregnant rats with no adverse effects noted in maternal or fetal rats. In one study, a calculated dose of 8 mg/kg per day was applied to the shaved skin of pregnant rats on days 1, 4, 7, 10, 13, 16, and 19 of gestation; more frequent application led to skin irritation in a pilot study (Burnett *et al.*, 1976). In the other study, 1 mg pyrogallol was applied to rats twice per week throughout mating, gestation, and lactation through weaning for 3 generations (CIR, 1991).

CARCINOGENICITY

Experimental Animals

Pyrogallol is a known cocarcinogen (Van Duuren, 1980). Pyrogallol acted as a potential or effective cocarcinogen in stomach and skin, but was not a promoter in the bladder. Pyrogallol did not increase the height of the mucosal epithelium by itself in rats fed 2% in the diet for 4 weeks, but it did potentiate the increase induced by sodium nitrite (Yoshida *et al.*, 1994). When 5 mg (0.04 mmol) pyrogallol and 0.005 mg benzo(*a*)pyrene were simultaneously applied to the clipped dorsal skin of mice three times per week for 440 days, squamous cell carcinomas were observed in 33 of 50 animals (Van Duuren and Goldschmidt, 1976). In an initiation/promotion study, pyrogallol did not significantly promote hyperplasia or papilloma of the urinary bladder in rats given 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine in the diet for 2 weeks followed by 0.5% pyrogallol in the diet for 22 weeks (Miyata *et al.*, 1985).

As with its cocarcinogenic effects, pyrogallol alone seems to induce cancer or precancerous lesions in some organs but not others. Exposure to feed containing 1% pyrogallol for 20 weeks induced mild hyperplasia of the forestomach in hamsters (Hirose *et al.*, 1986), but a 2% diet for 4 weeks did not have the same effect in rats (Yoshida *et al.*, 1994). Feeding pyrogallol at 0.5% in the diet for 22 weeks did not induce hyperplasia or papilloma of the urinary bladder in rats (Miyata *et al.*, 1985). However, subcutaneous injection of pyrogallol (100 mg/kg per day for 8 weeks followed by 14 mg/rat per day for 50 weeks) induced histiocytomas at the injection site in four out of 19 rats while none of the control rats developed tumors (Wang and Klemencic, 1979). In a lifetime dermal study, rabbits treated with 50% pyrogallol did not develop skin tumors; however one out of five animals developed a uterine tumor while none of the control animals or those treated with 5% or 25% pyrogallol developed any tumors (Stenbäck, 1977). In a 20-month dermal study, the incidences of commonly occurring tumors (lung adenoma, liver hemangioma, and malignant lymphoma) in mice treated with an oxidative dye containing 0.49% pyrogallol mixed with 6% peroxide solution before application were similar to those in control animals (Jacobs *et al.*, 1984).

Humans

No data on the epidemiology of pyrogallol exposure in humans were found in the literature.

GENETIC TOXICITY

Pyrogallol has been shown to induce DNA damage in acellular systems, and mutagenic and clastogenic responses in cell-based assays. The chemical was also reported to have some anticlastogenic and antimutagenic activity under certain experimental conditions.

Pyrogallol induced double-strand breaks in purified λ phage DNA and pBR322 plasmid DNA at 32 µg/mL (Yamada *et al.*, 1985). It also induced double-strand breaks in pBR322 plasmid DNA whether or not Fe^{++} was present (Lee *et al.*, 1995). Pyrogallol induced DNA strand breakage in calf thymus DNA in the presence, but not in the absence, of Cu^{++}; this latter effect was reversed when catalase or glutathione, but not superoxide dismutase, was added (Hayakawa *et al.*, 1997).

Pyrogallol has been tested extensively for mutagenicity in bacteria. Although many of the studies showed the compound to be mutagenic, some contradictory results within certain strains were reported. Specifically, in bacterial strains that mutate via frameshifting, pyrogallol was mutagenic to *Salmonella typhimurium* strain TA97 in the presence and absence of microsomal activation (S9) (Gocke *et al.*, 1981; Glatt *et al.*, 1989; Lin and Lee, 1992). Mixed results were reported with pyrogallol in *S. typhimurium* strain TA98, with some laboratories reporting mutations (Gocke *et al.*, 1981; Picciano *et al.*, 1983) and several others failing to demonstrate induction of mutation (Ben Gurion, 1979; Sakagami *et al.*, 1986; Glatt *et al.*, 1989; Watanabe *et al.*, 1991; Lin and Lee, 1992). Pyrogallol was mutagenic to *S. typhimurium* TA1537 with and without S9 (Ben-Gurion, 1979), but no mutagenicity was observed in strain TA1538 following exposure to pyrogallol, up to cytotoxic doses (Picciano *et al.*, 1983).

Mutagenicity test results reported for pyrogallol in *S. typhimurium* TA100, a strain that reverts via base substitution, were positive, with and without S9 (Ben-Gurion, 1979; Gocke *et al.*, 1981; Yamaguchi, 1981; Sakagami *et al.*, 1986; Glatt *et al.*, 1989; Lin and Lee, 1992). However, there is no evidence that pyrogallol was mutagenic to *S. typhimurium* TA1535, another base substitution strain, in either the presence or absence of S9 activation (Gocke *et al.*, 1981; Glatt *et al.*, 1989).

In *S. typhimurium* TA102, pyrogallol was found to induce revertants in the absence of S9 (Glatt *et al.*, 1989; Watanabe *et al.*, 1998), but with S9, no mutagenicity was detected (Glatt *et al.*, 1989). Pyrogallol was mutagenic to *S. typhimurium* strain TA2638 in the absence of metabolic activation; it was not tested with S9 (Watanabe *et al.*, 1998). It was also mutagenic to strain TA104 both in the presence and absence of metabolic activation (Glatt *et al.*, 1989).

Pyrogallol was mutagenic to *Escherichia coli* WP2 pKM101 and WP2 *uvrA* in the absence of metabolic activation by S9 mix (Watanabe *et al.*, 1998).

The genotoxicity of pyrogallol was tested at pH levels ranging from 5 to 10 in strain D7 of *Saccharomyces cerevisiae*. Pyrogallol induced significant mitotic gene conversion at alkaline pH but not at neutral pH (Rosin, 1984). Pyrogallol has been shown to induce sister chromatid exchanges and micronuclei in V79 cells (Glatt *et al.*, 1989). It also induced chromosomal aberrations in Chinese hamster ovary (CHO) cells in the absence and presence of S9 (Stich *et al.*, 1981) and in V79 cells, with S9 (Do Céu Silva *et al.*, 2003). Nakamura *et al.* (1997) were not able to demonstrate any clastogenic activity for pyrogallol in CHO cells, but in CHO cells treated with mitomycin-C, subsequent treatment with pyrogallol was shown to reduce the frequency of mitomycin-C-induced aberrant cells (anticlastogenic activity).

The inhibitory effect of pyrogallol on clastogen-induced chromosomal damage was also demonstrated *in vivo*. The incidence of benzo(*a*)pyrene-induced micronuclei in mouse bone marrow was decreased when benzo(*a*)pyrene was coadministered with pyrogallol (Paschin *et al.*, 1986). In contrast, Gocke *et al.* (1981) reported that pyrogallol induced micronuclei in mouse bone marrow and sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* strains Berlin K and Basc (Gocke *et al.*, 1981).

STUDY RATIONALE

Pyrogallol was nominated for testing based on its frequent occurrence in natural and manufactured products, including hair dyes, and scarcity of carcinogenicity data at the time of nomination. Testing was done in both sexes of F344/N rats and B6C3Fl/N mice through the dermal route because that is the primary route of exposure for humans.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Pyrogallol

Pyrogallol was obtained from Aceto Corporation (Lake Success, NY) in one lot (010326) which was used in the 3-month and 2-year animal studies. Two other lots (A008328201 and 83282/1) were purchased from Acros Organics (Pittsburgh, PA); these two lots were not used in the animal studies but were used in methods development and stability studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Midwest Research Institute (MRI; Kansas City, MO) (Appendix I). The study laboratory at Battelle Columbus Operations (Columbus, OH) conducted additional identity analyses, and MRI conducted bulk chemical stability analyses. Reports on analyses performed in support of the pyrogallol studies are on file at the National Institute of Environmental Health Sciences.

Lot 010326 of the chemical, a white crystalline powder, was identified as pyrogallol using melting point analyses, infrared, ultraviolet, proton nuclear magnetic resonance, and mass spectroscopy. Karl Fischer titration results were inconclusive due to a reaction of pyrogallol with the reagent used, and weight loss on drying could not be determined due to the sublimation of the test article. Thin-layer chromatography indicated a single major spot and no impurities. High-performance liquid chromatography with ultraviolet detection (HPLC/UV) indicated one major peak and no impurities with areas equal to or greater than 0.05% of the total peak areas in four of six containers tested; one container had one impurity, and another had two impurities that had peak areas greater than or equal to 0.05% of the total peak area. The overall purity of lot 010326 was determined to be 99% or greater.

Stability studies of lot A008328201 of the bulk chemical were performed using HPLC/UV. These studies indicated that pyrogallol was stable as a bulk chemical for at least 2 weeks when stored under an inert headspace, protected from light, at temperatures up to 60° C. To ensure stability, the bulk chemical was stored under an inert headspace, sealed in amber glass bottles kept at room temperature. Periodic reanalyses of the bulk chemical were performed during the 3-month and 2-year studies using HPLC/UV, and no degradation of the bulk chemical was detected.

Ethanol (95%)

USP-grade 95% ethanol was obtained from Spectrum Chemicals and Laboratory Products (Gardena, CA) in multiple lots that were used as the vehicle in the 3-month and 2-year studies. Identity and purity analyses were conducted by the study laboratory. The chemical, a clear liquid, was identified as ethanol containing approximately 5% to 10% water using infrared spectroscopy. The purity of each lot was determined using gas chromatography. No impurities (including benzene) were detected that exceeded a relative concentration of 0.1% in any lot. Periodic reanalyses of the 95% ethanol vehicle were performed by the study laboratory at approximately 6-month intervals during the 2-year studies and no degradation of the chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing pyrogallol and 95% ethanol to give the required concentrations (Table I3). The dose formulations were stored at approximately 5° C in amber glass bottles sealed with Teflon®-lined lids inside plastic bags (2-year studies only) for up to 42 days.

Stability studies of a 125 µg/mL formulation made with lot 83282/1 were performed by the analytical chemistry laboratory using HPLC/UV. Stability was confirmed for at least 42 days for formulations stored in amber glass containers sealed with Teflon®-lined lids at approximately 5° C and for at least 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of pyrogallol were conducted by the study laboratory using HPLC/UV. During the 3-month studies, the dose formulations were analyzed three times; animal room samples of these dose formulations were also analyzed (Table I4). All 15 dose formulations analyzed for rats and mice were within 10% of the target concentrations; all 15 animal room samples for rats and all 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose

formulations were analyzed approximately every 3 months; animal room samples were also analyzed (Table I5). Of the dose formulations analyzed, all 27 for rats and all 27 for mice were within 10% of the target concentrations; all nine animal room samples for rats and all nine for mice were within 10% of the target concentrations.

3-MONTH STUDIES

Three-month studies were conducted to evaluate the cumulative toxic effects of repeated exposures to pyrogallol and to determine the appropriate doses to be used in the 2-year studies. The selection of dose formulation concentrations for the 3-months studies in rats and mice was limited by the maximum solubility of pyrogallol in the ethanol vehicle and the fixed dosing volumes used. The maximum concentration determined for the test article solubility was 300 mg pyrogallol/mL 95% ethanol. Therefore, based on the solubility of pyrogallol in ethanol, the highest doses selected for rats and mice were 150 mg/kg and 600 mg/kg, respectively. Assessment of thyroid gland function was included in the 3-month rat study because pyrogallol is a potent inhibitor of thyroid peroxidase.

Male and female F344/N rats and B6C3F1/N mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 to 6 (rats) or 4 to 5 (mice) weeks old. Rats were quarantined for 11 (males) or 12 (females) days, and mice were quarantined for 13 (females) or 14 (males) days. Rats were 5 to 7 weeks old, and mice were 6 to 7 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At 4 weeks and at the end of the studies, serologic analyses were performed on five male and five female sentinel rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice received dermal applications of pyrogallol in 95% ethanol at doses of 9.5, 18.75, 37.5, 75, or 150 mg pyrogallol/kg body weight (rats) or 38, 75, 150, 300, or 600 mg/kg (mice) 5 days per week for 14 weeks. Groups of 10 vehicle control male and female rats and mice received the 95% ethanol vehicle alone. Groups of 10 male and 10 female special study rats were administered the same doses for 23 days. Dosing volumes were 0.5 mL/kg body weight for rats and 2.0 mL/kg for mice. A Corning Lambda (Corning, Inc., Corning, NY) single channel pipetter with a disposable polyethylene tip was used to administer each dose over the application site, which extended from the mid-back to the interscapular area. An area slightly larger than the application site was clipped 24 hours before the first dose and weekly thereafter.

Feed and water were available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded for core study animals initially, weekly, and at the end of the studies. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Blood was collected from the retroorbital sinus of special study rats on days 4 and 23 and mice at study termination and by cardiac puncture from core study rats at study termination for hematology (rats and mice), clinical chemistry (rats), and thyroid hormone (day 23 and core study rats) analyses. Animals were anesthetized with a mixture of CO_2/O_2. Blood samples were collected into microcollection serum separator tubes, and serum was obtained by centrifugation for clinical chemistry and thyroid hormones. Blood was also collected into microcollection tubes containing potassium EDTA as the anticoagulant for hematology. Using reagents obtained from the instrument manufacturer, clinical chemistry parameters and total thyroxine were measured using a Hitachi 911 chemistry analyzer (Roche Diagnostics Corp., Indianapolis, IN), hematology parameters were measured using an Advia 120 hematology analyzer (Siemens Healthcare Diagnostics, Deerfield, IL), and thyroid stimulating hormone and total triiodothyronine were performed by radioimmunoassay using a Packard Cobra II gammacounter (Packard Instrument Company, Meriden, CT). The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on rats in the vehicle control, 37.5, 75, and 150 mg/kg groups and mice in the vehicle control, 150, 300, and 600 mg/kg groups. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal kill, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was

made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, thymus, thyroid gland, and uterus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on core study vehicle control rats and mice, 150 mg/kg rats, and 600 mg/kg mice; tissues were examined to a no-effect level, except skin at the site of application was examined in all rats and mice and spleen was examined in all mice. Table 1 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, NTP pathologist, reviewing pathologist(s) and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats and mice received dermal applications of pyrogallol in 95% ethanol at doses of 5, 20, or 75 mg/kg, 5 days per week for up to 104 (rats) or 105 (mice) weeks. Groups of 50 male and 50 female vehicle control rats and mice received applications of the ethanol vehicle alone. Dosing volumes were 0.5 mL/kg for rats and 2.0 mL/kg for mice. Formulations were applied to the application site using an adjustable volume, single channel pipetter with a disposable polyethylene tip. An area slightly larger than the application site, from the mid-back to the interscapular area, was clipped 24 hours before the first dose and weekly thereafter.

Source and Specification of Animals

Male and female F344/N rats and B6C3F1/N mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-year studies. Rats were quarantined for 13 (males) or 14 (females) days, and mice were quarantined for 11 (females) or 12 (males) days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats were 6 to 7 weeks old and mice were 5 to 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Studies were approved by the Battelle Columbus Operations Animal Care and Use Committee and conducted in accordance with all relevant NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Rats and mice were housed individually. Feed and water were available *ad libitum*. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded monthly beginning at week 5. Body weights were recorded initially, weekly for 13 weeks, monthly thereafter, and at the end of the studies.

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, 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. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year study, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the skin of rats and mice and the adrenal cortex, bone marrow, Harderian gland, liver, mandibular lymph node, mammary gland, seminal vesicle, spleen, and urinary bladder of mice.

The quality assessment report and the reviewed slides were submitted to the NTP PWG coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Pyrogallol

3-Month Studies	2-Year Studies
Study Laboratory Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)
Strain and Species F344/N rats B6C3F1/N mice	F344/N rats B6C3F1/N mice
Animal Source Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies Rats: 11 (males) or 12 (females) days Mice: 13 (females) or 14 (males) days	Rats: 13 (males) or 14 (females) days Mice: 11 (females) or 12 (males) days
Average Age When Studies Began Rats: 5 to 7 weeks Mice: 6 to 7 weeks	Rats: 6 to 7 weeks Mice: 5 to 6 weeks
Date of First Dose Rats: November 3 (males) or 4 (females), 2003 Mice: November 5 (females) or 6 (males), 2003	Rats: September 15 (males) or 16 (females), 2004 Mice: September 20 (females) or 21 (males), 2004
Duration of Dosing 5 days/week for 14 weeks	5 days/week for 104 (rats) or 105 (mice) weeks
Date of Last Dose Rats: November 25 (males) or 26 (females), 2003 (special study) or February 2 (males) or 3 (females), 2004 (core study) Mice: February 4 (females) or 5 (males), 2004	Rats: September 11 (males) or 13 (females), 2006 Mice: September 18 (females) or 20 (males), 2006
Necropsy Dates Rats: February 3 (males) or 4 (females), 2004 Mice: February 5 (females) or 6 (males), 2004	Rats: September 11-12 (males) and 13-14 (females), 2006 Mice: September 18-19 (females) and 19-21 (males), 2006
Average Age at Necropsy Rats: 19 to 21 weeks Mice: 19 to 20 weeks	109 to 111 weeks
Size of Study Groups 10 males and 10 females	50 males and 50 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-month studies
Animals per Cage 1	1
Method of Animal Identification Tail tattoo	Tail tattoo

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Pyrogallol

3-Month Studies	2-Year Studies
Diet Irradiated NTP-2000 wafer feed (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as 3-month studies
Water Tap water (Columbus, OH, municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as 3-month studies
Cages Polycarbonate (Lab Products, Inc., Seaford, DE), changed weekly	Polycarbonate (Lab Products, Inc., Seaford, DE, and Allentown Caging Equipment Co., Inc., Allentown, NJ), changed weekly for 13 weeks then twice weekly (rats) or weekly (mice)
Bedding Irradiated Sani-Chips hardwood chips (P.J. Murphy Forest Products Corporation, Montville, NJ), changed weekly	Irradiated Sani-Chips hardwood chips (P.J. Murphy Forest Products Corporation, Montville, NJ), changed weekly for 13 weeks then twice weekly (rats) or weekly (mice)
Rack Filters Spun-bonded polyster (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 3-month studies
Racks Stainless steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks	Same as 3-month studies
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses Rats: 0, 9.5, 18.75, 37.5, 75, or 150 mg/kg in 95% ethanol (dosing volume 0.5 mL/kg) Mice: 0, 38, 75, 150, 300, or 600 mg/kg in 95% ethanol (dosing volume 2.0 mL/kg)	0, 5, 20, or 75 mg/kg in 95% ethanol (dosing volume 0.5 mL/kg for rats or 2.0 mL/kg for mice)
Type and Frequency of Observation Observed twice daily; animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded for core study animals initially, weekly, and at the end of the studies.	Observed twice daily; animals were weighed initially, weekly for 13 weeks, monthly thereafter, and at the end of the studies. Clinical findings were recorded monthly beginning at week 5.
Method of Kill Carbon dioxide asphyxiation	Same as 3-month studies
Necropsy Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, thymus, thyroid gland, and uterus.	Necropsies were performed on all animals.

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Pyrogallol

3-Month Studies	2-Year Studies
Thyroid Hormone Analysis Blood was collected from the retroorbital sinus of special study rats on day 23 and by cardiac puncture from core study rats at the end of the study for total thyroxine, total triiodothyronine, and thyroid stimulating hormone determinations.	None
Clinical Pathology Blood was collected from the retroorbital sinus of special study rats on days 4 and 23 and by cardiac puncture from core study rats and mice at the end of the study; blood was analyzed for hematology (rats and mice) and clinical chemistry (rats only). ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, bile acids; and glucose (core study rats)	None
Histopathology Complete histopathology was performed on vehicle control rats and mice, 150 mg/kg rats, and 600 mg/kg mice. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice only), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph node (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. The skin at the site of application was examined in all rats and mice, and the spleen was examined in the remaining groups of mice.	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, esophagus, eyes, gallbladder (mice only), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pharynx, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.
Sperm Motility and Vaginal Cytology At the end of the studies, spermatid and sperm samples were collected from male rats in the 0, 37.5, 75, and 150 mg/kg groups and from male mice in the 0, 150, 300, and 600 mg/kg groups. The following parameters were evaluated: spermatid heads per testis and per gram testis, sperm motility, and sperm per cauda epididymis and per gram cauda epididymis. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for 12 consecutive days prior to the end of the studies from female rats administered 0, 37.5, 75, or 150 mg/kg and female mice administered 0, 150, 300, or 600 mg/kg.	None

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes were censored; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B3, C1, C4, D1, and D3 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominators consist of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal kill.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal kill; if the animal died prior to terminal kill and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 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 dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, thyroid hormone, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by

the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Proportions of regular cycling females in each dosed group were compared to the control group using the Fisher exact test (Gart *et al.*, 1979). Tests for extended periods of estrus, diestrus, metestrus, and proestrus, as well as skipped estrus and skipped diestrus, were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each dose group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within each stage as well as for skipping estrus or diestrus within a cycle. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical 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 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 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 pyrogallol was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli*, micronucleated erythrocytes in mouse bone marrow, and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

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

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

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or

micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.*, 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies (Witt *et al.*, 2000). Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

RESULTS

RATS

3-MONTH STUDY

All rats survived until the end of the study except for one vehicle control female that died of chylothorax on day 43 of the study (Table 2). The mean body weight gain of 150 mg/kg females was less than that of the vehicle controls; otherwise, the final mean body weights and body weight gains of dosed groups of males and females were similar to those of the vehicle controls (Table 2 and Figure 1). Chemical-related clinical findings included brown staining and irritation of the skin at the site of application; at study termination, most of the pyrogallol-treated rats had brown staining and irritation at the site of application.

There were no changes in the hematology, serum clinical chemistry, or thyroid hormone values attributable to the dermal administration of pyrogallol (Table F1). No biologically significant organ weight changes were noted in males or females (Table G1). There were no significant differences in sperm parameters of male rats or the estrous cyclicity of female rats administered 37.5, 75, or 150 mg/kg pyrogallol when compared to the vehicle controls (Tables H1, H2, and H3; Figure H1).

TABLE 2
Survival and Body Weights of Rats in the 3-Month Dermal Study of Pyrogallol[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	116 ± 4	340 ± 4	224 ± 5	
9.5	10/10	115 ± 5	340 ± 8	225 ± 9	100
18.75	10/10	115 ± 5	346 ± 4	231 ± 6	102
37.5	10/10	116 ± 5	342 ± 5	226 ± 6	101
75	10/10	115 ± 4	330 ± 5	215 ± 6	97
150	10/10	116 ± 4	331 ± 4	215 ± 5	97
Female					
0	9/10[c]	104 ± 3	205 ± 3	103 ± 4	
9.5	10/10	103 ± 4	208 ± 2	105 ± 3	102
18.75	10/10	103 ± 4	205 ± 5	102 ± 3	100
37.5	10/10	103 ± 3	198 ± 3	95 ± 4	97
75	10/10	104 ± 4	201 ± 3	97 ± 4	98
150	10/10	104 ± 3	194 ± 3	90 ± 3*	95

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test

[a] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.

[b] Number of animals surviving at 14 weeks/number initially in group

[c] Week of death: 7

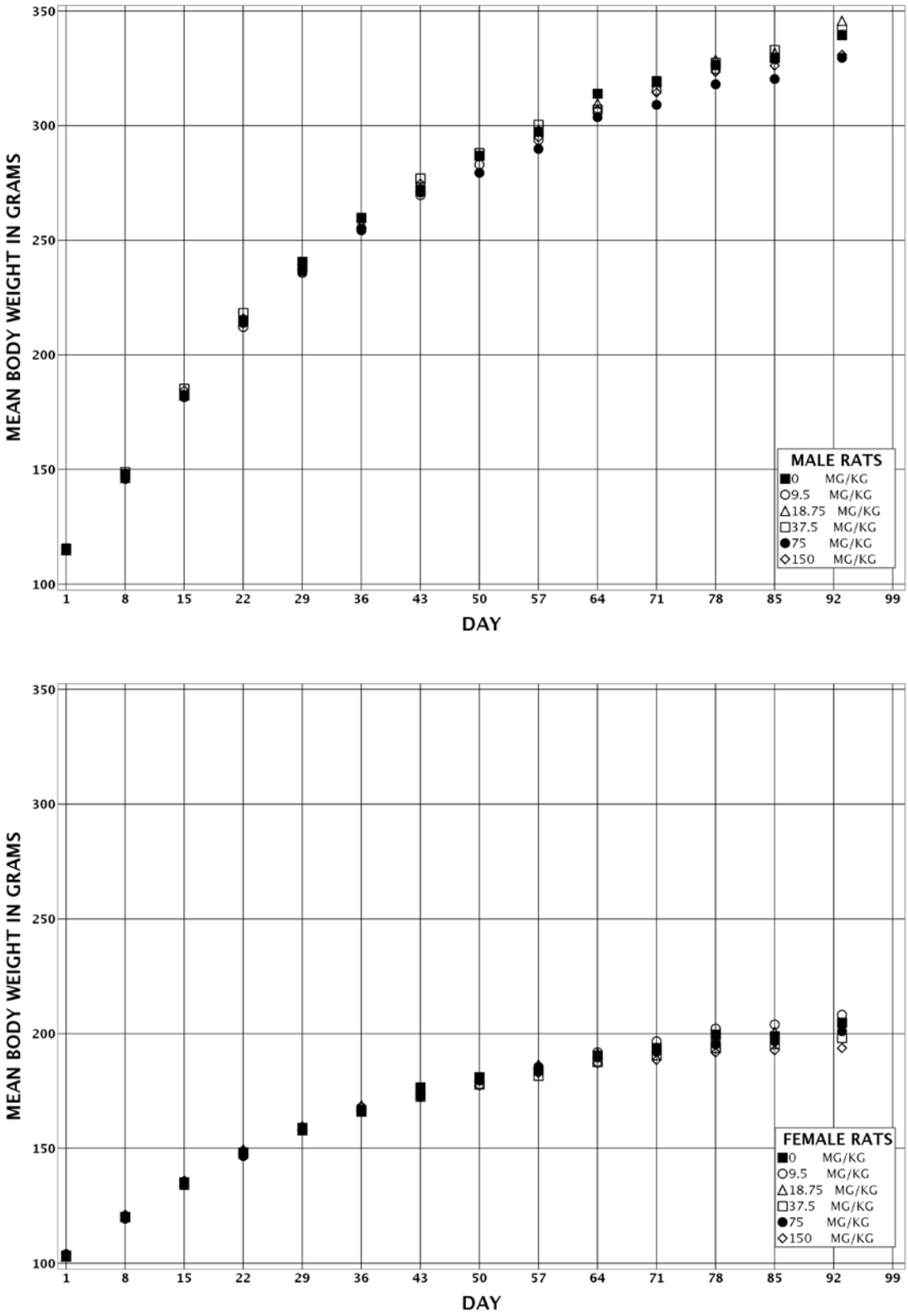


FIGURE 1
Growth Curves for Rats Administered Pyrogallol Dermally for 3 Months

Microscopically, the incidences of squamous hyperplasia, hyperkeratosis, and chronic active inflammation of the skin at the site of application were significantly increased in all dosed groups of males and females; these lesions occurred in nearly all of the treated rats (Table 3). The severities of these lesions ranged from minimal to moderate, and in general, increased with increasing dose. One male rat in the 150 mg/kg group had an ulcer.

The stratum corneum layer of the skin at the site of application in dosed rats often had a yellow-brown discoloration. This discoloration was attributed to absorption of the test article and was most evident at higher doses. Squamous hyperplasia consisted of an increase in the thickness of the epidermis from the normal one to two layers of epithelial cells to three to five layers (minimal) or six to eight layers (mild). Hyperkeratosis was characterized by pronounced thickening of the stratum corneum layer. Chronic active inflammation consisted of variable numbers of lymphocytes and macrophages (with fewer neutrophils) diffusely infiltrating the superficial dermis.

Dose Selection Rationale: The dermal toxicity observed in the 3-month study precluded the use of doses above 75 mg/kg. Use of 38 mg/kg as the high dose was discussed, but upon review of the skin lesions, 75 mg/kg was selected as a sufficiently challenging dose. The doses selected for the 2-year study in rats (5, 20, and 75 mg/kg) were spaced in a manner that would allow for a dose below the range of effects observed in the 3-month study.

TABLE 3
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in Rats in the 3-Month Dermal Study of Pyrogallol

	Vehicle Control	9.5 mg/kg	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg
Male						
Number Examined Microscopically	10	10	10	10	10	10
Squamous Hyperplasia[a]	1 (1.0)	10** (1.0)[b]	10** (1.0)	10** (1.0)	10** (1.5)	10** (1.4)
Hyperkeratosis	0	10** (1.1)	10** (1.3)	10** (1.7)	10** (2.2)	10** (2.0)
Inflammation, Chronic Active	0	9** (1.1)	9** (1.2)	10** (1.0)	10** (1.8)	10** (1.9)
Ulcer	0	0	0	0	0	1 (2.0)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Squamous Hyperplasia	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.3)	10** (1.1)
Hyperkeratosis	0	10** (1.0)	10** (1.4)	10** (1.4)	10** (1.9)	10** (2.1)
Inflammation, Chronic Active	0	8** (1.0)	10** (1.5)	9** (1.8)	10** (2.0)	10** (1.8)

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

2-YEAR STUDY

Survival

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

Body Weights and Clinical Findings

The mean body weights of dosed groups of male and female rats were similar to those of the vehicle control groups throughout the study (Tables 5 and 6; Figure 3). Irritation of the skin at the site of application was the only chemical-related clinical finding and occurred in the 20 and 75 mg/kg male and female groups.

TABLE 4
Survival of Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Male				
Animals initially in study	50	50	50	50
Moribund	17	14	15	17
Natural deaths	10	8	7	5
Animals surviving to study termination	23	28[d]	28	28
Percent probability of survival at end of study[a]	46	56	56	56
Mean survival (days)[b]	651	668	681	683
Survival analysis[c]	P=0.374N	P=0.419N	P=0.321N	P=0.217N
Female				
Animals initially in study	50	50	50	50
Accidental death[e]	0	1	0	0
Moribund	12	7	18	9
Natural deaths	9	9	6	10
Animals surviving to study termination	29	33	26[f]	31
Percent probability of survival at end of study	58	68	52	62
Mean survival (days)	670	689	672	679
Survival analysis	P=0.980N	P=0.339N	P=0.690	P=0.752N

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal kill).
[c] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dose group is indicated by **N.**
[d] Includes two animals that died during the last week of the study
[e] Censored from survival analyses
[f] Includes one animal that died during the last week of the study

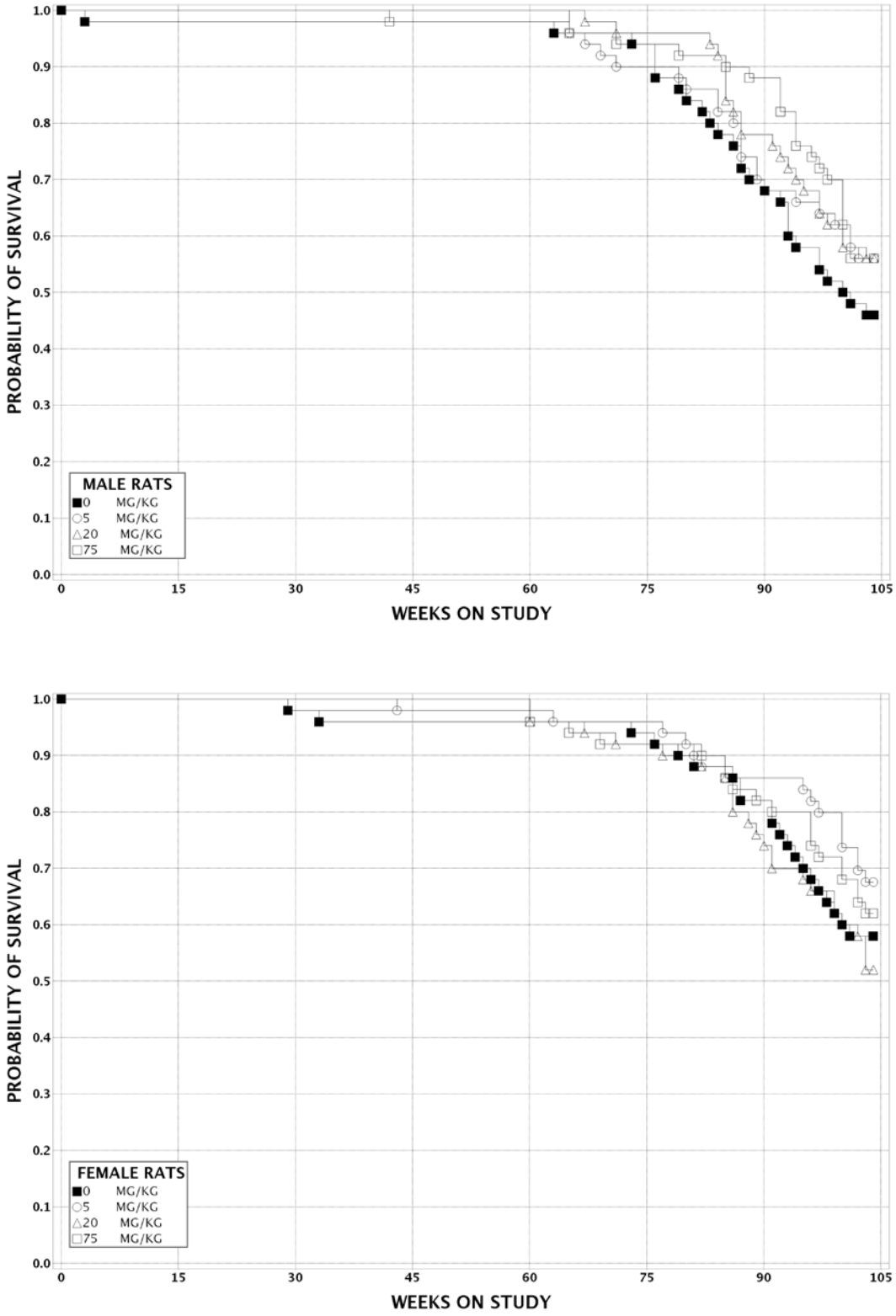


FIGURE 2
Kaplan-Meier Survival Curves for Rats Administered Pyrogallol Dermally for 2 Years

TABLE 5
Mean Body Weights and Survival of Male Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control		5 mg/kg			20 mg/kg			75 mg/kg		
Day	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	113	50	113	100	50	113	100	50	113	100	50
8	142	50	142	100	50	143	100	50	142	100	50
15	177	49	177	100	50	178	100	50	176	100	50
22	211	49	210	100	50	210	100	50	207	98	50
29	235	49	234	100	50	234	100	50	229	98	50
36	255	49	253	100	50	253	99	50	248	98	50
43	266	49	265	100	50	265	100	50	261	98	50
50	283	49	280	99	50	281	99	50	275	97	50
57	297	49	295	99	50	295	99	50	288	97	50
64	304	49	301	99	50	303	100	50	293	96	50
71	309	49	308	100	50	309	100	50	299	97	50
78	320	49	319	100	50	321	100	50	310	97	50
85	329	49	326	99	50	328	100	50	317	97	50
113	354	49	350	99	50	352	99	50	340	96	50
141	369	49	368	100	50	370	100	50	354	96	50
169	386	49	383	99	50	388	101	50	371	96	50
197	395	49	392	99	50	398	101	50	376	95	50
225	410	49	405	99	50	411	100	50	387	95	50
253	419	49	415	99	50	419	100	50	395	94	50
281	428	49	425	99	50	429	100	50	406	95	50
309	436	49	431	99	50	439	101	50	412	95	49
337	445	49	440	99	50	445	100	50	417	94	49
365	449	49	442	98	50	450	100	50	421	94	49
393	454	49	449	99	50	453	100	50	426	94	49
421	463	49	457	99	50	463	100	50	435	94	49
449	472	48	464	98	49	470	100	50	442	94	49
477	476	48	469	99	47	475	100	49	444	93	48
505	480	48	478	100	45	478	100	48	454	95	47
533	488	44	485	99	45	486	100	48	460	94	47
561	491	42	483	98	43	486	99	48	463	94	46
589	496	39	493	100	41	485	98	44	468	94	45
617	500	35	492	99	37	490	98	39	472	95	44
645	505	31	494	98	34	490	97	37	473	94	41
673	510	27	501	98	33	488	96	33	474	93	37
701	501	25	484	97	31	490	98	29	469	94	31
Mean for Weeks											
1-13	249		248	100		249	100		243	98	
14-52	405		401	99		406	100		384	95	
53-101	483		476	99		477	99		454	94	

TABLE 6
Mean Body Weights and Survival of Female Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control		5 mg/kg			20 mg/kg			75 mg/kg		
Day	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	101	50	101	100	50	101	100	50	101	100	50
8	118	50	118	100	50	118	101	50	117	100	50
15	133	50	134	101	50	135	101	50	133	100	50
22	146	50	147	101	50	147	101	50	145	99	50
29	157	50	157	101	50	157	101	50	155	99	50
36	163	50	165	101	50	165	101	50	162	99	50
43	173	50	174	100	50	174	101	50	171	99	50
50	177	50	178	100	50	179	101	50	175	99	50
57	182	50	184	101	50	184	101	50	180	99	50
64	186	50	187	101	50	187	101	50	182	98	50
71	191	50	191	100	50	192	101	50	187	98	50
78	194	50	195	101	50	196	101	50	191	98	50
85	195	50	197	101	50	198	102	50	193	99	50
113	210	50	213	101	50	214	102	50	207	99	50
141	219	50	223	102	50	224	102	50	215	98	50
169	228	50	234	102	50	234	102	50	225	98	50
197	234	50	240	103	50	239	102	50	231	99	49
225	241	49	247	102	50	247	102	50	236	98	49
253	250	48	255	102	50	254	102	50	245	98	49
281	257	48	262	102	50	261	102	50	250	97	49
309	262	48	270	103	49	269	103	50	257	98	49
337	271	48	279	103	49	279	103	50	265	98	49
365	278	48	286	103	49	285	103	50	270	97	49
393	284	48	294	103	49	290	102	50	275	97	49
421	295	48	302	103	49	301	102	48	287	97	48
449	303	48	312	103	48	312	103	48	296	97	47
477	312	48	319	103	48	318	102	47	304	98	47
505	318	47	324	102	48	322	101	46	309	97	46
533	325	46	329	101	48	329	101	46	316	97	46
561	332	44	335	101	45	332	100	45	321	97	46
589	338	44	339	100	44	338	100	44	329	97	45
617	341	41	348	102	43	340	100	38	330	97	41
645	342	38	347	101	42	342	100	35	338	99	40
673	345	34	350	102	40	348	101	33	340	99	37
701	351	30	345	98	36	345	98	30	337	96	34
Mean for Weeks											
1-13	163		164	101		164	101		161	99	
14-52	241		247	102		247	102		237	98	
53-101	320		325	102		323	101		312	97	

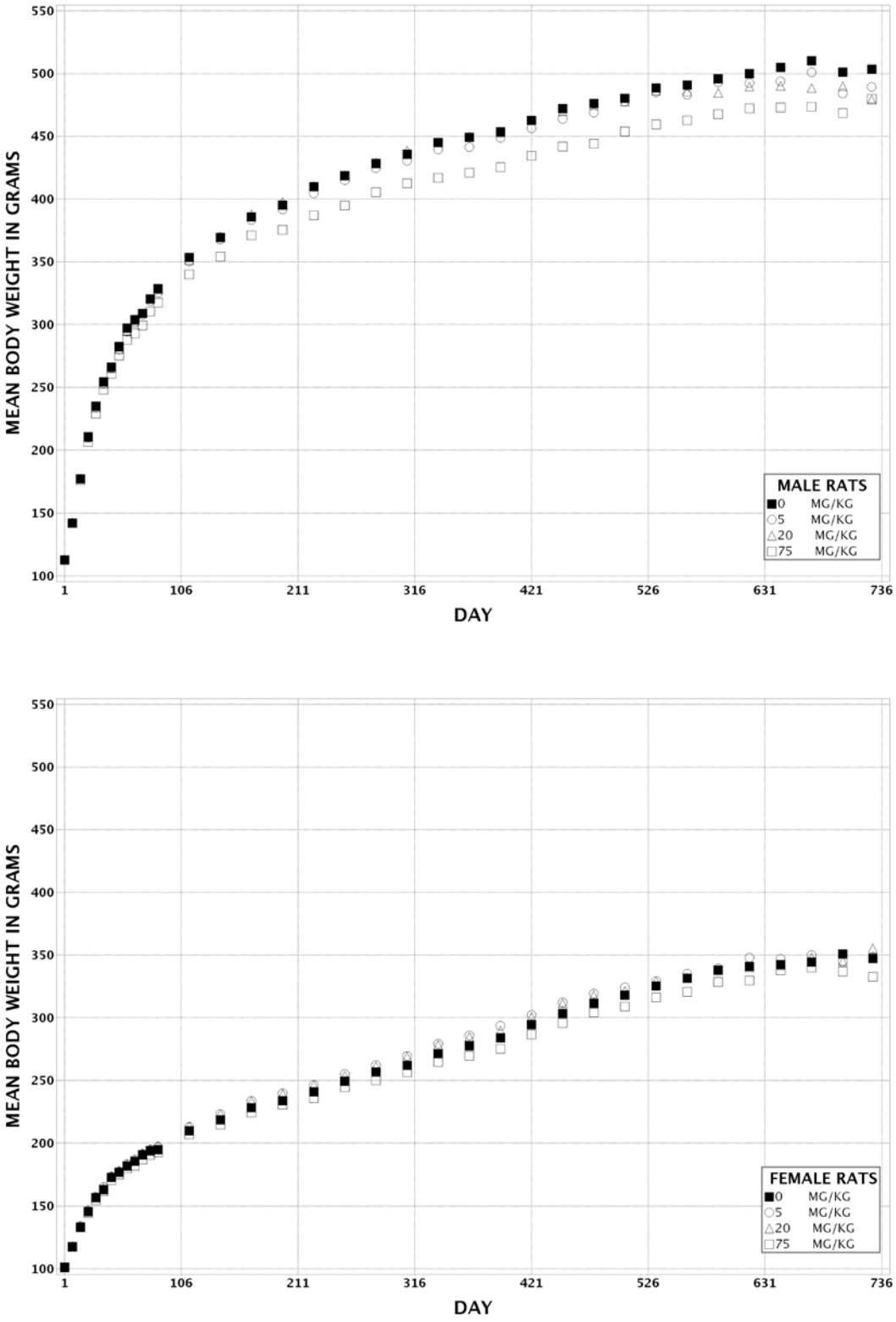


FIGURE 3
Growth Curves for Rats Administered Pyrogallol Dermally for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of malignant mesothelioma, mononuclear cell leukemia, and neoplasms and nonneoplastic lesions of the skin. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Skin, Site of Application: The incidences of hyperplasia and hyperkeratosis (except hyperkeratosis in 5 mg/kg males) in all dosed groups of male and female rats were significantly greater than those in the vehicle control groups (Tables 7, A4, and B3). The incidences of inflammation were significantly increased in 75 mg/kg males and 20 and 75 mg/kg females. The incidences of sebaceous gland hyperplasia were significantly increased in male and female rats administered 20 or 75 mg/kg.

The lesions at the site of application were morphologically similar to those observed in the 3-month study. Hyperplasia was characterized by thickening of the epidermis beyond the one to two cell layers typical of the vehicle control skin (Plates 1 and 2). Hyperplasia was considered minimal when the epidermis was thickened to three to four cell layers and mild when there was thickening of the epidermis to five to six cell layers (Plates 3 and 4). Hyperplasia was usually accompanied by varying degrees of hyperkeratosis, which was characterized by increased layers of keratin overlying the epidermis (Plate 4). Hyperkeratosis was considered minimal if the keratin overlying the stratum granulosum was thin and loosely packed and mild when there was a thick, dense, compact band of keratin above the stratum granulosum. Inflammation of predominantly minimal to mild severity was characterized by scattered aggregates of lymphocytes, macrophages, plasma cells, and neutrophils in the superficial dermis (Plate 4). Sebaceous gland hyperplasia was of minimal to mild severity and characterized by increased frequency and size of the sebaceous glands (Plate 4).

Skin: In male rats, the incidence of squamous cell papilloma in the 75 mg/kg group increased (1/50, 0/50, 0/50, 3/50) but was not significantly different from that in the vehicle control group (Tables A1 and A2). However, the incidence of squamous cell papilloma in 75 mg/kg males exceeded the historical control ranges for ethanol dermal studies and for all routes (Table A3a). In the 75 mg/kg group of males, single squamous cell papillomas occurred on the ear of one rat and on the dorsal surface of the nose of two rats. Because these lesions did not occur at the site of application, they were not considered to be related to treatment.

Other Findings: The incidences of malignant mesothelioma were increased in 5 and 75 mg/kg male rats (2/50, 5/50, 1/50, 4/50; Tables A1 and A2) and the incidences of mononuclear cell leukemia were increased in 20 and 75 mg/kg female rats (8/50, 9/50, 17/50, 11/50; Tables B1 and B2). Although the incidence of mesothelioma in 5 mg/kg males exceeded the historical control ranges for ethanol dermal studies and for all routes (Table A3b), the increase was not statistically significant (Table A2). In addition, the mesotheliomas were generally histologically similar regardless of the site of occurrence, including those observed on the epididymides and testes of two vehicle control rats. Although the increased incidence of mononuclear cell leukemia in 20 mg/kg females was statistically significant when compared to the vehicle controls (Table B2), it was still within the historical control ranges for ethanol dermal studies (16% to 36%) and for all routes (8% to 36%).

TABLE 7
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Hyperplasia[a]	0	6* (1.0)[b]	20** (1.0)	50** (1.2)
Hyperkeratosis	0	2 (1.0)	21** (1.0)	48** (1.6)
Inflammation	0	0	0	46** (1.3)
Sebaceous Gland, Hyperplasia	0	0	12** (1.0)	48** (1.1)
Female				
Number Examined Microscopically	50	50	50	50
Hyperplasia	0	9** (1.0)	11** (1.0)	49** (1.1)
Hyperkeratosis	0	6* (1.0)	23** (1.0)	49** (1.8)
Inflammation	0	3 (1.0)	6* (1.0)	49** (1.4)
Sebaceous Gland, Hyperplasia	0	0	5* (1.0)	41** (1.7)

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

MICE
3-MONTH STUDY

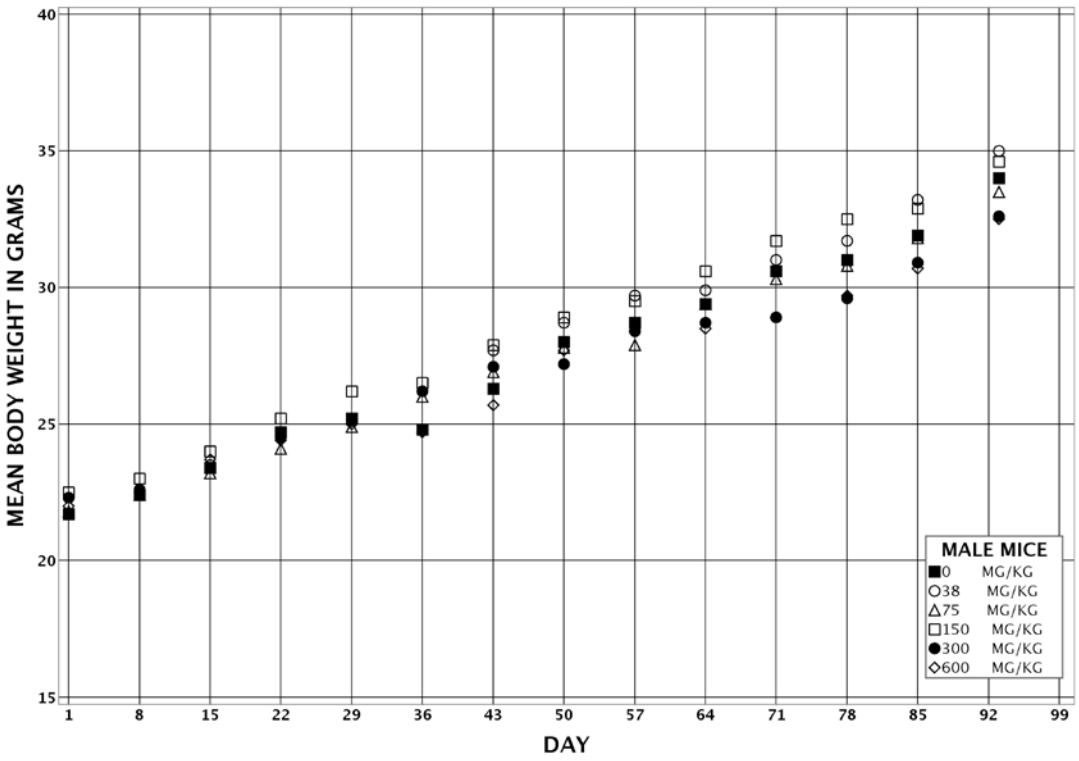
All mice survived until the end of the study (Table 8). The final mean body weights and body weight gains of dosed groups of males and females were similar to those of the vehicle controls (Table 8 and Figure 4). Chemical-related clinical findings included brown staining and irritation of the skin at the site of application; at study termination, most of the pyrogallol-treated mice had brown staining and irritation at the site of application.

There were no changes in the hematology values of mice attributable to the dermal administration of pyrogallol (Table F2). No biologically significant organ weight changes were noted in males or females (Table G2). There were no significant differences compared to the vehicle controls in sperm parameters of male mice receiving any dose or in the estrous cyclicity of female mice receiving 300 or 600 mg/kg pyrogallol (Tables H4, H5, and H6; Figure H2); the alteration in estrous cyclicity in the 150 mg/kg group was not considered biologically relevant.

TABLE 8
Survival and Body Weights of Mice in the 3-Month Dermal Study of Pyrogallol[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	21.7 ± 0.2	34.0 ± 0.7	12.3 ± 0.6	
38	10/10	22.3 ± 0.5	35.0 ± 0.8	12.7 ± 0.6	103
75	10/10	21.8 ± 0.3	33.5 ± 0.5	11.7 ± 0.6	99
150	10/10	22.5 ± 0.5	34.6 ± 0.8	12.1 ± 0.6	102
300	10/10	22.3 ± 0.5	32.6 ± 1.2	10.3 ± 0.9	96
600	10/10	22.0 ± 0.3	32.5 ± 0.7	10.5 ± 0.6	96
Female					
0	10/10	19.2 ± 0.2	30.4 ± 0.7	11.3 ± 0.6	
38	10/10	18.6 ± 0.2	31.3 ± 1.1	12.7 ± 1.1	103
75	10/10	18.8 ± 0.2	30.9 ± 0.9	12.0 ± 0.7	102
150	10/10	18.6 ± 0.2	29.9 ± 0.7	11.3 ± 0.6	98
300	10/10	18.8 ± 0.2	28.2 ± 0.8	9.4 ± 0.7	93
600	10/10	18.8 ± 0.3	28.6 ± 0.8	9.8 ± 0.6	94

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



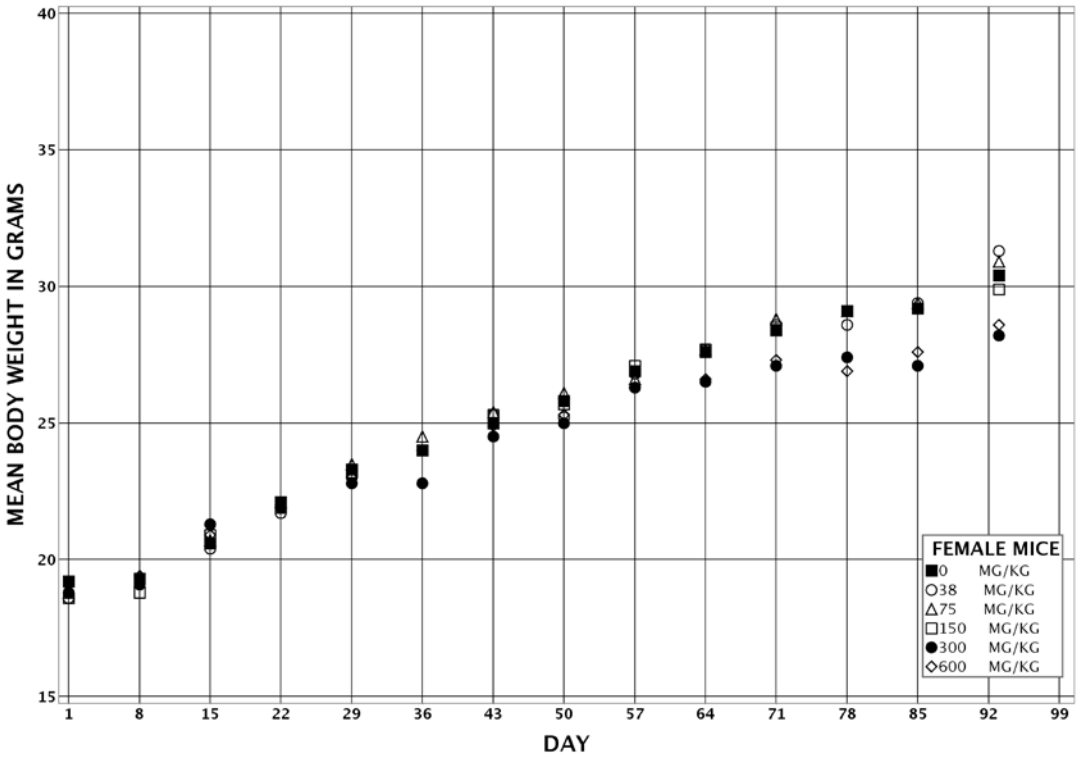


FIGURE 4
Growth Curves for Mice Administered Pyrogallol Dermally for 3 Months

Microscopically, the incidences of squamous hyperplasia, hyperkeratosis, and chronic active inflammation of the skin at the site of application were significantly increased in all dosed groups of males and females; these lesions occurred in nearly all of the treated mice (Table 9). The severities of these lesions ranged from minimal to mild, and in general, increased with increasing dose. These lesions were morphologically similar to those that occurred in rats. Ulcer (graded as mild) at the site of application occurred in one 300 mg/kg male, two 600 mg/kg males, and three 600 mg/kg females. One 600 mg/kg female had minimal epidermal necrosis at the site of application.

A significantly increased incidence of hematopoietic cell proliferation of the spleen occurred in 600 mg/kg male mice (Table 10).

Dose Selection Rationale: The dermal toxicity observed in the 3-month study precluded the use of doses above 75 mg/kg. Use of 38 mg/kg as the high dose was discussed, but upon review of the skin lesions, 75 mg/kg was selected as a sufficiently challenging dose. The doses selected for the 2-year study in mice (5, 20, and 75 mg/kg) were spaced in a manner that would allow for a dose below the range of effects observed in the 3-month study.

TABLE 9
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in Mice in the 3-Month Dermal Study of Pyrogallol

	Vehicle Control	38 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
Male						
Number Examined Microscopically	10	10	10	10	10	10
Squamous Hyperplasia[a]	0	10** (1.0)[b]	10** (1.0)	10** (1.1)	10** (1.6)	10** (2.2)
Hyperkeratosis	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.2)	10** (1.5)
Inflammation, Chronic Active	0	10** (1.3)	10** (1.4)	10** (1.5)	10** (1.7)	10** (2.2)
Ulcer	0	0	0	0	1 (2.0)	2 (2.0)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Squamous Hyperplasia	0	10** (1.0)	10** (1.1)	10** (1.0)	10** (1.4)	10** (1.8)
Hyperkeratosis	0	10** (1.0)	10** (1.0)	10** (1.0)	9** (1.1)	10** (1.4)
Inflammation, Chronic Active	0	10** (1.9)	10** (2.1)	10** (2.0)	10** (2.1)	10** (2.1)
Ulcer	0	0	0	0	0	3 (2.0)
Necrosis	0	0	0	0	0	1 (1.0)

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

TABLE 10
Incidences of Nonneoplastic Lesions of the Spleen in Mice in the 3-Month Dermal Study of Pyrogallol

	Vehicle Control	38 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
Male						
Number Examined Microscopically	10	10	10	10	10	10
Hematopoietic Cell Proliferation[a]	0	0	2 (1.0)[b]	2 (1.0)	3 (2.0)	6** (2.0)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Hematopoietic Cell Proliferation	3 (1.7)	6 (1.3)	6 (1.7)	4 (2.0)	7 (2.0)	6 (2.0)

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

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 11 and in the Kaplan-Meier survival curves (Figure 5). Survival of dosed groups of male mice was similar to that of the vehicle control group. Survival was significantly decreased in 75 mg/kg females; 23 of the 29 moribund kills were due to lesions diagnosed grossly as ulcers at the site of application.

Body Weights and Clinical Findings

The mean body weights of 75 mg/kg female mice were generally more than 10% less than those of the vehicle controls during year 2 of the study; otherwise, the mean body weights of dosed groups of male and female mice were similar to those of the vehicle control groups throughout the study (Figure 6; Tables 12 and 13). Irritation and/or ulceration of the skin at the site of application were the only chemical-related clinical findings and occurred predominantly in the 20 and 75 mg/kg male and female groups.

TABLE 11
Survival of Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental death[a]	0	0	0	1
Moribund	8	10	10	12
Natural deaths	5	4	6	6
Animals surviving to study termination	37	36	34	31
Percent probability of survival at end of study[b]	74	72	68	63
Mean survival (days)[c]	698	691	694	667
Survival analysis[d]	P=0.247	P=0.977	P=0.668	P=0.307
Female				
Animals initially in study	50	50	50	50
Moribund	9	15	7	29
Natural deaths	8	5	7	4
Animals surviving to study termination	33	30	36	17[e]
Percent probability of survival at end of study	66	60	72	34
Mean survival (days)	675	685	704	599
Survival analysis	P<0.001	P=0.710	P=0.565N	P=0.001

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal kill).
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A lower mortality in a dosed group is indicated by **N**.
[e] Includes one animal that died during the last week of the study

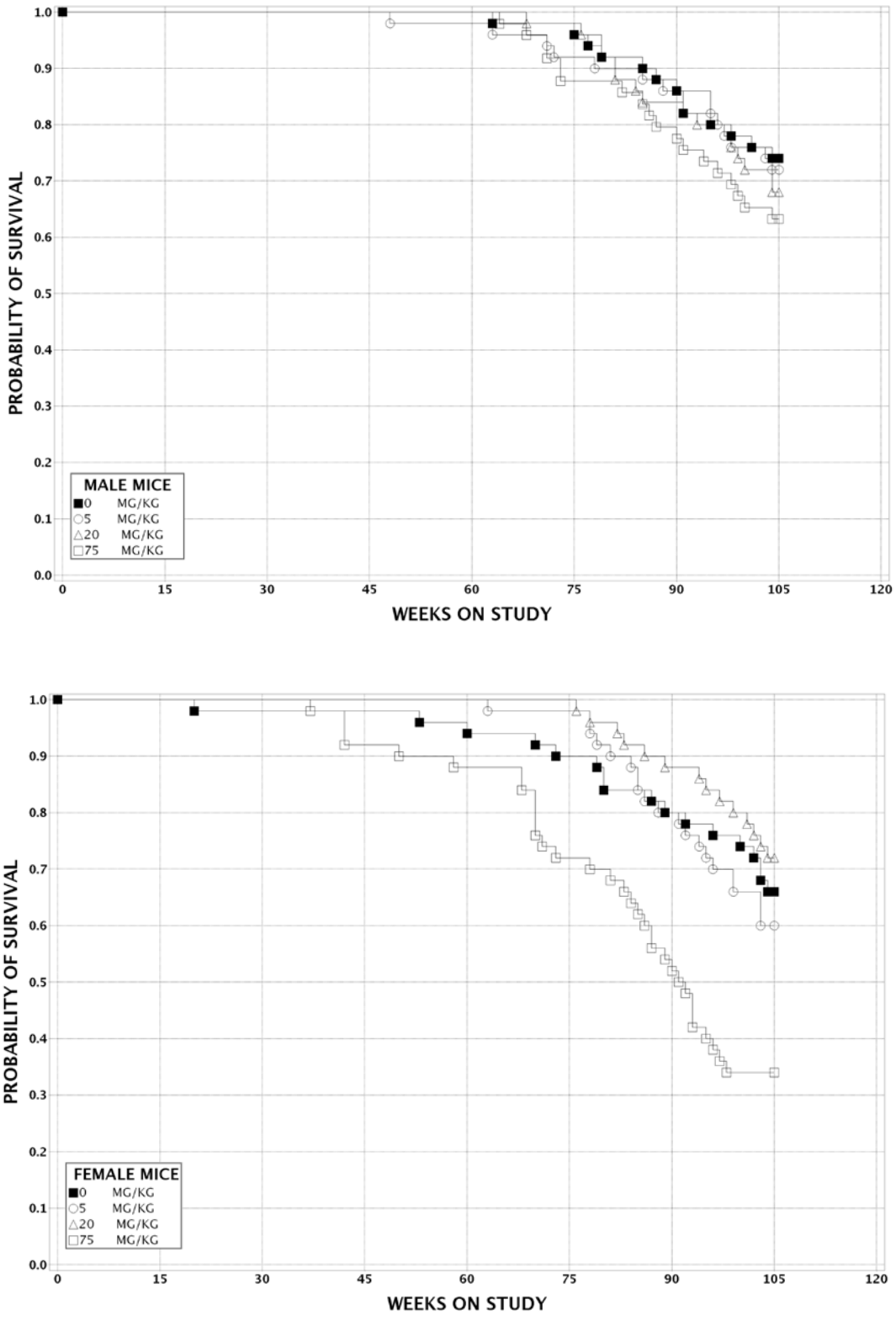


FIGURE 5
Kaplan-Meier Survival Curves for Mice Administered Pyrogallol Dermally for 2 Years

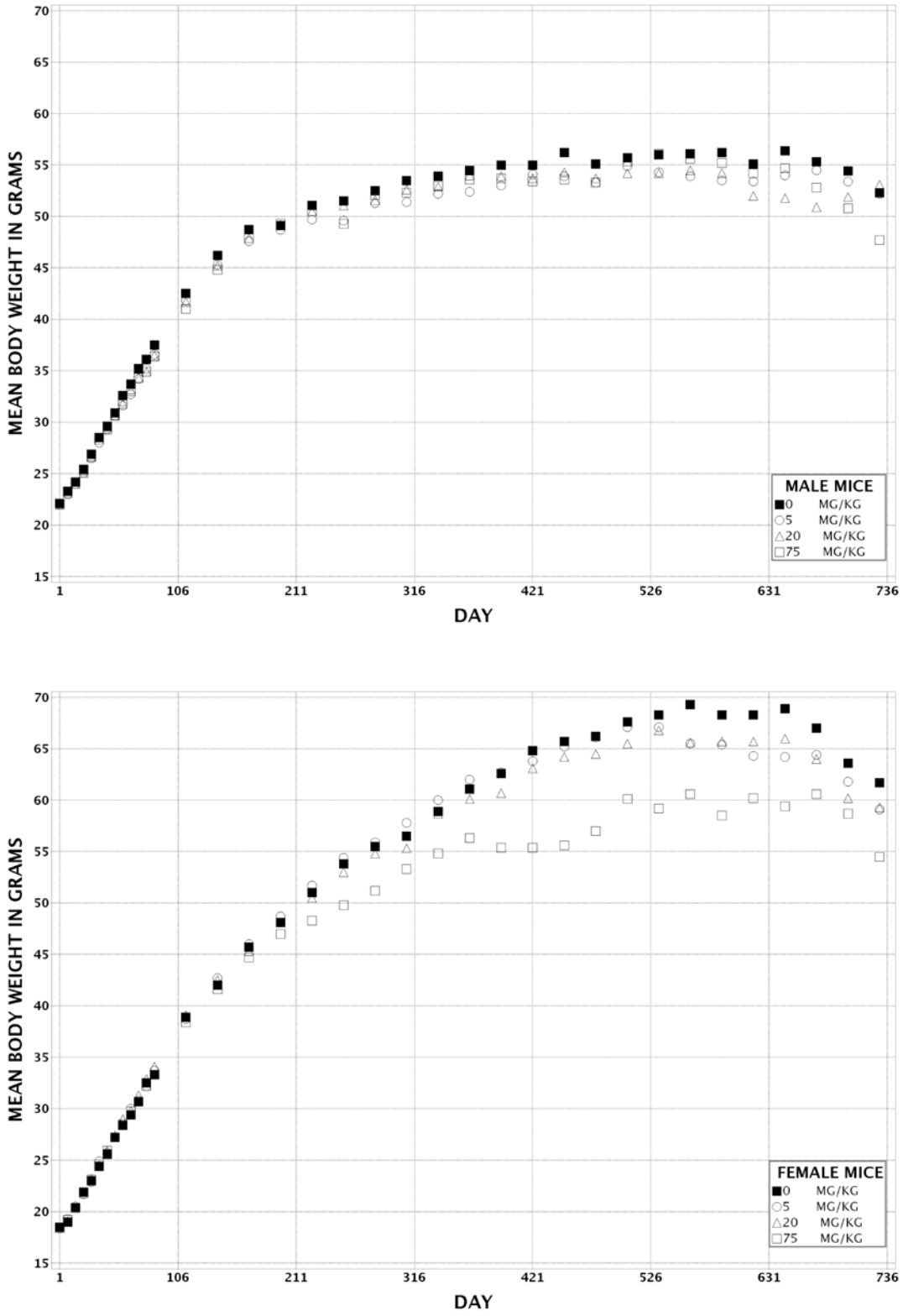


FIGURE 6
Growth Curves for Mice Administered Pyrogallol Dermally for 2 Years

TABLE 12
Mean Body Weights and Survival of Male Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control		5 mg/kg			20 mg/kg			75 mg/kg		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors
1	22.1	50	22.0	100	50	22.0	100	50	22.1	100	50
8	23.3	50	23.0	99	50	23.1	99	50	23.3	100	50
15	24.2	50	24.0	99	50	24.0	99	50	24.2	100	50
22	25.4	50	25.1	99	50	25.1	99	50	25.3	100	50
29	26.9	50	26.5	99	50	26.6	99	50	26.7	99	50
36	28.5	50	28.0	98	50	28.3	99	50	28.3	99	50
43	29.6	50	29.3	99	50	29.5	100	50	29.3	99	50
50	30.9	50	30.6	99	50	30.6	99	50	30.7	99	50
57	32.6	50	31.6	97	50	31.8	97	50	32.0	98	50
64	33.7	50	32.7	97	50	33.2	98	50	33.0	98	50
71	35.2	50	34.2	97	50	34.3	98	50	34.3	98	50
78	36.1	50	35.2	98	50	34.9	97	50	34.9	97	50
85	37.5	50	36.6	98	50	36.5	97	50	36.4	97	50
113	42.5	50	41.8	98	50	41.7	98	50	41.0	96	50
141	46.2	50	45.3	98	50	45.2	98	50	44.8	97	49
169	48.7	50	47.6	98	50	47.9	98	50	47.9	98	49
197	49.1	50	48.7	99	50	49.2	100	50	49.3	100	49
225	51.1	50	49.7	97	50	50.5	99	50	50.5	99	49
253	51.5	50	49.6	96	50	51.1	99	50	49.3	96	49
281	52.5	50	51.3	98	50	51.6	98	50	52.1	99	49
309	53.5	50	51.4	96	50	52.6	98	50	52.3	98	49
337	53.9	50	52.2	97	49	52.9	98	50	53.0	99	49
365	54.5	50	52.4	96	49	54.0	99	50	53.6	98	49
393	55.0	50	53.0	96	49	53.9	98	50	53.7	98	49
421	55.0	50	54.1	98	49	53.7	98	50	53.4	97	49
449	56.2	49	53.9	96	48	54.3	97	50	53.6	95	48
477	55.1	49	53.3	97	48	53.7	98	49	53.3	97	47
505	55.7	49	54.9	99	46	54.2	97	49	55.3	99	43
533	56.0	48	54.3	97	46	54.2	97	48	56.1	100	43
561	56.1	46	53.9	96	45	54.5	97	46	55.6	99	43
589	56.2	46	53.5	95	45	54.2	97	43	55.2	98	41
617	55.1	44	53.4	97	43	52.0	94	42	54.2	98	39
645	56.4	41	54.0	96	43	51.8	92	41	54.7	97	37
673	55.3	40	54.5	99	39	50.9	92	40	52.8	96	35
701	54.4	39	53.4	98	38	51.9	96	36	50.8	93	32
Mean for Weeks											
1-13	29.7		29.1	98		29.2	99		29.3	99	
14-52	49.9		48.6	97		49.2	98		48.9	98	
53-101	55.5		53.7	97		53.3	96		54.0	97	

TABLE 13
Mean Body Weights and Survival of Female Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control		5 mg/kg			20 mg/kg			75 mg/kg		
Day	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	18.5	50	18.4	100	50	18.5	100	50	18.4	100	50
8	19.0	50	19.3	102	50	19.1	101	50	19.2	101	50
15	20.4	50	20.5	101	50	20.4	100	50	20.4	100	50
22	21.9	50	21.7	99	50	21.9	100	50	21.8	100	50
29	23.0	50	22.9	100	50	23.2	101	50	23.1	101	50
36	24.4	50	24.9	102	50	24.5	100	50	24.6	101	50
43	25.6	50	25.7	100	50	25.9	101	50	25.9	101	50
50	27.2	50	27.2	100	50	27.4	101	50	27.2	100	50
57	28.4	50	28.5	101	50	29.0	102	50	28.5	101	50
64	29.4	50	30.0	102	50	29.8	102	50	29.7	101	50
71	30.7	50	30.8	100	50	31.3	102	50	30.7	100	50
78	32.5	50	32.2	99	50	32.9	101	50	32.2	99	50
85	33.3	50	33.7	101	50	34.1	102	50	33.3	100	50
113	38.9	50	38.7	100	50	39.1	101	50	38.4	99	50
141	42.0	49	42.7	102	50	42.5	101	50	41.6	99	50
169	45.7	49	46.0	101	50	45.3	99	50	44.7	98	50
197	48.1	49	48.7	101	50	48.1	100	50	47.0	98	50
225	51.0	49	51.7	101	50	50.5	99	50	48.3	95	50
253	53.8	49	54.4	101	50	53.0	99	50	49.8	93	50
281	55.5	49	55.9	101	50	54.8	99	50	51.2	92	49
309	56.5	49	57.8	102	50	55.3	98	50	53.3	94	46
337	58.9	49	60.0	102	50	58.7	100	50	54.8	93	46
365	61.1	48	62.0	102	50	60.1	98	50	56.3	92	45
393	62.6	48	62.7	100	50	60.7	97	50	55.4	89	45
421	64.8	47	63.8	99	50	63.1	98	50	55.4	86	44
449	65.7	47	65.2	99	49	64.2	98	50	55.6	85	44
477	66.2	47	66.1	100	49	64.5	97	50	57.0	86	42
505	67.6	46	67.1	99	49	65.5	97	50	60.1	89	37
533	68.3	45	67.1	98	49	66.8	98	49	59.2	87	36
561	69.3	42	65.5	95	45	65.6	95	48	60.6	87	34
589	68.3	42	65.4	96	43	65.7	96	46	58.5	86	32
617	68.3	40	64.3	94	40	65.7	96	45	60.2	88	27
645	68.9	39	64.2	93	38	66.0	96	44	59.4	86	23
673	67.0	38	64.4	96	35	64.0	96	42	60.6	91	19
701	63.6	37	61.8	97	33	60.2	95	40	58.7	92	17
Mean for Weeks											
1-13	25.7		25.8	101		26.0	101		25.8	100	
14-52	50.0		50.7	101		49.7	100		47.7	96	
53-101	66.3		64.6	98		64.0	97		58.2	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 skin, bone marrow, lymph nodes, adrenal cortex, and mammary 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 C for male mice and Appendix D for female mice.

Skin, Site of Application: In the 75 mg/kg females, the incidence of squamous cell carcinoma was significantly greater than that in the vehicle control group; squamous cell carcinomas have not been observed in historical control female mice in dermal studies with ethanol vehicle control groups (Tables 14, D1, and D2). Two 75 mg/kg males had squamous cell papillomas; squamous cell papillomas have not been observed in historical control male mice in ethanol dermal studies (Tables 14, C1, and C3). Squamous cell carcinomas were poorly demarcated, unencapsulated masses arising from the epidermis and invading the underlying dermis and subcutis (Plates 5 and 6); the affected epidermis was ulcerated in some cases. Squamous cell carcinomas were composed of pleomorphic, disorganized proliferations of neoplastic squamous epithelial cells forming irregular cords and islands (Plate 6). The neoplastic epithelial cells often surrounded concentrically arranged aggregates of keratin (keratin pearls) and were surrounded by fibrous connective tissue, which often contained neutrophils and other inflammatory cells. Squamous cell papilloma was a well-circumscribed, exophytic growth composed of an inner connective tissue core forming a stalk with superficial branching fronds that were covered by an outer layer of hyperplastic and hyperkeratotic squamous epithelium (Plates 7 and 8).

The incidences of hyperplasia and hyperkeratosis were significantly increased in all male and female dosed groups (Tables 14, C4, and D3). The incidences of inflammation, fibrosis, and pigmentation were significantly increased in groups of male and female mice administered 20 or 75 mg/kg. The incidences of sebaceous gland hyperplasia and ulcer were significantly increased in male and female mice administered 75 mg/kg. In general, these nonneoplastic lesions at the site of application tended to be more severe in female mice and were morphologically similar to those observed in the rat studies.

Hyperplasia was characterized by thickening of the epidermis beyond the one to two cell layers typical of the vehicle control skin (Plates 9 and 10). Hyperplasia was considered minimal when the epidermis was thickened to three to four cell layers, mild when there was thickening to five to six cell layers, moderate with thickening to seven to eight layers, and marked when the thickening was greater than eight cell layers (Plates 11 and 12). Hyperplasia was generally accompanied by hyperkeratosis, which was characterized by thickening of the stratum corneum that occurred as both orthokeratotic and, to a lesser extent, parakeratotic hyperkeratosis (Plate 12). Inflammation was characterized by infiltrates of lymphocytes, macrophages, plasma cells, neutrophils, and low numbers of mast cells in the dermis with occasional infiltration into the subcutis or epidermis. Fibrosis was diagnosed when there was an increased presence of bands of pale, plump fibroblasts in the dermis; the simple presence of mature collagen did not warrant a diagnosis. Pigmentation consisted of increased numbers of cells in the dermis containing abundant dark brown, granular, intracytoplasmic pigment considered to be melanin. Sebaceous gland hyperplasia was of minimal to mild severity and characterized by increased frequency and size of the sebaceous glands concomitant with more frequent hair follicles. Ulcer was characterized by full-thickness loss of epidermis and was invariably accompanied by necrosis and inflammation of the underlying dermis (Plates 13 and 14).

TABLE 14
Incidences of Neoplasms and Nonneoplastic Lesions of the Skin (Site of Application) in Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Hyperplasia[a]	8 (1.3)[b]	24** (1.2)	47** (1.6)	50** (2.5)
Hyperkeratosis	11 (1.5)	43** (1.3)	50** (1.9)	50** (2.6)
Inflammation	2 (1.5)	6 (1.7)	37** (1.2)	44** (2.4)
Fibrosis	3 (2.0)	6 (1.7)	28** (1.4)	47** (2.5)
Pigmentation	0	0	9** (1.0)	39** (1.5)
Sebaceous Gland, Hyperplasia	1 (2.0)	6 (1.3)	4 (1.3)	24** (1.5)
Ulcer	1 (2.0)	1 (3.0)	2 (2.5)	23** (3.0)
Squamous Cell Papilloma[c]				
Overall rate[d]	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
Adjusted rate[e]	0.0%	0.0%	0.0%	4.8%
Terminal rate[f]	0/37 (0%)	0/36 (0%)	0/34 (0%)	1/31 (3%)
First incidence (days)	—[h]	—	—	628
Poly-3 test[g]	P=0.037	—[i]	—	P=0.220
Female				
Number Examined Microscopically	50	50	50	50
Hyperplasia	20 (1.4)	31* (1.3)	49** (1.9)	49** (3.3)
Hyperkeratosis	24 (1.5)	38** (1.4)	49** (2.7)	49** (3.4)
Inflammation	12 (1.3)	14 (1.1)	42** (1.2)	48** (3.0)
Fibrosis	5 (1.8)	6 (1.3)	31** (1.3)	49** (3.1)
Pigmentation	0	0	35** (1.0)	40** (1.8)
Sebaceous Gland, Hyperplasia	1 (1.0)	2 (1.0)	6 (1.2)	34** (1.5)
Ulcer	2 (3.0)	0	3 (1.3)	33** (3.1)
Squamous Cell Carcinoma[j]				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	0.0%	0.0%	12.2%
Terminal rate	0/33 (0%)	0/30 (0%)	0/36 (0%)	2/17 (12%)
First incidence (days)	—	—	—	575
Poly-3 test	P<0.001	—	—	P=0.033

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year dermal studies with ethanol vehicle control groups (mean ± standard deviation): 0/200; all routes: 1/1,150 (0.1% ± 0.4%), range 0%-2%
[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] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[h] Not applicable; no neoplasms in animal group
[i] Value of statistic cannot be computed.
[j] Historical incidence for ethanol dermal studies: 0/200; all routes: 0/1,198

Skin: Although skin lesions were consistently found at the site of application, a few dosed mice also had morphologically similar lesions in the skin of the neck and back immediately adjacent to the site of application. The incidences of hyperplasia, hyperkeratosis, ulcer, inflammation, and fibrosis at these sites were significantly increased in 75 mg/kg male and female mice (Tables 15, C4, and D3). The incidences of sebaceous gland hyperplasia were also increased in 75 mg/kg mice, and the increase in females was significant. These lesions were considered related to the test material spreading to or beyond the margins of the clipped skin after application. One 75 mg/kg female had a squamous cell carcinoma of the skin of the right forelimb (Table D1).

TABLE 15
Incidences of Nonneoplastic Lesions of the Skin in Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control		5 mg/kg		20 mg/kg		75 mg/kg	
Male								
Number Examined Microscopically	50		50		50		50	
Hyperplasia[a]	1	(2.0)[b]	1	(3.0)	3	(3.7)	10**	(3.3)
Hyperkeratosis	1	(2.0)	1	(2.0)	3	(2.7)	10**	(3.1)
Ulcer	0		1	(2.0)	3	(4.0)	10**	(3.7)
Inflammation	1	(4.0)	1	(4.0)	3	(4.0)	10**	(3.5)
Fibrosis	1	(2.0)	1	(4.0)	3	(4.0)	10**	(3.6)
Sebaceous Gland, Hyperplasia	1	(1.0)	1	(1.0)	0		5	(1.8)
Female								
Number Examined Microscopically	50		50		50		50	
Hyperplasia	1	(4.0)	2	(3.0)	1	(4.0)	9**	(3.9)
Hyperkeratosis	1	(4.0)	2	(3.0)	1	(4.0)	9**	(3.3)
Ulcer	1	(3.0)	1	(2.0)	1	(4.0)	9**	(3.4)
Inflammation	1	(3.0)	0		0		9**	(3.7)
Fibrosis	1	(3.0)	2	(3.5)	1	(4.0)	9**	(4.0)
Sebaceous Gland, Hyperplasia	1	(2.0)	0		1	(1.0)	7*	(1.3)

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

Bone Marrow: The incidences of bone marrow hyperplasia were significantly increased in male mice administered 5 mg/kg and male and female mice administered 75 mg/kg (Tables 16, C4, and D3). Hyperplasia was characterized by increased numbers of hematopoietic cells in the marrow, with an overall increase in cellularity.

Lymph Nodes: In female mice exposed to 75 mg/kg, the incidences of lymphoid hyperplasia of the axillary, inguinal, and mandibular lymph nodes were significantly increased compared to those in the vehicle controls (Tables 16 and D3).

Adrenal Cortex: The incidence of hematopoietic cell proliferation in 75 mg/kg female mice was significantly increased compared to that in the vehicle control group (Tables 16 and D3).

Mammary Gland: The incidence of hyperplasia in 75 mg/kg female mice was significantly increased compared to that in the vehicle control group (Tables 16 and D3).

TABLE 16
Incidences of Selected Nonneoplastic Lesions in Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control		5 mg/kg		20 mg/kg		75 mg/kg	
Male								
Bone Marrow[a]	50		50		50		50	
Hyperplasia[b]	3	(2.0)[c]	10*	(1.4)	8	(2.3)	15**	(2.4)
Female								
Bone Marrow	50		50		50		50	
Hyperplasia	10	(1.9)	14	(2.0)	10	(1.7)	31**	(2.7)
Lymph Node	19		13		18		29	
Axillary, Hyperplasia, Lymphoid	0		0		0		8**	(3.8)
Inguinal, Hyperplasia, Lymphoid	4	(1.5)	2	(2.0)	7	(1.9)	17**	(2.4)
Lymph Node, Mandibular	50		50		50		50	
Hyperplasia, Lymphoid	14	(1.8)	19	(1.7)	23	(1.6)	27**	(2.1)
Adrenal Cortex	50		50		50		50	
Hematopoietic Cell Proliferation	3	(1.3)	4	(1.8)	4	(1.0)	14**	(1.4)
Mammary Gland	50		50		50		50	
Hyperplasia	5	(1.4)	9	(1.4)	3	(2.0)	16**	(1.6)

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

GENETIC TOXICOLOGY

Pyrogallol was tested in two independent bacterial mutation studies; both studies gave positive results in one or more strains of *Salmonella typhimurium* or *Escherichia coli.* In the first study (concentration range 3 to 333 μg/plate), positive results were seen in *S. typhimurium* strain TA100 with and without 30% S9 derived from hamster or rat liver; negative results were obtained in strain TA98 tested under the same conditions (Table E1). In the second study, which was conducted with the same lot of pyrogallol that was used in the 3-month and 2-year dermal studies, positive results were obtained over a concentration range of 10 to 1,000 μg/plate in *S. typhimurium* strains TA98 and TA100 and in *E. coli* strain WP2 *uvrA*/pKM101 in the absence of S9 (Table E2). With 10% rat liver S9, this sample of pyrogallol was mutagenic in the *E. coli* strain but gave equivocal responses in *S. typhimurium* strains TA98 and TA100 based on small increases in revertants that were not well correlated with dose. Thus, the results of studies in bacteria show that pyrogallol is a direct-acting mutagen.

In vivo, no significant increases in the frequencies of micronucleated polychromatic erythrocytes (reticulocytes) were observed in bone marrow of male B6C3F1/N mice injected intraperitoneally with pyrogallol (39 to 156 mg/kg) once daily for 3 days (Table E3). In a second *in vivo* test, no significant increases in the frequencies of micronucleated erythrocytes, an indicator of chromosomal damage, were observed in peripheral blood of female B6C3F1/N mice treated with pyrogallol (38 to 600 mg/kg) via dermal application for 3 months (Table E4). In male mice, however, results of this assay were judged to be equivocal, based on a significant increase in micronucleated erythrocytes observed at a single dose level (300 mg/kg) at the end of the 3-month study period. No significant alteration in the percentage of polychromatic erythrocytes in bone marrow or blood was observed in either study, suggesting that pyrogallol did not induce bone marrow toxicity over the dose ranges tested.

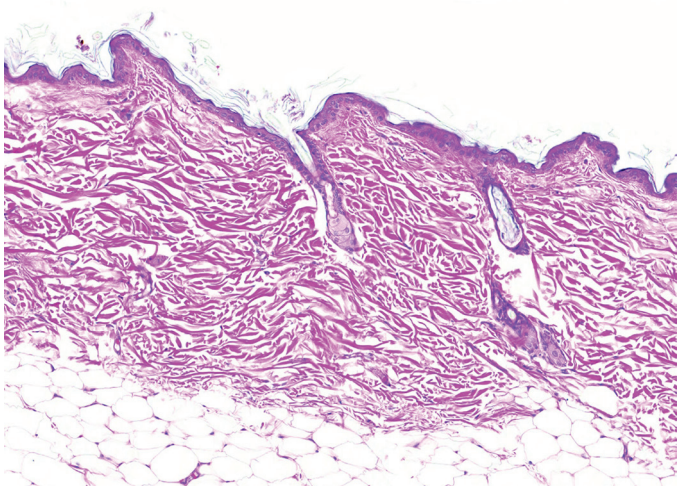


PLATE 1
Normal skin (site of application) of a male F344/N vehicle control rat in the 2-year dermal study of pyrogallol. H&E

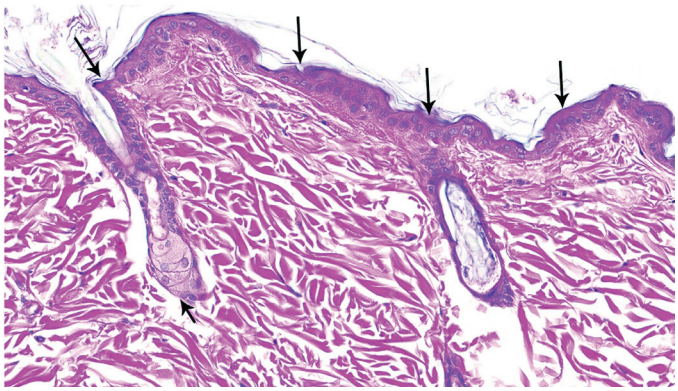


PLATE 2
Higher magnification of Plate 1. The epidermis consists of a single layer of epithelial cells (long arrows). Note the relative absence of a superficial layer of keratin and the sebaceous gland (short arrow) associated with a hair follicle. H&E

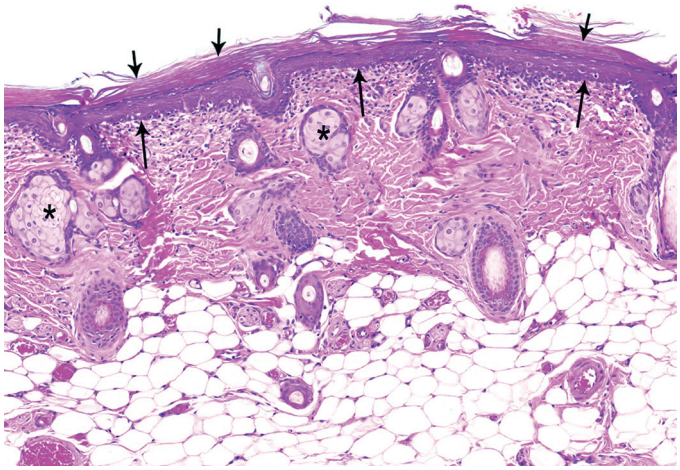


PLATE 3
Skin (site of application) of a male F344/N rat administered 75 mg/kg pyrogallol dermally for 2 years showing mild epidermal hyperplasia (long arrows), hyperkeratosis (short arrows), and hyperplastic sebaceous glands (asterisks). H&E

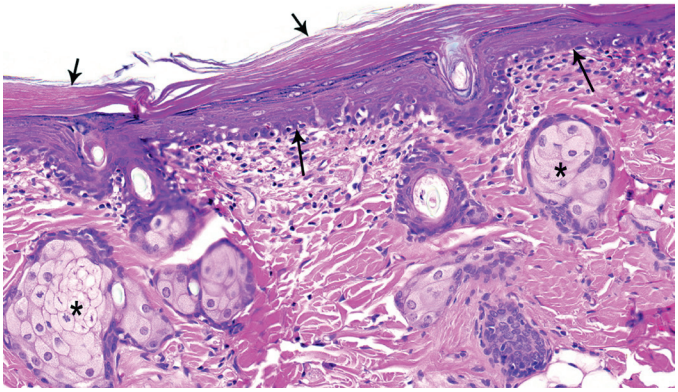


PLATE 4
Higher magnification of Plate 3 showing epidermal hyperplasia (long arrows), hyperkeratosis (short arrows), submucosal inflammation, and hyperplastic sebaceous glands (asterisks). H&E

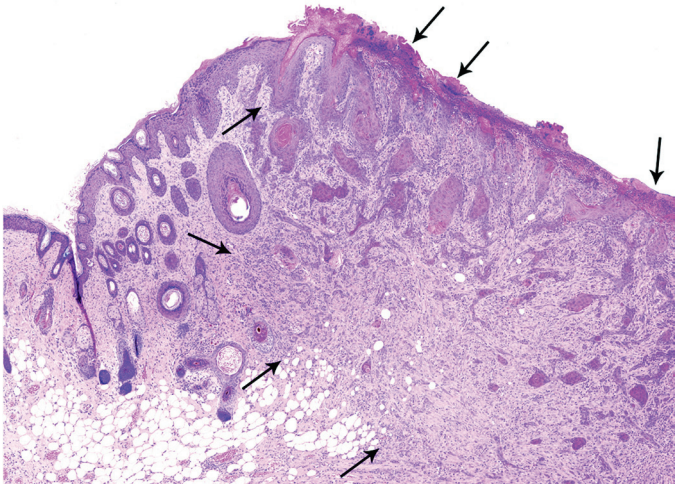


PLATE 5
Squamous cell carcinoma in the skin (site of application) of a female B6C3F1/N mouse administered 75 mg/kg pyrogallol dermally for 2 years. The neoplasm (arrows) has effaced the epidermis and invaded into the dermis and subcutis. H&E

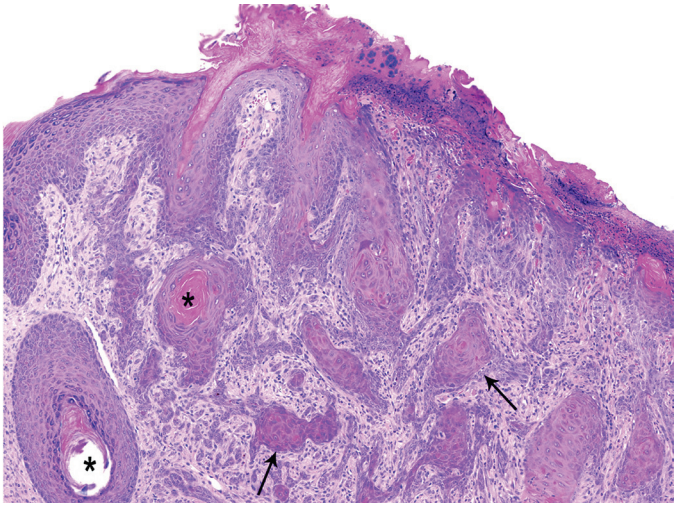


PLATE 6
Higher magnification of Plate 5. Note the cords and islands of well-differentiated but dysplastic squamous epithelium (arrows) some of which surround keratin pearls (asterisks). H&E

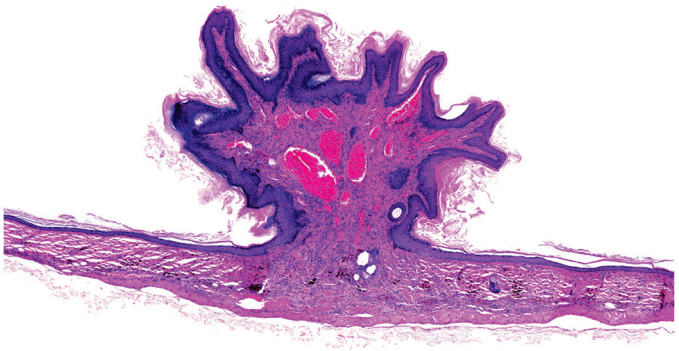


PLATE 7
Squamous cell papilloma in the skin (site of application) of a male B6C3F1/N mouse administered 75 mg/kg pyrogallol dermally for 2 years. The neoplasm is an exophytic growth composed of an inner connective tissue core forming a stalk with superficial branching fronds and covered by an outer layer of hyperplastic and hyperkeratotic squamous epithelium. H&E

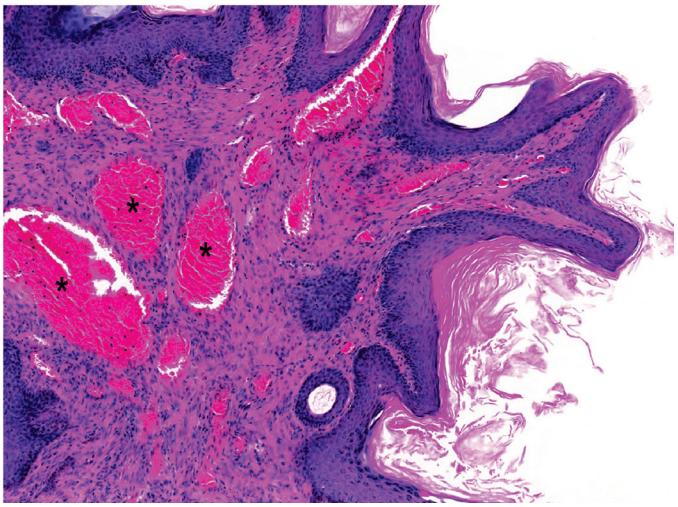


PLATE 8
Higher magnification of Plate 7 depicting the inner connective tissue core and superficial branching fronds covered by an outer layer of hyperplastic and hyperkeratotic squamous epithelium. Note the dilated blood vessels (asterisks) within the core. H&E

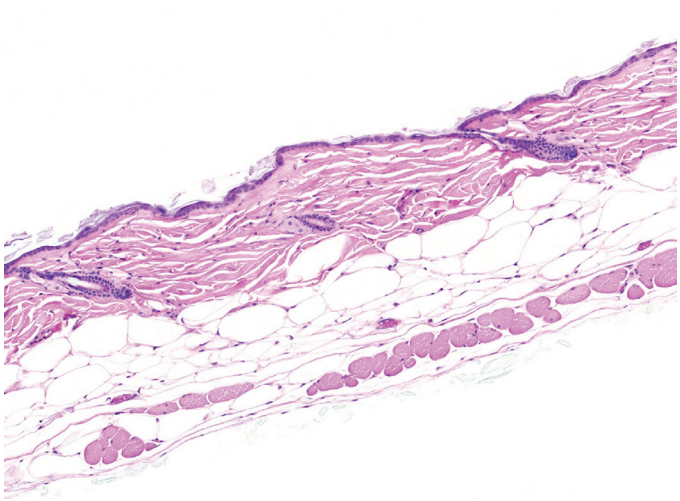


PLATE 9
Normal skin (site of application) of a male B6C3F1/N vehicle control mouse in the 2-year dermal study of pyrogallol. H&E

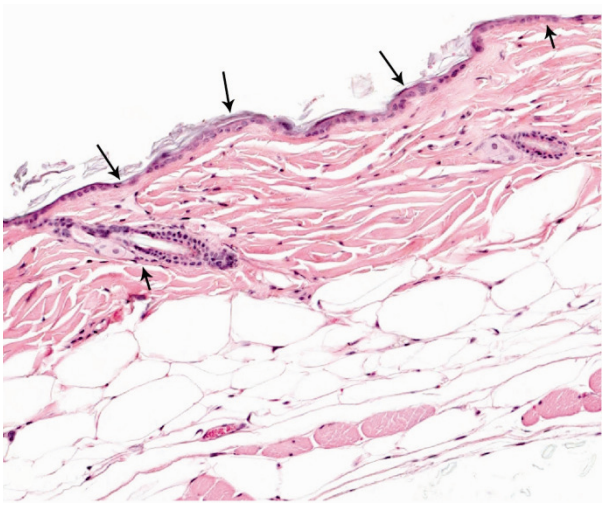


PLATE 10
Higher magnification of Plate 9. The epidermis consists of a single layer of epithelial cells (long arrows). Note the sebaceous gland (short arrow) associated with a hair follicle. H&E

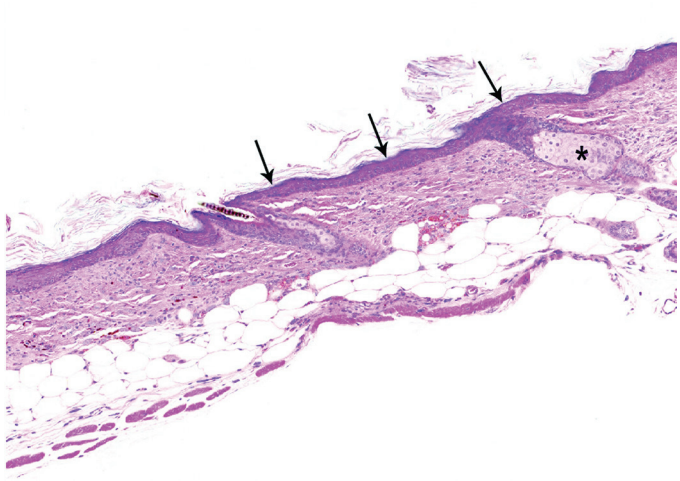


PLATE 11
Skin (site of application) of a male B6C3F1/N mouse administered 20 mg/kg pyrogallol dermally for 2 years depicting mild epidermal hyperplasia (arrows) and minimal hyperkeratosis. Note the hyperplastic sebaceous gland (asterisk) and brown pigment within the dermis. H&E

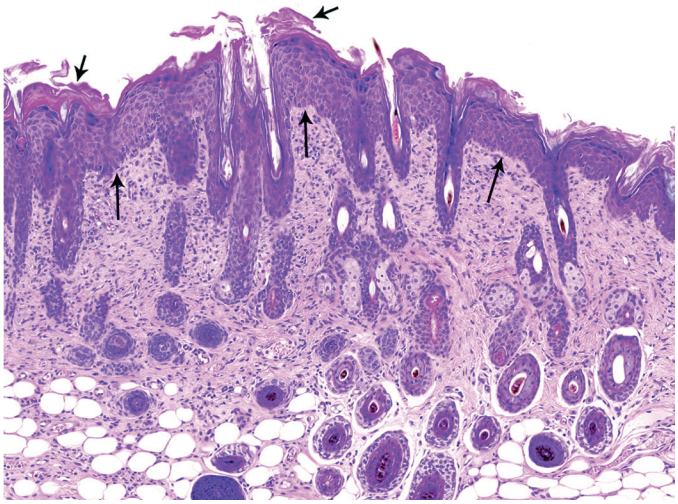


PLATE 12
Skin (site of application) of a male B6C3F1/N mouse administered 20 mg/kg pyrogallol dermally for 2 years depicting moderate epidermal hyperplasia (long arrows) and hyperkeratosis (short arrows). Note the inflammatory cells within the dermis. H&E

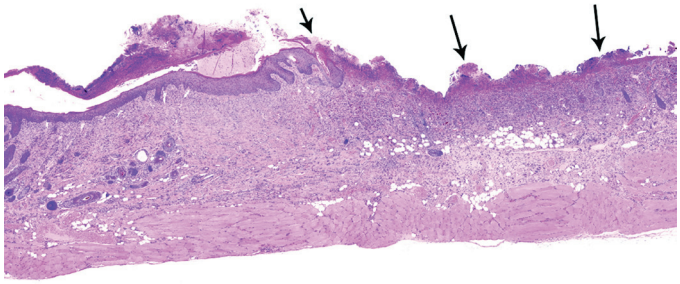


PLATE 13
Ulcers (arrows) in the skin (site of application) of a male B6C3F1/N mouse administered 75 mg/kg pyrogallol dermally for 2 years. H&E

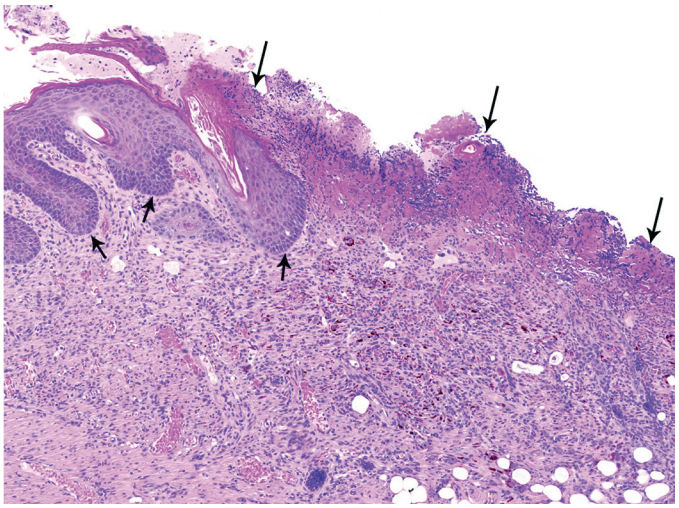


PLATE 14
Higher magnification of Plate 13. Note the complete loss of the epidermis (long arrows) with superficial necrosis, diffuse inflammation, and pigment within the dermis. The adjacent intact epidermis is hyperplastic (short arrows). H&E

DISCUSSION AND CONCLUSIONS

Pyrogallol has a long history of use as a hair dye, textile dye, and photographic developer. It was commonly used as a modifier in oxidation hair dyes sold in the United States until the early 1990s (CIR, 1991; Leston, 2000; *Merck*, 2006) and may still be in use in other countries (Mazzei *et al.*, 2007). Currently pyrogallol is mostly used in the United States to produce other chemicals (Leston, 2000) and in the formulation of the fine arts photographic developer known as PMK or Gordon Hutchings formula (Bergger, 2011). The general population may be exposed to pyrogallol naturally present in foods (Lang *et al.*, 2006), especially those processed by roasting or smoking (Knowles *et al.*, 1975; Ohshima *et al.*, 1989), and by endogenous metabolism of gallic acid and other polyphenols (Meselhy *et al.*, 1997; Daykin *et al.*, 2005; Schantz *et al.*, 2010).

Pyrogallol was nominated to the NTP for testing due to its widespread occurrence in natural and manufactured products and the lack of studies assessing its carcinogenicity potential. In the studies presented here, testing of rats and mice was done through the dermal route because that is the most common exposure route for humans. The selection of dose formulation concentrations for the 3-month studies in rats and mice was limited by the maximum solubility of pyrogallol in the ethanol vehicle and the fixed dosing volumes used.

The studies presented in this report show that the skin at the site of application is the primary site of toxicity for pyrogallol. Although the incidences of most lesions at the site of application were similar in all dosed groups (i.e., a no-effect level was not observed), the severity of the lesions was dose-dependent.

The 3-month studies in F344/N rats and B6C3F1/N mice had very similar results. There were no chemical-related deaths; final body weights, body weight gains, and organ weights of treated animals were comparable to those of the vehicle controls. The only chemical-related clinical findings were brown staining and irritation of the skin at the site of application, and by study termination, most animals had both. Concomitant microscopic lesions (squamous hyperplasia, hyperkeratosis, and chronic active inflammation) were also observed in most dosed rats and mice with incidences significantly greater than those in the vehicle controls. The severity of these lesions increased with increasing dose. Mild ulcers occurred in a few mice in the 300 or 600 mg/kg groups. The incidence of hematopoietic cell proliferation in the spleen was significantly increased in 600 mg/kg male mice. No treatment-related effects on the thyroid gland or thyroid hormone levels were seen in the rat or mouse studies.

The dermal toxicity observed in the 3-month studies precluded the use of doses greater than 75 mg/kg. Therefore, 75 mg/kg was selected as the high dose, 20 mg/kg as the mid dose, and 5 mg/kg as the low dose for the 2-year studies.

In general, mice were more sensitive to the effects of pyrogallol than rats during the 2-year studies. Survival and mean body weights of rats and male mice administered pyrogallol were generally similar to those of the vehicle controls, but survival of female mice administered 75 mg/kg was significantly less than that of the vehicle controls. By the end of the study, only 34% of 75 mg/kg females were alive, compared with 66% of the vehicle controls, and mean body weight averaged 88% of that of the vehicle controls during the second year of the study.

In the 2-year study, irritation of the skin at the site of application was the only clinical finding observed in rats administered 20 or 75 mg/kg pyrogallol. Irritation and/or ulceration at the site of application were observed in 2-year mice predominantly in the 20 and 75 mg/kg groups. Ulceration was especially severe in 75 mg/kg female mice, and this lesion was the cause of decreased survival in this group. By the end of the study, 86% of the moribund kills of females were due to severe ulceration.

Several types of microscopic lesions seen in the 3-month studies in the skin at the site of application were also observed in the 2-year studies in rats and mice, including hyperplasia, hyperkeratosis, inflammation, and ulcer. In addition, fibrosis, pigmentation, and sebaceous gland hyperplasia were seen in mice after 2 years of treatment. Incidences of inflammation and ulcer of the skin at sites adjacent to the site of application were also significantly increased in 75 mg/kg male and female mice.

Two types of neoplastic skin lesions of concern occurred at the site of application in mice: Squamous cell papilloma in males and squamous cell carcinoma in

females. The incidence of squamous cell papilloma at the site of application in 75 mg/kg male mice (2/50, 4%) was not statistically different from that in the vehicle control group (0/50); however, it exceeded the historical control ranges for 2-year ethanol dermal studies (0/200) and for all routes (1/1,150). The incidence of squamous cell carcinoma of the skin at the site of application was significantly increased in 75 mg/kg female mice (4/50, 8%) when compared to the vehicle controls (0/50) and exceeded the historical control ranges for 2-year ethanol dermal studies (0/200) and for all routes (0/1,198).

Occurrences of nonneoplastic lesions in non-skin tissue were common only in mice, and they occurred at higher rates in females than in males. The significantly increased incidences included hematopoietic cell proliferation in the adrenal cortex (female mice), lymphoid hyperplasia in lymph nodes (female mice), and hyperplasia in the bone marrow (male and female mice) and mammary gland (female mice). Most of these lesions (except those in mammary gland) were considered secondary to more severe skin lesions such as ulcers. These findings were interpreted to be a physiologic response to inflammatory lesions in the skin and not related to any direct systemic response to pyrogallol.

CONCLUSIONS

Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenic activity** of pyrogallol in male or female F344/N rats administered 5, 20, or 75 mg/kg. There was *equivocal evidence of carcinogenic activity* of pyrogallol in male B6C3F1/N mice based on increased incidences of squamous cell papilloma of the skin at the site of application. There was *some evidence of carcinogenic activity* of pyrogallol in female B6C3F1/N mice based on increased incidences of squamous cell carcinoma of the skin at the site of application.

Dermal administration of pyrogallol caused increased incidences of nonneoplastic lesions of the skin at the site of application in male and female rats and mice, skin adjacent to the site of application in male and female mice, and mammary gland in female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Peer Review Panel's comments and the public discussion on this Technical Report appears on page 13.

REFERENCES

The Aldrich Library of ^{13}C and ^{1}H FT-NMR Spectra (1993). 1st ed. (C.J. Pouchert and J. Behnke, Eds.), Vol. 2, p. 299, spectrum A. Aldrich Chemical Company, Inc., Milwaukee, WI.

The Aldrich Library of FT-IR Spectra (1997). 2nd ed. (C.J. Pouchert, Ed.), Vol. 2, p. 1887. Aldrich Chemical Company, Inc., Milwaukee, WI.

Algaier, J., Barnes, S.M., Canham, B.A., Decker, K., Porter, A.J., Harris, R.K., Clark, A.P., Overstreet, D., and Smith, C.S. (2003). Stabilization and analysis of pyrogallol (PG) in rat blood and in receptor fluid media. *Toxicologist* **114**, 46-47 (Abstr. No. 224).

Allen, A. (1907). Gallic Acid and Its Allies. In: *Commercial Organic Analysis*, 3rd ed., Volume II – Part III, Aromatic Acids, pp. 115-126. P. Blakiston's Son & Co., Philadelphia.

Angel, A., and Rogers, K.J. (1968). Convulsant action of polyphenols. *Nature* **217**, 84-85.

Angel, A., Lemon, R.N., Rogers, K.J., and Banks, P. (1969). The effect of polyhydroxyphenols on brain ATP in the mouse. *Exp. Brain Res.* **7**, 250-257.

Archer, D.L., Smith, B.G., and Bukovic-Wess, J.A. (1978). Use of an *in vitro* antibody-producing system for recognizing potentially immunosuppressive compounds. *Int. Arch. Allergy Appl. Immunol.* **56**, 90-93.

Arnott, D.G., and Doniach, I. (1952). The effect of compounds allied to resorcinol upon the uptake of radioactive iodine (^{131}I) by the thyroid of the rat. *Biochem. J.* **50**, 473-479.

Ashby, J., and Tennant, R.W. (1991). Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* **257**, 229-306.

Bailer, A.J., and Portier, C.J. (1988). Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. *Biometrics* **44**, 417-431.

Bakke, O.M. (1970). *O*-methylation of simple phenols in the rat. *Acta Pharmacol. Toxicol.* **28**, 28-38.

Ben-Gurion, R. (1979). Mutagenic and colicine-inducing activity of two antioxidants: Pyrogallol and purpurogallin. *Mutat. Res.* **68**, 201-205.

Bergger Products, Inc. (2011). Chemicals: PMK Liquid Developer. Bergger Products, Inc., Rockford, IL. <http://bergger.com/inter/pmk.html> Accessed November 28, 2011.

Bhalla, T.N., Sinha, J.N., Tangri, K.K., and Bhargava, K.P. (1970). Role of catecholamines in inflammation. *Eur. J. Pharmacol.* **13**, 90-96.

Bieler, G.S., and Williams, R.L. (1993). Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity. *Biometrics* **49**, 793-801.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Booth, A.N., Masri, M.S., Robbins, D.J., Emerson, O.H., Jones, F.T., and DeEds, F. (1959). The metabolic fate of gallic acid and related compounds. *J. Biol. Chem.* **234**, 3014-3016.

Burnett, C., Goldenthal, E.I., Harris, S.B., Wazeter, F.X., Strausburg, J., Kapp, R., and Voelker, R. (1976). Teratology and percutaneous toxicity studies on hair dyes. *J. Toxicol. Environ. Health* **1**, 1027-1040.

Cerilliant Corporation. (2010). Pyrogallol: Analytical Report. Cerilliant Corporation, Round Rock, TX. <http://www.cerilliant.com/products/coa/PHY89772.pdf> Accessed November 28, 2011.

Chemical Sources International, Inc. (CSI) (2011). Chem Sources: Pyrogallol, CI 76515. <http://db2.chemsources.com> Accessed November 28, 2011.

Code of Federal Regulations (CFR) **21**, Part 58.

Code of Federal Regulations (CFR) **21**, Part 73.

Code of Federal Regulations (CFR) **21**, § 73.1375.

Code of Federal Regulations (CFR) **21**, Part 74.

Code of Federal Regulations (CFR) **21**, § 101.108.

Cooksey, R.C., Gaitan, E., Lindsay, R.H., Hill, J.B., and Kelly, K. (1985). Humic substances, a possible source of environmental goitrogens. *Org. Geochem.* **8**, 77-80.

Cosmetic Ingredient Review (CIR) Expert Panel (1991). Final report on the safety assessment of pyrogallol. *Int. J. Toxicol.* **10**, 67-85.

Cox, D.R. (1972). Regression models and life-tables. *J. R. Stat. Soc.* **B34**, 187-220.

Crawford, B.D. (1985). Perspectives on the somatic mutation model of carcinogenesis. In *Advances in Modern Environmental Toxicology. Mechanisms and Toxicity of Chemical Carcinogens and Mutagens* (M.A. Mehlman, W.G. Flamm, and R.J. Lorentzen, Eds.), pp. 13-59. Princeton Scientific Publishing Co., Inc., Princeton, NJ.

CRC Handbook of Chemistry and Physics (2011). 92nd ed. (D.R. Lide, Ed.). CRC Press, Taylor & Francis, Inc., Boca Raton, FL. <www.hbcpnetbase.com> Accessed November 28, 2011.

Creelman, R.A., Adam, L.J., Riechmann, J.L., Heard, J.E., Pineda, O., Jiang, C.-Z., Ratcliffe, O.J., and Reuber, T.L. (2010). Transcription factors for increasing yield. United States Patent Application 20110119789.

Daykin, C.A., Van Duynhoven, J.P.M., Groenewegen, A., Dachtler, M., Van Amelsvoort, J.M.M., and Mulder, T.P.J. (2005). Nuclear magnetic resonance spectroscopic based studies of the metabolism of black tea polyphenols in humans. *J. Agric. Food Chem.* **53**, 1428-1434.

Dixon, W.J., and Massey, F.J., Jr. (1957). *Introduction to Statistical Analysis*, 2nd ed., pp. 276-278, 412. McGraw-Hill Book Company, Inc., New York.

Do Céu Silva, M., Gaspar, J., Silva, I.D., Leão, D., and Rueff, J. (2003). Induction of chromosomal aberrations by phenolic compounds: Possible role of reactive oxygen species. *Mutat. Res.* **540**, 29-42.

Dollahite, J.W., Pigeon, R.F., and Camp, B.J. (1962). The toxicity of gallic acid, pyrogallol, tannic acid and *Quercus havardi* in the rabbit. *Am. J. Vet. Res.* **23**, 1264-1267.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Eccleston, D., and Ritchie, I.M. (1973). Sulphate ester formation from catecholamine metabolites and pyrogallol in rat brain *in vivo*. *J. Neurochem.* **21**, 635-646.

Federal Register (2004). Allethrin, Bendiocarb, *Burkholderia cepacia*, Fenridazon potassium, and Molinate; Tolerance Actions. Vol. 69, No. 188, pp. 58079-58083. U.S. Environmental Protection Agency, Washington, DC.

Frosch, P.J., Burrows, D., Camarasa, J.G., Dooms-Goossens, A., Ducombs, G., Lahti, A., Menné, T., Rycroft, R.J.G., Shaw, S., White, I.R., and Wilkinson, J.D., on behalf of The European Environmental and Contact Dermatitis Research Group (1993). Allergic reactions to a hairdressers' series: Results from 9 European centres. *Contact Dermatitis* **28**, 180-183.

Gaitan, E. (1983). Endemic goiter in western Colombia. *Ecol. Dis.* **2**, 295-308.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *JNCI* **62**, 957-974.

Girard, D.M., and Sager, D.B. (1987). The use of Markov chains to detect subtle variation in reproductive cycling. *Biometrics* **43**, 225-234.

Glatt, H., Padykula, R., Berchtold, G.A., Ludewig, G., Platt, K.L., Klein, J., and Oesch, F. (1989). Multiple activation pathways of benzene leading to products with varying genotoxic characteristics. *Environ. Health Perspect.* **82**, 81-89.

Gocke, E., King, M.T., Eckhardt, K., and Wild, D. (1981). Mutagenicity of cosmetics ingredients licensed by the European Communities. *Mutat. Res.* **90**, 91-109.

Gosselin, R.E., Smith, R.P., Hodge, H.C., and Braddock, J.E. (1984). *Clinical Toxicology of Commercial Products*, 5th ed., p. II-190. Williams & Wilkins, Baltimore, MD.

Guerra, L., Tosti, A., Bardazzi, F., Pigatto, P., Lisi, P., Santucci, B., Valsecchi, R., Schena, D., Angelini, G., Sertoli, A., Ayala, F., Kokeli, F., and Gruppo Italiano Ricerca Dermatiti da Contatto e Ambientali (1992a). Contact dermatitis in hairdressers: The Italian experience. *Contact Dermatitis* **26**, 101-107.

Guerra, L., Bardazzi, F., and Tosti, A. (1992b). Contact dermatitis in hairdressers' clients. *Contact Dermatitis* **26**, 108-111.

Guldberg, H.C., and Marsden, C.A. (1975). Catechol-O-methyl transferase: Pharmacological aspects and physiological role. *Pharmacol. Rev.* **27**, 135-206.

Hasegawa, K., Nishi, T., Kinsho, T., and Tachibana, S. (2011). Lactone-containing compound, polymer, resist composition, and patterning process. United States Patent 7,871,752.

Haslam, E. (2003). Thoughts on thearubigins. *Phytochemistry* **64**, 61-73.

Hayakawa, F., Kimura, T., Maeda, T., Fujita, M., Sohmiya, H., Fujii, M., and Ando, T. (1997). DNA cleavage reaction and linoleic acid peroxidation induced by tea catechins in the presence of cupric ion. *Biochim. Biophys. Acta* **1336**, 123-131.

Heddle, J.A., Hite, M., Kirkhart, B., Mavournin, K., MacGregor, J.T., Newell, G.W., and Salamone, M.F. (1983). The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* **123**, 61-118.

Hillen, U., Grabbe, S., and Uter, W. (2007). Patch test results in patients with scalp dermatitis: Analysis of data of the Information Network of Departments of Dermatology. *Contact Dermatitis* **56**, 87-93.

Hirose, M., Inoue, T., Asamoto, M., Tagawa, Y., and Ito, N. (1986). Comparison of the effects of 13 phenolic compounds in induction of proliferative lesions of the forestomach and increase in the labelling indices of the glandular stomach and urinary bladder epithelium of Syrian golden hamsters. *Carcinogenesis* **7**, 1285-1289.

Jacobs, M.M., Burnett, C.M., Penicnak, A.J., Herrera, J.A., Morris, W.E., Shubik, P., Apaja, M., and Granroth, G. (1984). Evaluation of the toxicity and carcinogenicity of hair dyes in Swiss mice. *Drug Chem. Toxicol.* **7**, 573-586.

Joharapurkar, A.A., Wanjari, M.M., Dixit, P.V., Zambad, S.P., and Umathe, S.N. (2004). Pyrogallol: A novel tool for screening immunomodulators. *Indian J. Pharmacol.* **36**, 355-359.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* **53**, 457-481.

Keil, H. (1962). Group reactions in contact dermatitis due to resorcinol. *Arch. Dermatol.* **86**, 212-216.

Knowles, M.E., Gilbert, J., and McWeeny, D.J. (1975). Phenols in smoked, cured meats: Nitrosation of phenols in liquid smokes and in smoked bacon. *J. Sci. Food Agric.* **26**, 267-276.

Kortüm, G., Vogel, W., and Andrussow, K., on behalf of the International Union of Pure and Applied Chemistry (1961). Dissociation constants of organic acids in aqueous solution. Butterworths, London.

Lang, R., Mueller, C., and Hofmann, T. (2006). Development of a stable isotope dilution analysis with liquid chromatography-tandem mass spectrometry detection for the quantitative analysis of di- and trihydroxybenzenes in foods and model systems. *J. Agric. Food Chem.* **54**, 5755-5762.

Lautala, P., Ulmanen, I., and Taskinen, J. (2001). Molecular mechanisms controlling the rate and specificity of catechol O-methylation by human soluble catechol O-methyltransferase. *Mol. Pharmacol.* **59**, 393-402.

Lee, S.-F., and Lin, J.-K. (1994). Generation of hydrogen peroxide, superoxide anion and the hydroxyl free radical from polyphenols and active benzene metabolites: Their possible role in mutagenesis. *J. Biomed. Sci.* **1**, 125-130.

Lee, S.-F., Liang, Y.C., and Lin, J.-K. (1995). Inhibition of 1,2,4-benzenetriol-generated active oxygen species and induction of phase II enzymes by green tea polyphenols. *Chem. Biol. Interact.* **98**, 283-301.

Leston, G. (2000). Polyhydroxybenzenes. In *Kirk-Othmer Encyclopedia of Chemical Technology* (Wiley online library <http://onlinelibrary.wiley.com/doi/10.1002/0471238961>), pp. 1-38.

Lin, J.-K., and Lee, S.-F. (1992). Enhancement of the mutagenicity of polyphenols by chlorination and nitrosation in *Salmonella typhimurium*. *Mutat. Res.* **269**, 217-224.

Lynch, B.S., Delzell, E.S., and Bechtel, D.H. (2002). Toxicology review and risk assessment of resorcinol: Thyroid effects. *Regul. Toxicol. Pharmacol.* **36**, 198-210.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.

MacGregor, J.T., Wehr, C.M., Henika, P.R., and Shelby, M.D. (1990). The *in vivo* erythrocyte micronucleus test: Measurement at steady state increase assay efficiency and permits integration with toxicity studies. *Fundam. Appl. Toxicol.* **14**, 513-522.

Marklund, S., and Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.* **47**, 469-474.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

Masri, M.S., Robbins, D.J., Emerson, O.H., and Deeds, F. (1964). Selective *para*- or *meta*-*O*-methylation with catechol *O*-methyl transferase from rat liver. *Nature* **202**, 878-879.

Mazzei, J.L., da Silva, D.N., Oliveira, V., Hosomi, R.Z., do Val, R.R., Pestana, C.B., and Felzenszwalb, I. (2007). Absence of mutagenicity of acid pyrogallol-containing hair gels. *Food Chem. Toxicol.* **45**, 643-648.

Meiser, H., Hagedorn, H.W., and Schulz, R. (2000). Pyrogallol concentrations in rumen content, liver and kidney of cows at pasture [in German]. *Berl. Munch. Tierarztl. Wochenschr.* **113**, 108-111.

Mercado Común de Sur (MERCOSUR) (2005). Reglamento Técnico MERCOSUR sobre lista de sustancias que los productos de higiene personal, cosméticos y perfumes no deben contener excepto en las condiciones y con las arestricciones establecidas. MECOSUR, Montevideo, Uruguay.

The Merck Index (1968). 8th ed. (P.G. Strecher, Ed.), pp. 894-898. Merck & Co, Inc., Whitehall, NJ.

The Merck Index (1983). 11th ed. (S. Budavari, Ed.), p. 1154. Merck & Co., Inc., Rahway, NJ.

The Merck Index (1996). 12th ed. (S. Budavari, Ed.), pp. 1375-1376. Merck & Company, Inc., Whitehouse Station, NJ.

The Merck Index (2006). 14th ed. (M.J. O'Neil, Ed.), p. 1376. Merck Research Laboratories, Division of Merck & Co., Inc., Whitehouse Station, NJ.

Meselhy, M.R., Nakamura, N., and Hattori, M. (1997). Biotransformation of (–)-epicatechin 3-*O*-gallate by human intestinal bacteria. *Chem. Pharm. Bull.* (Tokyo) **45**, 888-893.

Miller, J.A., and Miller, E.C. (1977). Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer* (H.H. Hiatt, J.D. Watson, and J.A. Winsten, Eds.), pp. 605-627. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Ministry of Health and Welfare (MHW) (2000). Standards for Cosmetics, Ministry of Health and Welfare Notification No. 331 (English translation). Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labor, and Welfare, Japan.

Miyata, Y., Fukushima, S., Hirose, M., Masui, T., and Ito, N. (1985). Short-term screening of promoters of bladder carcinogenesis in N-butyl-N-(4-hydroxybutyl)nitrosamine-initiated, unilaterally ureter-ligated rats. *Jpn. J. Cancer Res.* **76**, 828-834.

Miyazaki, K., Arai, S., Iwamoto, T., Takasaki, M., and Tomoda, A. (2004). Metabolism of pyrogallol to purpurogallin by human erythrocytic hemoglobin. *Tohoku J. Exp. Med.* **203**, 319-330.

Nakamura, T., Nakazawa, Y., Onizuka, S., Satoh, S., Chiba, A., Sekihashi, K., Miura, A., Yasugahira, N., and Sasaki, Y.F. (1997). Antimutagenicity of Tochu tea (an aqueous extract of *Eucommia ulmoides* leaves): 1. The clastogen-suppressing effects of Tochu tea in CHO cells and mice. *Mutat. Res.* **388**, 7-20.

National Center for Biotechnology Information (NCBI) (2011). Pyrogallol – Compound Summary (CID 1057). NCBI PubChem Public Chemical Compound Database. <pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=1057&loc=ec_rcs> Accessed October 14, 2011.

National Institute for Occupational Safety and Health (NIOSH) (1990). National Occupational Exposure Survey (1981-1983), unpublished provisional data as of July 1, 1990. NIOSH, Cincinnati, OH.

National Toxicology Program (NTP) (2006). Immunotoxicity Study Report M960044. Assessment of Contact Hypersensitivity to Pyrogallol in Female BALB/c Mice. Research Triangle Park, NC.

Ohshima, H., Friesen, M., Malaveille, C., Brouet, I., Hautefeuille, A., and Bartsch, H. (1989). Formation of direct-acting genotoxic substances in nitrosated smoked fish and meat products: Identification of simple phenolic precursors and phenyldiazonium ions as reactive products. *Food Chem. Toxicol.* **27**, 193-203.

Paschin, Iu.V., Bakhitova, L.M., and Benthen, T.I. (1986). Increased antimutagenic activity of simple substituted phenols mixed with the hindered phenolic antioxidant dibunol. *Food Chem. Toxicol.* **24**, 881-883.

Patty's Industrial Hygiene and Toxicology (1981). 3rd ed. (G.D. Clayton and F.E. Clayton, Eds.), Revised Vol. 2A, p. 2594. John Wiley & Sons, New York.

Pewny, R. (1925). A fatal case of pyrogallic acid poisoning. *Med. Klin.* **21**, 970.

Picciano, J.C., Morris, W.E., Kwan, S., and Wolf, B.A. (1983). Evaluation of the teratogenic and mutagenic potential of the oxidative dyes, 4-chlororesorcinol, *m*-phenylenediamine, and pyrogallol. *Int. J. Toxicol.* **2**, 325-333.

Piegorsch, W.W., and Bailer, A.J. (1997). *Statistics for Environmental Biology and Toxicology*, Section 6.3.2. Chapman and Hall, London.

Portier, C.J., and Bailer, A.J. (1989). Testing for increased carcinogenicity using a survival-adjusted quantal response test. *Fundam. Appl. Toxicol.* **12**, 731-737.

Portier, C.J., Hedges, J.C., and Hoel, D.G. (1986). Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program's carcinogenicity experiments. *Cancer Res.* **46**, 4372-4378.

Rao, G.N. (1996). New diet (NTP-2000) for rats in the National Toxicology Program toxicity and carcinogenicity studies. *Fundam. Appl. Toxicol.* **32**, 102-108.

Rao, G.N. (1997). New nonpurified diet (NTP-2000) for rodents in the National Toxicology Program's toxicology and carcinogenesis studies. *J. Nutr.* **127**, 842s-846s.

Reed, J.D. (1995). Nutritional toxicology of tannins and related polyphenols in forage legumes. *J. Anim. Sci.* **73**, 1516-1528.

Reuber, T.L., Ratcliffe, O., Heard, J., Riechmann, J.L., Pineda, O., and Adam, L. (2010). Transcription factors for increasing yield. United States Patent 7,858,848.

Rogers, K.J., Angel, A., and Butterfield, L. (1968). The penetration of catechol and pyrogallol into mouse brain and the effect on cerebral monoamine levels. *J. Pharm. Pharmacol.* **20**, 727-729.

Rosin, M.P. (1984). The influence of pH on the convertogenic activity of plant phenolics. *Mutat. Res.* **135**, 109-113.

Rovito, R.J., Rieker, J.M., and Peters, D.W. (2010). Aqueous buffered fluoride-containing etch residue removers and cleaners. United States Patent 7,807,613.

Russell, W.R., and Schultz, J.A. (2011). Process for preparing stable photoresist compositions. United States Patent 7,862,983.

Sakagami, Y., Yokoyama, H., Ose, Y., and Sato, T. (1986). Screening test for carcinogenicity of chlorhexidine digluconate and its metabolites. *J. Hyg. Chem.* **32**, 171-175.

Schantz, M., Erk, T., and Richling, E. (2010). Metabolism of green tea catechins by the human small intestine. *Biotechnol. J.* **5**, 1050-1059.

Scheline, R.R. (1966). The decarboxylation of some phenolic acids by the rat. *Acta Pharmacol. Toxicol.* **24**, 275-285.

Scheline, R.R. (1968). The metabolism of drugs and other organic compounds by the intestinal microflora. *Acta Pharmacol. Toxicol.* **26**, 332-342.

Schmid, W. (1975). The micronucleus test. *Mutat. Res.* **31**, 9-15.

Scholander, P.F., Van Dam, L., Lloyd Claff, C., and Kanwisher, J.W. (1955). Micro gasometric determination of dissolved oxygen and nitrogen. *Biol. Bull.* **109**, 328-334.

Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) Intended for Consumers of the European Commission (2003). The SCCNFP's Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation. Report No. SCCNFP/0690/03, p. 86. European Commission, Brussels.

Seffner, W., Schiller, F., Heinze, R., and Breng, R. (1995). Subchronic application of humic acids and associated compounds provokes histological changes of goitre in the rat. *Exp. Toxicol. Pathol.* **47**, 63-70.

Shelby, M.D., and Witt, K.L. (1995). Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. *Environ. Mol. Mutagen.* **25**, 302-313.

Shelby, M.D., Erexson, G.L., Hook, G.J., and Tice, R.R. (1993). Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. *Environ. Mol. Mutagen.* **21**, 160-179.

Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.

Sigma-Aldrich Corporation. (2011). Sigma-Aldrich Catalog: Pyrogallol, Catalog No. 16040. <http://www.sigmaaldrich.com/catalog/Lookup.do?N5=All&N3=mode+matchpartialmax&N4=pyrogallol&D7=0&D10=pyrogallol&N1=S_ID&ST=RS&N25=0&F=PR> Accessed November 28, 2011.

Stenbäck, F. (1977). Local and systemic effects of commonly used cutaneous agents: Lifetime studies of 16 compounds in mice and rabbits. *Acta Pharmacol. Toxicol.* **41**, 417-431.

Stenbäck, F., and Shubik, P. (1974). Lack of toxicity and carcinogenicity of some commonly used cutaneous agents. *Toxicol. Appl. Pharmacol.* **30**, 7-13.

Stich, H.F., Rosin, M.P., Wu, C.H., and Powrie, W.D. (1981). The action of transition metals on the genotoxicity of simple phenols, phenolic acids and cinnamic acids. *Cancer Lett.* **14**, 251-260.

Straus, D.S. (1981). Somatic mutation, cellular differentiation, and cancer causation. *JNCI* **67**, 233-241.

Suzuki, T., and Otake, A. (2010). Cleaning composition comprising a chelant and quaternary ammonium hydroxide mixture. United States Patent 7,825,079.

Tan, K.H. (2003). Humic Matter in Soil and the Environment: Principles and Controversies. Marcel Dekker, Inc., New York.

Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* **62**, 679-682.

Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B., and Minor, R. (1987). Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. *Science* **236**, 933-941.

United States Environmental Protection Agency (USEPA) (1999). R.E.D. Facts: Bendiocarb. E.P.A. Report No. 738-F-99-010. U.S. Environmental Protection Agency, Office of Pesticide Programs, Special Review and Reregistration Division, Washington, DC. <www.epa.gov/oppsrrd1/REDs/factsheets/0409fact.pdf> Accessed November 28, 2011.

Van Duuren, B.L. (1980). Carcinogenicity of hair dye components. *J. Environ. Pathol. Toxicol.* **3**, 237-251.

Van Duuren, B.L., and Goldschmidt, B.M. (1976). Cocarcinogenic and tumor-promoting agents in tobacco carcinogenesis. *J. Natl. Cancer Inst.* **56**, 1237-1242.

Von Oettingen, W.F. (1949). Phenol and its derivatives: The relation between their chemical constitution and their effect on the organism. National Institutes of Health Bulletin No. 190. National Institutes of Health, Experimental Biology and Medicine Institute, Laboratory of Physical Biology, Washington, D.C.

Wang, C.Y., and Klemencic, J.M. (1979). Mutagenicity and carcinogenicity of polyhydric phenols. *Proc. Am. Assoc. Cancer Res.* **20**, 117 (Abstr.).

Wang, M.Z., Farmer, S.A., Richardson, D.M., and Davis, M.D.P. (2011). Patch-testing with hairdressing chemicals. *Dermatitis* **22**, 16-26.

Watanabe, K., Sakamoto, K., and Sasaki, T. (1998). Comparisons on chemically-induced mutation among four bacterial strains, *Salmonella typhimurium* TA102 and TA2638, and E*scherichia coli* WP2/pKM101 and WP2 *uvrA*/pKM101: Collaborative study II. *Mutat. Res.* **412**, 17-31.

Watanabe, T., Kusumoto, M., Ishihara, M., Okumura, H., Takase, M., Wakisaka, H., and Hirayama, T. (1991). The modulating effect of hair dye components on the formation of mutagenic oxidized products from *m*-phenylenediamine with hydrogen peroxide. *Eisei Kagaku* **37**, 512-521.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

Williams, D.A. (1986). A note on Shirley's nonparametric test for comparing several dose levels with a zero-dose control. *Biometrics* **42**, 183-186.

Willsteed, E., and Regan, W. (1985). Psoriasis, pyrogallol and skin cancer. *Aust. J. Dermatol.* **26**, 144-145.

Winter, R. (2009). *A Consumer's Dictionary of Cosmetic Ingredients*, 7th ed., p. 440. Three Rivers Press, New York.

Witt, K.L., Knapton, A., Wehr, C.M., Hook, G.J., Mirsalis, J., Shelby, M.D., and MacGregor, J.T. (2000). Micronucleated erythrocyte frequency in peripheral blood of B6C3F1 mice from short-term, prechronic, and chronic studies of the NTP Carcinogenesis Bioassay Program. *Environ. Mol. Mutagen.* **36**, 163-194.

Yamada, K., Shirahata, S., Murakami, H., Nishiyama, K., Shinohara, K., and Omura, H. (1985). DNA breakage by phenyl compounds. *Agric. Biol. Chem.* **49**, 1423-1428.

Yamaguchi, T. (1981). Mutagenicity of low molecular substances in various superoxide generating systems. *Agric. Biol. Chem.* **45**, 327-330.

Yoshida, Y., Hirose, M., Takaba, K., Kimura, J., and Ito, N. (1994). Induction and promotion of forestomach tumors by sodium nitrite in combination with ascorbic acid or sodium ascorbate in rats with or without *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine pre-treatment. *Int. J. Cancer* **56** (1), 124-128.

Zeiger, E., Haseman, J.K., Shelby, M.D., Margolin, B.H., and Tennant, R.W. (1990). Evaluation of four in vitro genetic toxicity tests for predicting rodent carcinogenicity: Confirmation of earlier results with 41 additional chemicals. *Environ. Mol. Mutagen.* **16** (Suppl. 18), 1-14.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1992). *Salmonella* mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ. Mol. Mutagen.* **19** (Suppl. 21), 2-141.

APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR DERMAL STUDY OF PYROGALLOL

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

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	17	14	15	17
Natural deaths	10	8	7	5
Survivors				
Died last week of study		2		
Terminal kill	23	26	28	28
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(49)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	1 (2%)		1 (2%)	1 (2%)
Hepatocellular adenoma, multiple			1 (2%)	
Hepatocellular carcinoma		1 (2%)		
Mesentery	(9)	(2)	(3)	(5)
Fibrous histiocytoma	1 (11%)			
Oral mucosa	(2)	(1)	(4)	(3)
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)			
Schwannoma malignant	1 (2%)		1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma		1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		1 (2%)
Carcinosarcoma	1 (2%)			
Leiomyosarcoma		1 (2%)		
Tongue	(1)	(0)	(0)	(2)
Squamous cell carcinoma	1 (100%)			
Squamous cell papilloma				1 (50%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Carcinoma, metastatic, urethra				1 (2%)
Schwannoma malignant	2 (4%)	1 (2%)	1 (2%)	
Thymoma malignant, metastatic, thymus			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	3 (6%)	5 (10%)	7 (14%)	3 (6%)
Pheochromocytoma malignant	1 (2%)			1 (2%)
Bilateral, pheochromocytoma benign	1 (2%)	1 (2%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	5 (10%)	4 (8%)	5 (10%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Endocrine System (continued)				
Parathyroid gland	(50)	(49)	(47)	(49)
Pituitary gland	(50)	(49)	(50)	(50)
Pars distalis, adenoma	33 (66%)	33 (67%)	34 (68%)	27 (54%)
Pars intermedia, adenoma	1 (2%)	1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Bilateral, C-cell, adenoma	1 (2%)	1 (2%)	1 (2%)	
C-cell, adenoma	8 (16%)	8 (16%)	6 (12%)	6 (12%)
C-cell, carcinoma		1 (2%)		1 (2%)
Follicular cell, adenoma			1 (2%)	
Follicular cell, carcinoma			1 (2%)	1 (2%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Penis	(0)	(0)	(1)	(0)
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Hemangioma	1 (2%)			
Bilateral, interstitial cell, adenoma	26 (52%)	19 (38%)	28 (56%)	20 (40%)
Interstitial cell, adenoma	11 (22%)	12 (24%)	14 (28%)	17 (34%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(10)	(8)	(4)	(7)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Thymus	(47)	(48)	(48)	(47)
Carcinoma, metastatic, thyroid gland				1 (2%)
Thymoma malignant			1 (2%)	
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Adenocarcinoma	1 (2%)			
Fibroadenoma		2 (4%)	1 (2%)	1 (2%)
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma				1 (2%)
Keratoacanthoma	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Squamous cell papilloma	1 (2%)			3 (6%)
Control, subcutaneous tissue, fibrous histiocytoma		1 (2%)		
Subcutaneous tissue, fibroma	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Subcutaneous tissue, fibrosarcoma			1 (2%)	
Subcutaneous tissue, fibrous histiocytoma		1 (2%)	1 (2%)	
Subcutaneous tissue, hemangiosarcoma		1 (2%)		
Subcutaneous tissue, lipoma		1 (2%)		1 (2%)
Subcutaneous tissue, schwannoma malignant		1 (2%)	3 (6%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma		1 (2%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant			1 (2%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)		
Alveolar/bronchiolar carcinoma	3 (6%)	2 (4%)	2 (4%)	
Carcinoma, metastatic, urethra				1 (2%)
Carcinoma, metastatic, Zymbal's gland	1 (2%)			
Osteosarcoma, metastatic, bone		1 (2%)		
Pheochromocytoma malignant, metastatic, adrenal medulla				1 (2%)
Thymoma malignant, metastatic, thymus			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(1)	(0)	(0)	(0)
Carcinoma	1 (100%)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Urethra	(0)	(0)	(0)	(1)
Urinary bladder	(50)	(50)	(50)	(50)
Transitional epithelium, papilloma	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Leukemia mononuclear	11 (22%)	12 (24%)	11 (22%)	10 (20%)
Mesothelioma malignant	2 (4%)	5 (10%)	1 (2%)	4 (8%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	50	50	48
Total primary neoplasms	122	124	127	107
Total animals with benign neoplasms	47	49	50	45
Total benign neoplasms	96	95	103	89
Total animals with malignant neoplasms	22	24	22	15
Total malignant neoplasms	26	29	24	18
Total animals with metastatic neoplasms	1	2	1	2
Total metastatic neoplasms	1	4	2	4

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

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	4/50 (8%)	6/50 (12%)	7/50 (14%)	3/50 (6%)
Adjusted rate[b]	10.3%	14.6%	16.5%	6.9%
Terminal rate[c]	3/23 (13%)	5/28 (18%)	6/28 (21%)	1/28 (4%)
First incidence (days)	694	599	647	681
Poly-3 test[d]	P=0.210N	P=0.403	P=0.311	P=0.440N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	5/50 (10%)	6/50 (12%)	7/50 (14%)	4/50 (8%)
Adjusted rate	12.7%	14.6%	16.5%	9.2%
Terminal rate	3/23 (13%)	5/28 (18%)	6/28 (21%)	2/28 (7%)
First incidence (days)	532	599	647	681
Poly-3 test	P=0.286N	P=0.528	P=0.430	P=0.441N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	2/50 (4%)	0/50 (0%)
Adjusted rate	7.7%	4.9%	4.8%	0.0%
Terminal rate	2/23 (9%)	1/28 (4%)	2/28 (7%)	0/28 (0%)
First incidence (days)	702	619	727 (T)	—[e]
Poly-3 test	P=0.092N	P=0.475N	P=0.463N	P=0.101N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	10.3%	7.3%	4.8%	0.0%
Terminal rate	3/23 (13%)	2/28 (7%)	2/28 (7%)	0/28 (0%)
First incidence (days)	702	619	727 (T)	—
Poly-3 test	P=0.043N	P=0.470N	P=0.299N	P=0.047N
Pancreatic Islets: Adenoma				
Overall rate	3/50 (6%)	5/50 (10%)	4/50 (8%)	5/50 (10%)
Adjusted rate	7.7%	12.3%	9.4%	11.4%
Terminal rate	2/23 (9%)	5/28 (18%)	2/28 (7%)	3/28 (11%)
First incidence (days)	681	727 (T)	673	552
Poly-3 test	P=0.480	P=0.379	P=0.547	P=0.424
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	33/50 (66%)	33/49 (67%)	34/50 (68%)	27/50 (54%)
Adjusted rate	73.0%	71.3%	71.7%	58.1%
Terminal rate	14/23 (61%)	17/27 (63%)	18/28 (64%)	14/28 (50%)
First incidence (days)	527	449	466	496
Poly-3 test	P=0.051N	P=0.521N	P=0.540N	P=0.093N
Skin: Squamous Cell Papilloma				
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.6%	0.0%	0.0%	7.0%
Terminal rate	0/23 (0%)	0/28 (0%)	0/28 (0%)	3/28 (11%)
First incidence (days)	615	—	—	727 (T)
Poly-3 test	P=0.052	P=0.493N	P=0.485N	P=0.340
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	3/50 (6%)	1/50 (2%)	2/50 (4%)	5/50 (10%)
Adjusted rate	7.6%	2.5%	4.7%	11.6%
Terminal rate	1/23 (4%)	1/28 (4%)	1/28 (4%)	5/28 (18%)
First incidence (days)	608	727 (T)	583	727 (T)
Poly-3 test	P=0.126	P=0.295N	P=0.466N	P=0.404

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Basal Cell Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	2/50 (4%)	6/50 (12%)
Adjusted rate	7.6%	2.5%	4.7%	13.9%
Terminal rate	1/23 (4%)	1/28 (4%)	1/28 (4%)	5/28 (18%)
First incidence (days)	608	727 (T)	583	696
Poly-3 test	P=0.055	P=0.295N	P=0.466N	P=0.286
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Fibrous Histiocytoma				
Overall rate	1/50 (2%)	3/50 (6%)	4/50 (8%)	1/50 (2%)
Adjusted rate	2.6%	7.4%	9.5%	2.3%
Terminal rate	0/23 (0%)	2/28 (7%)	4/28 (14%)	0/28 (0%)
First incidence (days)	652	702	727 (T)	589
Poly-3 test	P=0.323N	P=0.320	P=0.202	P=0.736N
Skin (Subcutaneous Tissue): Malignant Schwannoma				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	2.4%	6.9%	0.0%
Terminal rate	0/23 (0%)	0/28 (0%)	0/28 (0%)	0/28 (0%)
First incidence (days)	—	609	580	—
Poly-3 test	P=0.406N	P=0.512	P=0.139	—[f]
Testes: Adenoma				
Overall rate	37/50 (74%)	31/50 (62%)	42/50 (84%)	37/50 (74%)
Adjusted rate	84.6%	71.2%	89.0%	80.6%
Terminal rate	22/23 (96%)	22/28 (79%)	26/28 (93%)	25/28 (89%)
First incidence (days)	507	469	491	589
Poly-3 test	P=0.537	P=0.079N	P=0.359	P=0.406N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	9/50 (18%)	9/50 (18%)	7/50 (14%)	6/50 (12%)
Adjusted rate	22.5%	21.4%	16.4%	13.8%
Terminal rate	4/23 (17%)	5/28 (18%)	6/28 (21%)	3/28 (11%)
First incidence (days)	603	559	589	654
Poly-3 test	P=0.186N	P=0.558N	P=0.338N	P=0.225N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	9/50 (18%)	10/50 (20%)	7/50 (14%)	7/50 (14%)
Adjusted rate	22.5%	23.5%	16.4%	16.1%
Terminal rate	4/23 (17%)	5/28 (18%)	6/28 (21%)	4/28 (14%)
First incidence (days)	603	559	589	654
Poly-3 test	P=0.249N	P=0.559	P=0.338N	P=0.320N
All Organs: Malignant Mesothelioma				
Overall rate	2/50 (4%)	5/50 (10%)	1/50 (2%)	4/50 (8%)
Adjusted rate	5.1%	12.3%	2.4%	9.3%
Terminal rate	1/23 (4%)	5/28 (18%)	1/28 (4%)	3/28 (11%)
First incidence (days)	626	727 (T)	727 (T)	696
Poly-3 test	P=0.475	P=0.230	P=0.474N	P=0.384
All Organs: Mononuclear Cell Leukemia				
Overall rate	11/50 (22%)	12/50 (24%)	11/50 (22%)	10/50 (20%)
Adjusted rate	26.8%	27.7%	24.7%	22.0%
Terminal rate	4/23 (17%)	5/28 (18%)	4/28 (14%)	1/28 (4%)
First incidence (days)	507	469	580	451
Poly-3 test	P=0.319N	P=0.562	P=0.509N	P=0.396N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
All Organs: Benign Neoplasms				
Overall rate	47/50 (94%)	49/50 (98%)	50/50 (100%)	45/50 (90%)
Adjusted rate	98.5%	99.0%	100.0%	93.9%
Terminal rate	23/23 (100%)	28/28 (100%)	28/28 (100%)	27/28 (96%)
First incidence (days)	507	449	466	496
Poly-3 test	P=0.035N	P=0.854	P=0.701	P=0.228N
All Organs: Malignant Neoplasms				
Overall rate	22/50 (44%)	24/50 (48%)	22/50 (44%)	16/50 (32%)
Adjusted rate	49.1%	53.1%	47.2%	34.9%
Terminal rate	7/23 (30%)	12/28 (43%)	9/28 (32%)	5/28 (18%)
First incidence (days)	440	469	491	451
Poly-3 test	P=0.048N	P=0.430	P=0.510N	P=0.120N
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	50/50 (100%)	50/50 (100%)	48/50 (96%)
Adjusted rate	100.0%	100.0%	100.0%	98.4%
Terminal rate	23/23 (100%)	28/28 (100%)	28/28 (100%)	28/28 (100%)
First incidence (days)	440	449	466	451
Poly-3 test	P=0.303N	P=1.000	P=1.000	P=0.633N

(T) Terminal kill

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, lung, pancreatic islets, 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 vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE A3a
Historical Incidence of Squamous Cell Papilloma of the Skin in Control Male F344/N Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Ethanol Vehicle Dermal Studies	
bis(2-Chloroethoxy)methane (September 2002)	0/50
1,2-Dibromo-2,4-dicyanobutane (July 2002)	0/50
Methyl *trans*-styryl ketone (April 2004)	1/50
Pyrogallol (September 2004)	1/50
Total (%)	2/200 (1.0%)
Mean ± standard deviation	1.0% ± 1.2%
Range	0%-2%
Overall Historical Incidence: All Routes	
Total (%)	8/1,249 (0.6%)
Mean ± standard deviation	0.6% ± 1.1%
Range	0%-4%

[a] Data as of May 18, 2011

TABLE A3b
Historical Incidence of Malignant Mesothelioma in Control Male F344/N Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Ethanol Vehicle Dermal Studies	
bis(2-Chloroethoxy)methane (September 2002)	2/50
1,2-Dibromo-2,4-dicyanobutane (July 2002)	0/50
Methyl *trans*-styryl ketone (April 2004)	4/50
Pyrogallol (September 2004)	2/50
Total (%)	8/200 (4.0%)
Mean ± standard deviation	4.0% ± 3.3%
Range	0%-8%
Overall Historical Incidence: All Routes	
Total (%)	40/1,249 (3.2%)
Mean ± standard deviation	3.2% ± 2.8%
Range	0%-8%

[a] Data as of May 18, 2011

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

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	17	14	15	17
Natural deaths	10	8	7	5
Survivors				
Died last week of study		2		
Terminal kill	23	26	28	28
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Periesophageal tissue, hemorrhage	1 (2%)			
Periesophageal tissue, inflammation	1 (2%)			
Intestine large, cecum	(49)	(50)	(50)	(50)
Inflammation		2 (4%)		
Necrosis		1 (2%)		
Thrombosis		1 (2%)	1 (2%)	
Intestine large, colon	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid		1 (2%)		
Parasite metazoan	2 (4%)		1 (2%)	6 (12%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan	4 (8%)	5 (10%)	3 (6%)	4 (8%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Inflammation			1 (2%)	
Ulcer			1 (2%)	
Intestine small, ileum	(50)	(50)	(50)	(50)
Inflammation			1 (2%)	
Epithelium, dysplasia	1 (2%)			
Intestine small, jejunum	(50)	(50)	(50)	(50)
Inflammation				1 (2%)
Peyer's patch, inflammation, granulomatous		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis	3 (6%)	2 (4%)		1 (2%)
Basophilic focus	8 (16%)	8 (16%)	11 (22%)	8 (16%)
Clear cell focus	18 (36%)	17 (34%)	21 (42%)	21 (42%)
Congestion, acute	1 (2%)			
Degeneration, cystic	1 (2%)	6 (12%)	1 (2%)	1 (2%)
Eosinophilic focus	3 (6%)	2 (4%)		2 (4%)
Fatty change	1 (2%)	3 (6%)	1 (2%)	3 (6%)
Hematopoietic cell proliferation				2 (4%)
Hemorrhage			1 (2%)	
Hepatodiaphragmatic nodule	3 (6%)	2 (4%)	5 (10%)	4 (8%)
Inflammation	19 (38%)	17 (34%)	17 (34%)	17 (34%)
Mixed cell focus	8 (16%)	6 (12%)	6 (12%)	5 (10%)
Bile duct, cyst			1 (2%)	
Bile duct, hyperplasia	28 (56%)	24 (48%)	27 (54%)	26 (52%)
Centrilobular, fibrosis	1 (2%)		1 (2%)	
Hepatocyte, atrophy		1 (2%)	1 (2%)	
Hepatocyte, degeneration	1 (2%)	1 (2%)		
Hepatocyte, necrosis	1 (2%)	5 (10%)	4 (8%)	5 (10%)
Hepatocyte, regeneration	1 (2%)		1 (2%)	
Hepatocyte, vacuolization cytoplasmic	19 (38%)	20 (40%)	24 (48%)	18 (36%)
Kupffer cell, pigmentation				1 (2%)
Portal, fibrosis	3 (6%)	6 (12%)	9 (18%)	6 (12%)
Vein, thrombosis		1 (2%)		

[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 Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Alimentary System (continued)				
Mesentery	(9)	(2)	(3)	(5)
Necrosis	7 (78%)	2 (100%)	3 (100%)	4 (80%)
Oral mucosa	(2)	(1)	(4)	(3)
Gingival, inflammation	2 (100%)	1 (100%)	2 (50%)	3 (100%)
Pharyngeal, hyperplasia			2 (50%)	
Pancreas	(50)	(50)	(50)	(50)
Acinus, atrophy	28 (56%)	31 (62%)	28 (56%)	31 (62%)
Acinus, hyperplasia	3 (6%)	8 (16%)	4 (8%)	1 (2%)
Duct, cyst	5 (10%)	4 (8%)	3 (6%)	4 (8%)
Salivary glands	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Inflammation			1 (2%)	1 (2%)
Duct, cyst			1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Inflammation	2 (4%)	1 (2%)	1 (2%)	5 (10%)
Perforation		1 (2%)		
Ulcer	4 (8%)	6 (12%)	7 (14%)	4 (8%)
Epithelium, hyperplasia	2 (4%)	2 (4%)	4 (8%)	6 (12%)
Stomach, glandular	(50)	(50)	(50)	(50)
Congestion		1 (2%)		
Erosion	2 (4%)	4 (8%)		3 (6%)
Inflammation		4 (8%)		3 (6%)
Ulcer			1 (2%)	
Epithelium, hyperplasia		2 (4%)	1 (2%)	
Glands, cyst		1 (2%)	1 (2%)	
Tongue	(1)	(0)	(0)	(2)
Epithelium, hyperplasia				1 (50%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	46 (92%)	45 (90%)	46 (92%)	47 (94%)
Atrium, thrombosis		2 (4%)		1 (2%)
Myocardium, inflammation				1 (2%)
Valve, thrombosis				1 (2%)
Ventricle, thrombosis				2 (4%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Degeneration, cystic		1 (2%)	1 (2%)	
Hematopoietic cell proliferation			1 (2%)	
Hemorrhage		1 (2%)	1 (2%)	
Hyperplasia	19 (38%)	21 (42%)	29 (58%)	20 (40%)
Hypertrophy	7 (14%)	10 (20%)	10 (20%)	4 (8%)
Mineralization				1 (2%)
Necrosis			1 (2%)	1 (2%)
Vacuolization cytoplasmic	27 (54%)	18 (36%)	23 (46%)	19 (38%)
Capsule, fibrosis, focal				1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Endocrine System (continued)				
Adrenal medulla	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Hemorrhage		1 (2%)		1 (2%)
Hyperplasia	16 (32%)	18 (36%)	20 (40%)	18 (36%)
Infiltration cellular, mononuclear cell				1 (2%)
Mineralization				1 (2%)
Necrosis			1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia				1 (2%)
Parathyroid gland	(50)	(49)	(47)	(49)
Cyst		1 (2%)		
Hyperplasia		3 (6%)		
Pituitary gland	(50)	(49)	(50)	(50)
Hemorrhage		1 (2%)		2 (4%)
Pigmentation, hemosiderin				1 (2%)
Pars distalis, atrophy	1 (2%)			3 (6%)
Pars distalis, cyst		2 (4%)		4 (8%)
Pars distalis, hyperplasia	11 (22%)	10 (20%)	12 (24%)	13 (26%)
Pars intermedia, hyperplasia		1 (2%)	1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Cyst		1 (2%)		
Inflammation				1 (2%)
C-cell, hyperplasia	18 (36%)	19 (38%)	21 (42%)	18 (36%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Inflammation		1 (2%)	2 (4%)	
Penis	(0)	(0)	(1)	(0)
Congestion			1 (100%)	
Preputial gland	(50)	(50)	(50)	(50)
Inflammation	2 (4%)	8 (16%)	5 (10%)	7 (14%)
Duct, hyperplasia, squamous				1 (2%)
Prostate	(50)	(50)	(50)	(50)
Inflammation	44 (88%)	45 (90%)	48 (96%)	44 (88%)
Epithelium, hyperplasia	4 (8%)	8 (16%)	5 (10%)	5 (10%)
Epithelium, metaplasia, squamous			1 (2%)	1 (2%)
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation		1 (2%)		
Bilateral, atrophy			1 (2%)	
Testes	(50)	(50)	(50)	(50)
Cyst			2 (4%)	1 (2%)
Mineralization			2 (4%)	
Arteriole, inflammation		1 (2%)		
Bilateral, germinal epithelium, atrophy		3 (6%)		1 (2%)
Germinal epithelium, atrophy	5 (10%)	4 (8%)	7 (14%)	9 (18%)
Interstitial cell, hyperplasia	11 (22%)	13 (26%)	5 (10%)	8 (16%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Hyperplasia	10 (20%)	11 (22%)	11 (22%)	9 (18%)
Infiltration cellular, histiocyte				1 (2%)
Myelofibrosis		1 (2%)	1 (2%)	
Lymph node	(10)	(8)	(4)	(7)
Degeneration, cystic			1 (25%)	
Deep cervical, degeneration, cystic		1 (13%)		
Deep cervical, hemorrhage				2 (29%)
Mediastinal, degeneration, cystic	2 (20%)	1 (13%)		
Mediastinal, hemorrhage		2 (25%)		
Mediastinal, hyperplasia		1 (13%)		
Mediastinal, hyperplasia, lymphoid		1 (13%)		
Mediastinal, hyperplasia, plasma cell	2 (20%)	1 (13%)	1 (25%)	
Pancreatic, degeneration, cystic	1 (10%)			
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Degeneration, cystic		4 (8%)	1 (2%)	5 (10%)
Hemorrhage				2 (4%)
Hyperplasia, histiocytic			2 (4%)	
Hyperplasia, plasma cell			1 (2%)	
Infiltration cellular, histiocyte				1 (2%)
Necrosis, lymphoid		2 (4%)		
Spleen	(50)	(50)	(50)	(50)
Congestion			1 (2%)	
Hematopoietic cell proliferation				1 (2%)
Hyperplasia, lymphoid	3 (6%)	3 (6%)	1 (2%)	2 (4%)
Infarct	2 (4%)	2 (4%)	3 (6%)	1 (2%)
Necrosis, lymphoid		1 (2%)		
Necrosis, focal		1 (2%)		
Thrombosis				1 (2%)
Capsule, fibrosis		1 (2%)		1 (2%)
Capsule, inflammation				1 (2%)
Lymphoid follicle, atrophy		1 (2%)	2 (4%)	
Lymphoid follicle, depletion cellular		1 (2%)		
Thymus	(47)	(48)	(48)	(47)
Cyst			1 (2%)	
Ectopic parathyroid gland				1 (2%)
Fibrosis			1 (2%)	
Epithelial cell, hyperplasia				1 (2%)
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Inflammation		1 (2%)		
Duct, cyst	1 (2%)			
Duct, dilatation	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Abscess		1 (2%)		
Fibrosis				1 (2%)
Hyperkeratosis				1 (2%)
Hyperplasia				2 (4%)
Inflammation				1 (2%)
Ulcer			1 (2%)	1 (2%)
Control, hyperkeratosis				1 (2%)
Control, hyperplasia				1 (2%)
Control, inflammation				1 (2%)
Control, sebaceous gland, hyperplasia				1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Integumentary System (continued)				
Skin (continued)	(50)	(50)	(50)	(50)
Sebaceous gland, site of application, hyperplasia			12 (24%)	48 (96%)
Site of application, hyperkeratosis		2 (4%)	21 (42%)	48 (96%)
Site of application, hyperplasia		6 (12%)	20 (40%)	50 (100%)
Site of application, inflammation				46 (92%)
Site of application, ulcer				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrosis				1 (2%)
Hemorrhage		1 (2%)		
Osteopetrosis				1 (2%)
Epiphysis, femur, cyst		1 (2%)		
Mandible, cyst			1 (2%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage	3 (6%)	4 (8%)		2 (4%)
Hydrocephalus	2 (4%)	3 (6%)	4 (8%)	
Cerebrum, inflammation				1 (2%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	1 (2%)		1 (2%)	
Hemorrhage	1 (2%)	3 (6%)		
Hyperplasia			1 (2%)	
Inflammation	6 (12%)	12 (24%)	13 (26%)	8 (16%)
Metaplasia, squamous			1 (2%)	
Pigmentation, hemosiderin		1 (2%)		
Thrombosis	2 (4%)	1 (2%)		
Alveolar epithelium, hyperplasia	9 (18%)	7 (14%)	10 (20%)	6 (12%)
Alveolus, infiltration cellular, histiocyte	6 (12%)	6 (12%)	5 (10%)	4 (8%)
Bronchiole, fibrosis			1 (2%)	
Serosa, hyperplasia	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Inflammation	21 (42%)	28 (56%)	21 (42%)	18 (36%)
Metaplasia, squamous	1 (2%)			
Polyp, inflammatory		1 (2%)		
Thrombosis		2 (4%)		
Nasolacrimal duct, inflammation				1 (2%)
Trachea	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell		1 (2%)		
Inflammation	1 (2%)		2 (4%)	
Metaplasia, squamous			1 (2%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract		2 (4%)		1 (2%)
Anterior chamber, inflammation	1 (2%)			
Cornea, inflammation	1 (2%)			
Retina, atrophy	1 (2%)	3 (6%)		1 (2%)
Retina, developmental malformation			1 (2%)	
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)	2 (4%)	
Inflammation	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Zymbal's gland	(1)	(0)	(0)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst, multiple				1 (2%)
Hyperplasia, tubular			2 (4%)	3 (6%)
Inflammation	1 (2%)	1 (2%)		2 (4%)
Nephropathy	45 (90%)	47 (94%)	49 (98%)	48 (96%)
Thrombosis			1 (2%)	
Pelvis, calculus microscopic observation only				1 (2%)
Transitional epithelium, hyperplasia				1 (2%)
Urethra	(0)	(0)	(0)	(1)
Bulbourethral gland, hyperplasia				1 (100%)
Urinary bladder	(50)	(50)	(50)	(50)
Hemorrhage				1 (2%)
Inflammation		1 (2%)		
Transitional epithelium, hyperplasia			1 (2%)	1 (2%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR DERMAL STUDY OF PYROGALLOL

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study of Pyrogallol[a]

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death		1		
Moribund	12	7	18	9
Natural deaths	9	9	6	10
Survivors				
Died last week of study			1	
Terminal kill	29	33	25	31
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	1 (2%)	
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	2 (4%)			
Hepatocellular adenoma, multiple		1 (2%)		
Hepatocellular carcinoma		1 (2%)		
Hepatocellular carcinoma, multiple				1 (2%)
Sarcoma stromal, metastatic, uterus			1 (2%)	
Mesentery	(8)	(11)	(13)	(10)
Schwannoma malignant	2 (25%)			
Oral mucosa	(2)	(2)	(0)	(0)
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Sarcoma stromal, metastatic, uterus			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)		1 (2%)	
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	2 (4%)			1 (2%)
Parathyroid gland	(49)	(49)	(50)	(49)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	25 (50%)	29 (58%)	28 (56%)	27 (54%)
Pars distalis, carcinoma			1 (2%)	
Pars intermedia, adenoma			1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	7 (14%)	6 (12%)	6 (12%)	7 (14%)
Follicle, adenoma		1 (2%)		
Follicular cell, adenoma	1 (2%)			1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma	3 (6%)		3 (6%)	
Carcinoma	1 (2%)	3 (6%)		1 (2%)
Ovary	(50)	(50)	(50)	(50)
Granulosa cell tumor benign	1 (2%)			
Uterus	(50)	(50)	(50)	(50)
Leiomyoma			1 (2%)	
Leiomyosarcoma		1 (2%)		
Polyp stromal	7 (14%)	6 (12%)	5 (10%)	3 (6%)
Cervix, polyp stromal		1 (2%)		
Cervix, sarcoma	1 (2%)			
Cervix, sarcoma stromal			1 (2%)	
Vagina	(0)	(1)	(0)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(3)	(2)	(4)	(2)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Thymus	(50)	(50)	(49)	(50)
Thymoma benign	1 (2%)			
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma	2 (4%)		1 (2%)	
Carcinoma	1 (2%)	1 (2%)		2 (4%)
Fibroadenoma	22 (44%)	12 (24%)	18 (36%)	11 (22%)
Fibroadenoma, multiple	2 (4%)	3 (6%)	3 (6%)	2 (4%)
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma	1 (2%)			
Keratoacanthoma				1 (2%)
Site of application, subcutaneous tissue, Schwannoma benign				1 (2%)
Subcutaneous tissue, fibroma	1 (2%)			
Subcutaneous tissue, fibrosarcoma			1 (2%)	
Subcutaneous tissue, lipoma			1 (2%)	
Subcutaneous tissue, schwannoma malignant	1 (2%)			1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, osteoma	1 (2%)			
Skeletal muscle	(0)	(0)	(2)	(0)
Sarcoma			1 (50%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Respiratory System				
Larynx	(0)	(1)	(0)	(0)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		1 (2%)		
Alveolar/bronchiolar carcinoma		1 (2%)		1 (2%)
Sarcoma stromal, metastatic, uterus			1 (2%)	
Squamous cell carcinoma				1 (2%)
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Lipoma	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(49)
Sarcoma stromal, metastatic, uterus			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		1 (2%)
Leukemia mononuclear	8 (16%)	9 (18%)	17 (34%)	11 (22%)
Lymphoma malignant				1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	45	42	47	44
Total primary neoplasms	96	79	92	77
Total animals with benign neoplasms	43	40	42	34
Total benign neoplasms	81	62	71	57
Total animals with malignant neoplasms	12	17	20	18
Total malignant neoplasms	15	17	21	20
Total animals with metastatic neoplasms		1	1	
Total metastatic neoplasms		7	4	

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

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Clitoral Gland: Adenoma				
Overall rate[a]	3/50 (6%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate[b]	7.2%	0.0%	7.2%	0.0%
Terminal rate[c]	3/29 (10%)	0/33 (0%)	1/26 (4%)	0/31 (0%)
First incidence (days)	728 (T)	—[e]	590	—
Poly-3 test[d]	P=0.196N	P=0.109N	P=0.659N	P=0.113N
Clitoral Gland: Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	2.4%	6.7%	0.0%	2.3%
Terminal rate	1/29 (3%)	1/33 (3%)	0/26 (0%)	1/31 (3%)
First incidence (days)	728 (T)	572	—	728 (T)
Poly-3 test	P=0.442N	P=0.331	P=0.502N	P=0.754N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rate	9.6%	6.7%	7.2%	2.3%
Terminal rate	4/29 (14%)	1/33 (3%)	1/26 (4%)	1/31 (3%)
First incidence (days)	728 (T)	572	590	728 (T)
Poly-3 test	P=0.157N	P=0.462N	P=0.496N	P=0.169N
Mammary Gland: Fibroadenoma				
Overall rate	24/50 (48%)	15/50 (30%)	21/50 (42%)	13/50 (26%)
Adjusted rate	56.0%	33.7%	48.8%	29.2%
Terminal rate	19/29 (66%)	11/33 (33%)	14/26 (54%)	6/31 (19%)
First incidence (days)	633	661	590	590
Poly-3 test	P=0.034N	P=0.025N	P=0.321N	P=0.008N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	25/50 (50%)	15/50 (30%)	21/50 (42%)	13/50 (26%)
Adjusted rate	57.8%	33.7%	48.8%	29.2%
Terminal rate	19/29 (66%)	11/33 (33%)	14/26 (54%)	6/31 (19%)
First incidence (days)	608	661	590	590
Poly-3 test	P=0.026N	P=0.016N	P=0.261N	P=0.004N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	2/50 (4%)
Adjusted rate	7.1%	2.3%	2.4%	4.6%
Terminal rate	0/29 (0%)	1/33 (3%)	0/26 (0%)	1/31 (3%)
First incidence (days)	608	728 (T)	632	574
Poly-3 test	P=0.638N	P=0.292N	P=0.313N	P=0.490N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	26/50 (52%)	16/50 (32%)	21/50 (42%)	15/50 (30%)
Adjusted rate	59.8%	35.9%	48.8%	33.3%
Terminal rate	19/29 (66%)	12/33 (36%)	14/26 (54%)	7/31 (23%)
First incidence (days)	608	661	590	574
Poly-3 test	P=0.046N	P=0.017N	P=0.202N	P=0.009N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	25/50 (50%)	29/50 (58%)	28/50 (56%)	27/50 (54%)
Adjusted rate	54.1%	60.4%	62.0%	61.1%
Terminal rate	11/29 (38%)	17/33 (52%)	18/26 (69%)	20/31 (65%)
First incidence (days)	530	439	414	594
Poly-3 test	P=0.384	P=0.340	P=0.285	P=0.318

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	25/50 (50%)	29/50 (58%)	29/50 (58%)	27/50 (54%)
Adjusted rate	54.1%	60.4%	64.2%	61.1%
Terminal rate	11/29 (38%)	17/33 (52%)	18/26 (69%)	20/31 (65%)
First incidence (days)	530	439	414	594
Poly-3 test	P=0.390	P=0.340	P=0.216	P=0.318
Thyroid Gland (C-Cell): Adenoma				
Overall rate	7/50 (14%)	6/50 (12%)	6/50 (12%)	7/50 (14%)
Adjusted rate	16.7%	13.6%	14.4%	16.3%
Terminal rate	6/29 (21%)	6/33 (18%)	3/26 (12%)	6/31 (19%)
First incidence (days)	674	728 (T)	617	712
Poly-3 test	P=0.510	P=0.461N	P=0.502N	P=0.595N
Uterus: Stromal Polyp				
Overall rate	7/50 (14%)	7/50 (14%)	5/50 (10%)	3/50 (6%)
Adjusted rate	16.8%	15.9%	11.8%	7.0%
Terminal rate	7/29 (24%)	7/33 (21%)	2/26 (8%)	2/31 (7%)
First incidence (days)	728 (T)	728 (T)	574	721
Poly-3 test	P=0.104N	P=0.571N	P=0.365N	P=0.143N
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	7/50 (14%)	7/50 (14%)	6/50 (12%)	3/50 (6%)
Adjusted rate	16.8%	15.9%	14.0%	7.0%
Terminal rate	7/29 (24%)	7/33 (21%)	2/26 (8%)	2/31 (7%)
First incidence (days)	728 (T)	728 (T)	574	721
Poly-3 test	P=0.102N	P=0.571N	P=0.477N	P=0.143N
All Organs: Mononuclear Cell Leukemia				
Overall rate	8/50 (16%)	9/50 (18%)	17/50 (34%)	11/50 (22%)
Adjusted rate	19.0%	20.3%	38.2%	24.5%
Terminal rate	6/29 (21%)	7/33 (21%)	7/26 (27%)	6/31 (19%)
First incidence (days)	646	661	495	449
Poly-3 test	P=0.430	P=0.548	P=0.038	P=0.362
All Organs: Benign Neoplasms				
Overall rate	43/50 (86%)	40/50 (80%)	42/50 (84%)	34/50 (68%)
Adjusted rate	89.6%	83.1%	88.4%	75.0%
Terminal rate	26/29 (90%)	27/33 (82%)	24/26 (92%)	24/31 (77%)
First incidence (days)	230	439	414	590
Poly-3 test	P=0.042N	P=0.256N	P=0.555N	P=0.046N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
All Organs: Malignant Neoplasms				
Overall rate	12/50 (24%)	17/50 (34%)	20/50 (40%)	18/50 (36%)
Adjusted rate	28.0%	37.8%	44.1%	39.4%
Terminal rate	6/29 (21%)	13/33 (39%)	7/26 (27%)	11/31 (36%)
First incidence (days)	646	572	495	449
Poly-3 test	P=0.310	P=0.224	P=0.085	P=0.180
All Organs: Benign or Malignant Neoplasms				
Overall rate	45/50 (90%)	42/50 (84%)	47/50 (94%)	44/50 (88%)
Adjusted rate	93.1%	86.4%	95.6%	91.7%
Terminal rate	26/29 (90%)	28/33 (85%)	24/26 (92%)	28/31 (90%)
First incidence (days)	230	439	414	449
Poly-3 test	P=0.504	P=0.221N	P=0.461	P=0.555N

(T) Terminal kill

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for clitoral gland, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.

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

[c] Observed incidence at terminal kill

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

[e] Not applicable; no neoplasms in animal group

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of Pyrogallol[a]

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death		1		
Moribund	12	7	18	9
Natural deaths	9	9	6	10
Survivors				
Died last week of study			1	
Terminal kill	29	33	25	31
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(50)	(50)	(50)	(50)
Erosion	1 (2%)			
Inflammation	1 (2%)		1 (2%)	2 (4%)
Thrombosis			1 (2%)	
Ulcer	1 (2%)	1 (2%)	1 (2%)	
Intestine large, colon	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Parasite metazoan	2 (4%)	5 (10%)	2 (4%)	1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan	3 (6%)	8 (16%)	5 (10%)	5 (10%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Parasite metazoan	1 (2%)			
Intestine small, ileum	(50)	(50)	(50)	(50)
Inflammation		1 (2%)	1 (2%)	
Parasite metazoan			1 (2%)	
Intestine small, jejunum	(50)	(50)	(50)	(50)
Peyer's patch, hyperplasia	1 (2%)			
Serosa, fibrosis	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	2 (4%)		3 (6%)
Atypia cellular, focal			1 (2%)	
Basophilic focus	31 (62%)	34 (68%)	28 (56%)	34 (68%)
Clear cell focus	9 (18%)	1 (2%)	2 (4%)	4 (8%)
Congestion, diffuse				1 (2%)
Degeneration, cystic		1 (2%)	1 (2%)	
Eosinophilic focus	2 (4%)	4 (8%)	2 (4%)	1 (2%)
Fatty change	4 (8%)	1 (2%)	6 (12%)	4 (8%)
Hematopoietic cell proliferation		1 (2%)		
Hemorrhage			1 (2%)	1 (2%)
Hepatodiaphragmatic nodule	1 (2%)	6 (12%)	3 (6%)	7 (14%)
Inflammation	23 (46%)	26 (52%)	10 (20%)	16 (32%)
Mixed cell focus	5 (10%)	5 (10%)	3 (6%)	4 (8%)
Bile duct, hyperplasia	10 (20%)	7 (14%)	7 (14%)	7 (14%)
Hepatocyte, hypertrophy		1 (2%)		
Hepatocyte, necrosis	1 (2%)	1 (2%)	3 (6%)	
Hepatocyte, regeneration			1 (2%)	1 (2%)
Hepatocyte, vacuolization cytoplasmic	12 (24%)	15 (30%)	8 (16%)	6 (12%)
Hepatocyte, midzonal, degeneration	1 (2%)			
Portal, fibrosis	4 (8%)	1 (2%)	3 (6%)	1 (2%)
Serosa, fibrosis				1 (2%)
Mesentery	(8)	(11)	(13)	(10)
Necrosis	6 (75%)	11 (100%)	13 (100%)	10 (100%)

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

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Alimentary System (continued)				
Oral mucosa	(2)	(2)	(0)	(0)
Gingival, cyst	1 (50%)	1 (50%)		
Gingival, inflammation	1 (50%)	1 (50%)		
Pancreas	(50)	(50)	(50)	(50)
Basophilic focus		1 (2%)		
Acinus, atrophy	16 (32%)	13 (26%)	17 (34%)	15 (30%)
Acinus, hyperplasia	3 (6%)	8 (16%)	1 (2%)	5 (10%)
Duct, cyst	4 (8%)	2 (4%)	2 (4%)	2 (4%)
Salivary glands	(50)	(50)	(50)	(50)
Hyperplasia			1 (2%)	
Inflammation			1 (2%)	
Mineralization			1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation	1 (2%)		2 (4%)	3 (6%)
Ulcer	5 (10%)	3 (6%)	7 (14%)	3 (6%)
Epithelium, hyperplasia	3 (6%)		1 (2%)	3 (6%)
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion	1 (2%)	1 (2%)	1 (2%)	
Inflammation	2 (4%)		1 (2%)	2 (4%)
Ulcer			1 (2%)	
Epithelium, cyst				1 (2%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Thrombosis			1 (2%)	
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	44 (88%)	45 (90%)	46 (92%)	45 (90%)
Inflammation	1 (2%)			
Atrium, thrombosis	1 (2%)	1 (2%)	1 (2%)	
Myocardium, inflammation		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Atrophy			1 (2%)	1 (2%)
Atypia cellular			1 (2%)	
Degeneration, cystic	8 (16%)	4 (8%)	4 (8%)	2 (4%)
Hematopoietic cell proliferation	1 (2%)			1 (2%)
Hemorrhage				2 (4%)
Hyperplasia	29 (58%)	24 (48%)	26 (52%)	29 (58%)
Hypertrophy	17 (34%)	15 (30%)	24 (48%)	21 (42%)
Necrosis			1 (2%)	
Thrombosis			1 (2%)	
Vacuolization cytoplasmic	14 (28%)	13 (26%)	13 (26%)	18 (36%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	5 (10%)	1 (2%)	6 (12%)	4 (8%)
Infiltration cellular, mononuclear cell				1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Parathyroid gland	(49)	(49)	(50)	(49)
Hyperplasia			2 (4%)	

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Endocrine System (continued)				
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Cyst			1 (2%)	1 (2%)
Hemorrhage	1 (2%)			2 (4%)
Pars distalis, cyst	4 (8%)	8 (16%)	7 (14%)	7 (14%)
Pars distalis, hyperplasia	19 (38%)	16 (32%)	17 (34%)	19 (38%)
Pars distalis, inflammation		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Cyst				1 (2%)
C-cell, hyperplasia	22 (44%)	31 (62%)	23 (46%)	31 (62%)
Follicle, cyst		1 (2%)		
Follicular cell, hyperplasia				1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Fibrosis			1 (2%)	
Hyperplasia	3 (6%)	1 (2%)	4 (8%)	1 (2%)
Inflammation	3 (6%)	4 (8%)	6 (12%)	6 (12%)
Duct, dilatation	1 (2%)		2 (4%)	
Ovary	(50)	(50)	(50)	(50)
Cyst	3 (6%)	3 (6%)	6 (12%)	1 (2%)
Uterus	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Hyperplasia, adenomatous				1 (2%)
Inflammation	3 (6%)			1 (2%)
Cervix, fibrosis	1 (2%)			
Endometrium, cyst			1 (2%)	
Endometrium, edema			1 (2%)	
Endometrium, hyperplasia, cystic	5 (10%)	5 (10%)	4 (8%)	
Vagina	(0)	(1)	(0)	(0)
Inflammation		1 (100%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Hyperplasia	10 (20%)	6 (12%)	10 (20%)	10 (20%)
Myelofibrosis			2 (4%)	1 (2%)
Lymph node	(3)	(2)	(4)	(2)
Hemorrhage			1 (25%)	
Deep cervical, hyperplasia, plasma cell	1 (33%)			
Mediastinal, congestion		1 (50%)		
Mediastinal, hemorrhage	2 (67%)		1 (25%)	
Mediastinal, hyperplasia, plasma cell	1 (33%)	1 (50%)	1 (25%)	
Pancreatic, hemorrhage			1 (25%)	
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Degeneration, cystic	3 (6%)			1 (2%)
Hyperplasia, lymphoid				1 (2%)
Hyperplasia, plasma cell			1 (2%)	1 (2%)
Infiltration cellular, histiocyte		1 (2%)		
Inflammation		2 (4%)		

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Hematopoietic System (continued)				
Spleen	(50)	(50)	(50)	(50)
Accessory spleen			1 (2%)	1 (2%)
Hematopoietic cell proliferation		3 (6%)	1 (2%)	
Hyperplasia, lymphoid		1 (2%)	1 (2%)	2 (4%)
Infarct		2 (4%)	4 (8%)	1 (2%)
Pigmentation, hemosiderin		1 (2%)		1 (2%)
Capsule, hyperplasia			1 (2%)	
Capsule, inflammation			1 (2%)	
Lymphoid follicle, atrophy		1 (2%)	1 (2%)	1 (2%)
Thymus	(50)	(50)	(49)	(50)
Cyst				1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	4 (8%)		1 (2%)
Duct, cyst	1 (2%)	1 (2%)	1 (2%)	5 (10%)
Duct, hyperplasia, cystic	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Hyperkeratosis		2 (4%)		
Hyperplasia				1 (2%)
Ulcer				1 (2%)
Control, hyperplasia			1 (2%)	
Control, inflammation			1 (2%)	
Sebaceous gland, site of application, hyperplasia			5 (10%)	41 (82%)
Site of application, hyperkeratosis		6 (12%)	23 (46%)	49 (98%)
Site of application, hyperplasia		9 (18%)	11 (22%)	49 (98%)
Site of application, inflammation		3 (6%)	6 (12%)	49 (98%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteopetrosis			1 (2%)	
Osteosclerosis	1 (2%)			
Fibula, tibia, fracture	1 (2%)			
Skeletal muscle	(0)	(0)	(2)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)	1 (2%)		1 (2%)
Hydrocephalus	1 (2%)	3 (6%)	3 (6%)	3 (6%)
Thrombosis			1 (2%)	
Cerebellum, gliosis	1 (2%)			
Cerebrum, necrosis	1 (2%)			
Respiratory System				
Larynx	(0)	(1)	(0)	(0)
Foreign body		1 (100%)		
Inflammation		1 (100%)		
Lung	(50)	(50)	(50)	(50)
Congestion		1 (2%)	1 (2%)	1 (2%)
Fibrosis	2 (4%)			
Hemorrhage		2 (4%)		
Inflammation	8 (16%)	9 (18%)	2 (4%)	10 (20%)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Respiratory System (continued)				
Lung (continued)	(50)	(50)	(50)	(50)
Thrombosis	1 (2%)			
Alveolar epithelium, hyperplasia	6 (12%)	11 (22%)	5 (10%)	12 (24%)
Alveolus, infiltration cellular, histiocyte	2 (4%)	3 (6%)	2 (4%)	6 (12%)
Alveolus, pigmentation, hemoglobin				1 (2%)
Bronchiole, hyperplasia				1 (2%)
Serosa, fibrosis		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Inflammation	11 (22%)	10 (20%)	12 (24%)	8 (16%)
Metaplasia, squamous	1 (2%)	1 (2%)		
Thrombosis	1 (2%)	1 (2%)	1 (2%)	
Goblet cell, hyperplasia	2 (4%)	2 (4%)		
Nasolacrimal duct, inflammation		1 (2%)		
Nasolacrimal duct, squamous epithelium, hyperplasia	1 (2%)			
Nasopharyngeal duct, inflammation			2 (4%)	
Olfactory epithelium, hyperplasia			1 (2%)	
Respiratory epithelium, necrosis		1 (2%)		
Squamous epithelium, necrosis		1 (2%)		
Vomeronasal organ, necrosis		1 (2%)		
Trachea	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	1 (2%)			
Inflammation	1 (2%)		1 (2%)	
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	3 (6%)	4 (8%)	1 (2%)	1 (2%)
Synechia	1 (2%)	1 (2%)		
Anterior chamber, exudate				1 (2%)
Bilateral, retina, atrophy		1 (2%)		
Optic nerve, atrophy	1 (2%)		1 (2%)	
Posterior chamber, exudate				1 (2%)
Retina, atrophy	3 (6%)	3 (6%)	2 (4%)	
Retina, developmental malformation	1 (2%)			
Retina, dysplasia		1 (2%)		1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	2 (4%)	4 (8%)	3 (6%)
Inflammation	2 (4%)	4 (8%)		4 (8%)
Metaplasia, squamous	1 (2%)			
Pigmentation, porphyrin		3 (6%)	6 (12%)	1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet		2 (4%)		
Atrophy			1 (2%)	
Cyst		1 (2%)	1 (2%)	
Hypertrophy			1 (2%)	
Infiltration cellular, lipocyte		1 (2%)		
Inflammation	1 (2%)		3 (6%)	
Nephropathy	46 (92%)	42 (84%)	42 (84%)	44 (88%)
Papilla, necrosis			1 (2%)	
Transitional epithelium, hyperplasia			1 (2%)	
Urinary bladder	(50)	(50)	(50)	(49)
Transitional epithelium, hyperplasia		1 (2%)	1 (2%)	

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR DERMAL STUDY OF PYROGALLOL

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of Pyrogallol[a]

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death				1
Moribund	8	10	10	12
Natural deaths	5	4	6	6
Survivors				
Terminal kill	37	36	34	31
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(49)	(49)	(50)	(46)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)			1 (2%)
Liver	(50)	(50)	(50)	(50)
Hemangioma			1 (2%)	
Hemangiosarcoma	3 (6%)	5 (10%)	2 (4%)	2 (4%)
Hemangiosarcoma, metastatic, spleen				1 (2%)
Hepatoblastoma	4 (8%)	1 (2%)	3 (6%)	3 (6%)
Hepatoblastoma, multiple			1 (2%)	
Hepatocellular adenoma	10 (20%)	17 (34%)	16 (32%)	12 (24%)
Hepatocellular adenoma, multiple	29 (58%)	19 (38%)	22 (44%)	22 (44%)
Hepatocellular carcinoma	13 (26%)	15 (30%)	17 (34%)	15 (30%)
Hepatocellular carcinoma, multiple	7 (14%)	3 (6%)	3 (6%)	6 (12%)
Mast cell tumor malignant, metastatic, bone marrow		1 (2%)		
Mesentery	(8)	(6)	(8)	(10)
Hepatoblastoma, metastatic, liver				1 (10%)
Mast cell tumor malignant, metastatic, bone marrow		1 (17%)		
Sarcoma	1 (13%)			
Sarcoma, metastatic, skin		1 (17%)		
Oral mucosa	(0)	(0)	(0)	(1)
Pharyngeal, squamous cell carcinoma				1 (100%)
Pancreas	(50)	(50)	(50)	(50)
Mast cell tumor malignant, metastatic, bone marrow		1 (2%)		
Plasma cell tumor malignant, metastatic, lymph node, mesenteric		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Mast cell tumor malignant, metastatic, skin	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma		1 (2%)		
Squamous cell papilloma, multiple	1 (2%)			
Stomach, glandular	(50)	(50)	(50)	(50)
Mast cell tumor malignant, metastatic, bone marrow		1 (2%)		
Tooth	(36)	(30)	(24)	(22)
Odontoma	1 (3%)	3 (10%)	3 (13%)	1 (5%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Sarcoma, metastatic, skin		1 (2%)		
Subcapsular, adenoma	8 (16%)	7 (14%)	6 (12%)	2 (4%)
Subcapsular, adenoma, multiple	1 (2%)	1 (2%)		1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign				1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Carcinoma	1 (2%)	1 (2%)		
Parathyroid gland	(41)	(44)	(42)	(47)
Pituitary gland	(50)	(50)	(50)	(50)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, carcinoma		1 (2%)		
General Body System				
Peritoneum	(0)	(1)	(1)	(1)
Genital System				
Coagulating gland	(0)	(2)	(1)	(0)
Carcinoma			1 (100%)	
Sarcoma, metastatic, skin		1 (50%)		
Epididymis	(50)	(50)	(50)	(50)
Sarcoma, metastatic, skin		1 (2%)		
Penis	(1)	(0)	(0)	(0)
Preputial gland	(50)	(50)	(50)	(50)
Hemangioma	1 (2%)			
Hemangiosarcoma	1 (2%)			
Squamous cell carcinoma		1 (2%)		
Prostate	(50)	(50)	(50)	(50)
Sarcoma, metastatic, skin		1 (2%)		
Seminal vesicle	(50)	(50)	(50)	(50)
Sarcoma, metastatic, skin		1 (2%)		
Testes	(50)	(50)	(50)	(50)
Hemangioma		1 (2%)		
Hemangiosarcoma		1 (2%)		
Interstitial cell, adenoma			1 (2%)	2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma		2 (4%)		
Hemangiosarcoma, metastatic, spleen		1 (2%)		1 (2%)
Mast cell tumor malignant		1 (2%)		
Lymph node	(10)	(12)	(9)	(16)
Axillary, hemangiosarcoma		1 (8%)		
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung			1 (11%)	
Lumbar, sarcoma, metastatic, skin		1 (8%)		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Hematopoietic System (continued)				
Lymph node, mandibular	(50)	(50)	(50)	(50)
Mast cell tumor malignant, metastatic, skin	1 (2%)			
Lymph node, mesenteric	(50)	(49)	(50)	(49)
Plasma cell tumor malignant		1 (2%)		
Sarcoma, metastatic, skin		1 (2%)		
Spleen	(50)	(49)	(50)	(50)
Hemangiosarcoma	1 (2%)	2 (4%)		1 (2%)
Mast cell tumor malignant, metastatic, bone marrow		1 (2%)		
Thymus	(48)	(50)	(46)	(48)
Mast cell tumor malignant, metastatic, bone marrow		1 (2%)		
Mast cell tumor malignant, metastatic, skin	1 (2%)			
Integumentary System				
Mammary gland	(0)	(0)	(0)	(1)
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma		1 (2%)		
Fibrous histiocytoma		3 (6%)	1 (2%)	
Trichoepithelioma		1 (2%)		
Control, mast cell tumor malignant	1 (2%)			
Site of application, hamartoma		1 (2%)		
Site of application, squamous cell papilloma				2 (4%)
Site of application, subcutaneous tissue, hemangiosarcoma	1 (2%)			
Site of application, subcutaneous tissue, lipoma				1 (2%)
Subcutaneous tissue, fibrous histiocytoma	1 (2%)			
Subcutaneous tissue, sarcoma		1 (2%)		
Subcutaneous tissue, schwannoma malignant			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Mast cell tumor malignant, metastatic, bone marrow		1 (2%)		
Skeletal muscle	(0)	(0)	(1)	(2)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	10 (20%)	6 (12%)	8 (16%)	6 (12%)
Alveolar/bronchiolar adenoma, multiple	2 (4%)	1 (2%)		
Alveolar/bronchiolar carcinoma	4 (8%)	7 (14%)	5 (10%)	8 (16%)
Alveolar/bronchiolar carcinoma, multiple		1 (2%)		
Hepatocellular carcinoma, metastatic, liver	8 (16%)	7 (14%)	10 (20%)	5 (10%)
Sarcoma, metastatic, skin		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Mast cell tumor malignant, metastatic, bone marrow		1 (2%)		
Trachea	(50)	(50)	(50)	(50)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	6 (12%)	3 (6%)	7 (14%)	6 (12%)
Adenoma, multiple				1 (2%)
Carcinoma	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Mast cell tumor malignant, metastatic, bone marrow		1 (2%)		
Renal tubule, adenoma			1 (2%)	
Urethra	(0)	(1)	(1)	(1)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)		2 (4%)	2 (4%)
Lymphoma malignant	2 (4%)	3 (6%)	3 (6%)	3 (6%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	47	47	46	45
Total primary neoplasms	114	116	105	100
Total animals with benign neoplasms	42	43	42	40
Total benign neoplasms	70	61	65	57
Total animals with malignant neoplasms	33	32	27	33
Total malignant neoplasms	44	55	40	43
Total animals with metastatic neoplasms	9	11	11	7
Total metastatic neoplasms	11	27	11	8

[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 C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Adrenal Cortex: Adenoma				
Overall rate[a]	9/50 (18%)	8/50 (16%)	6/50 (12%)	3/50 (6%)
Adjusted rate[b]	20.0%	18.0%	13.4%	7.3%
Terminal rate[c]	9/37 (24%)	7/36 (19%)	5/34 (15%)	3/31 (10%)
First incidence (days)	729 (T)	673	595	729 (T)
Poly-3 test[d]	P=0.064N	P=0.508N	P=0.290N	P=0.080N
Harderian Gland: Adenoma				
Overall	6/50 (12%)	3/50 (6%)	7/50 (14%)	7/50 (14%)
Adjusted rate	13.3%	6.8%	15.8%	16.8%
Terminal rate	5/37 (14%)	3/36 (8%)	7/34 (21%)	5/31 (16%)
First incidence (days)	635	729 (T)	729 (T)	633
Poly-3 test	P=0.230	P=0.252N	P=0.482	P=0.437
Harderian Gland: Adenoma or Carcinoma				
Overall rate	8/50 (16%)	5/50 (10%)	8/50 (16%)	8/50 (16%)
Adjusted rate	17.4%	11.3%	18.0%	19.2%
Terminal rate	5/37 (14%)	5/36 (14%)	7/34 (21%)	6/31 (19%)
First incidence (days)	521	729 (T)	681	633
Poly-3 test	P=0.339	P=0.301N	P=0.576	P=0.523
Liver: Hemangiosarcoma				
Overall rate	3/50 (6%)	5/50 (10%)	2/50 (4%)	2/50 (4%)
Adjusted rate	6.6%	11.3%	4.5%	4.9%
Terminal rate	2/37 (5%)	4/36 (11%)	2/34 (6%)	1/31 (3%)
First incidence (days)	632	724	729 (T)	722
Poly-3 test	P=0.322N	P=0.345	P=0.511N	P=0.542N
Liver: Hepatocellular Adenoma				
Overall rate	39/50 (78%)	36/50 (72%)	38/50 (76%)	34/50 (68%)
Adjusted rate	82.2%	77.0%	79.3%	76.1%
Terminal rate	32/37 (87%)	28/36 (78%)	27/34 (79%)	25/31 (81%)
First incidence (days)	521	502	531	493
Poly-3 test	P=0.357N	P=0.348N	P=0.459N	P=0.311N
Liver: Hepatocellular Carcinoma				
Overall rate	20/50 (40%)	18/50 (36%)	20/50 (40%)	21/50 (42%)
Adjusted rate	42.9%	39.8%	43.3%	47.8%
Terminal rate	15/37 (41%)	13/36 (36%)	12/34 (35%)	13/31 (42%)
First incidence (days)	547	610	548	493
Poly-3 test	P=0.295	P=0.461N	P=0.570	P=0.401
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	43/50 (86%)	40/50 (80%)	42/50 (84%)	41/50 (82%)
Adjusted rate	89.7%	85.0%	86.8%	89.8%
Terminal rate	34/37 (92%)	30/36 (83%)	29/34 (85%)	29/31 (94%)
First incidence (days)	521	502	531	493
Poly-3 test	P=0.428	P=0.344N	P=0.443N	P=0.643
Liver: Hepatoblastoma				
Overall rate	4/50 (8%)	1/50 (2%)	4/50 (8%)	3/50 (6%)
Adjusted rate	8.8%	2.3%	9.0%	7.3%
Terminal rate	3/37 (8%)	1/36 (3%)	3/34 (9%)	3/31 (10%)
First incidence (days)	521	729 (T)	648	729 (T)
Poly-3 test	P=0.517	P=0.187N	P=0.630	P=0.555N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	21/50 (42%)	19/50 (38%)	21/50 (42%)	24/50 (48%)
Adjusted rate	44.5%	42.0%	45.5%	54.6%
Terminal rate	15/37 (41%)	14/36 (39%)	13/34 (38%)	16/31 (52%)
First incidence (days)	521	610	548	493
Poly-3 test	P=0.138	P=0.486N	P=0.545	P=0.223
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	43/50 (86%)	40/50 (80%)	42/50 (84%)	41/50 (82%)
Adjusted rate	89.7%	85.0%	86.8%	89.8%
Terminal rate	34/37 (92%)	30/36 (83%)	29/34 (85%)	29/31 (94%)
First incidence (days)	521	502	531	493
Poly-3 test	P=0.428	P=0.344N	P=0.443N	P=0.643
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	12/50 (24%)	7/50 (14%)	8/50 (16%)	6/50 (12%)
Adjusted rate	26.7%	15.8%	18.1%	14.5%
Terminal rate	12/37 (32%)	6/36 (17%)	8/34 (24%)	5/31 (16%)
First incidence (days)	729 (T)	724	729 (T)	681
Poly-3 test	P=0.209N	P=0.157N	P=0.235N	P=0.127N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	4/50 (8%)	8/50 (16%)	5/50 (10%)	8/50 (16%)
Adjusted rate	8.9%	17.5%	11.1%	19.3%
Terminal rate	4/37 (11%)	4/36 (11%)	2/34 (6%)	7/31 (23%)
First incidence (days)	729 (T)	502	636	681
Poly-3 test	P=0.204	P=0.186	P=0.499	P=0.138
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	15/50 (30%)	14/50 (28%)	13/50 (26%)	13/50 (26%)
Adjusted rate	33.4%	30.5%	29.0%	31.4%
Terminal rate	15/37 (41%)	10/36 (28%)	10/34 (29%)	12/31 (39%)
First incidence (days)	729 (T)	502	636	681
Poly-3 test	P=0.536N	P=0.474N	P=0.411N	P=0.514N
Skin: Fibrous Histiocytoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.2%	6.7%	2.3%	0.0%
Terminal rate	1/37 (3%)	2/36 (6%)	1/34 (3%)	0/31 (0%)
First incidence (days)	729 (T)	686	729 (T)	—[e]
Poly-3 test	P=0.189N	P=0.302	P=0.757	P=0.517N
Skin: Fibrous Histiocytoma or Sarcoma				
Overall rate	1/50 (2%)	4/50 (8%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.2%	9.0%	2.3%	0.0%
Terminal rate	1/37 (3%)	3/36 (8%)	1/34 (3%)	0/31 (0%)
First incidence (days)	729 (T)	686	729 (T)	—
Poly-3 test	P=0.140N	P=0.175	P=0.757	P=0.517N
Tooth: Odontoma				
Overall rate	1/50 (2%)	3/50 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.2%	6.7%	6.7%	2.4%
Terminal rate	1/37 (3%)	2/36 (6%)	2/34 (6%)	1/31 (3%)
First incidence (days)	729 (T)	686	564	729 (T)
Poly-3 test	P=0.435N	P=0.302	P=0.304	P=0.741

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
All Organs: Hemangiosarcoma				
Overall rate	5/50 (10%)	6/50 (12%)	2/50 (4%)	3/50 (6%)
Adjusted rate	11.0%	13.5%	4.5%	7.2%
Terminal rate	4/37 (11%)	5/36 (14%)	2/34 (6%)	1/31 (3%)
First incidence (days)	632	724	729 (T)	667
Poly-3 test	P=0.285N	P=0.485	P=0.226N	P=0.405N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	6/50 (12%)	7/50 (14%)	3/50 (6%)	3/50 (6%)
Adjusted rate	13.3%	15.5%	6.8%	7.2%
Terminal rate	5/37 (14%)	5/36 (14%)	3/34 (9%)	1/31 (3%)
First incidence (days)	632	437	729 (T)	667
Poly-3 test	P=0.184N	P=0.497	P=0.253N	P=0.287N
All Organs: Malignant Lymphoma				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	3/50 (6%)
Adjusted rate	4.4%	6.7%	6.7%	7.1%
Terminal rate	1/37 (3%)	2/36 (6%)	1/34 (3%)	1/31 (3%)
First incidence (days)	437	673	567	446
Poly-3 test	P=0.467	P=0.488	P=0.491	P=0.463
All Organs: Benign Neoplasms				
Overall rate	42/50 (84%)	43/50 (86%)	42/50 (84%)	40/50 (80%)
Adjusted rate	87.9%	90.4%	87.1%	88.8%
Terminal rate	34/37 (92%)	33/36 (92%)	30/34 (88%)	29/31 (94%)
First incidence (days)	521	437	531	493
Poly-3 test	P=0.589	P=0.475	P=0.576N	P=0.580
All Organs: Malignant Neoplasms				
Overall rate	33/50 (66%)	32/50 (64%)	27/50 (54%)	33/50 (66%)
Adjusted rate	68.0%	67.2%	57.3%	73.1%
Terminal rate	24/37 (65%)	21/36 (58%)	16/34 (47%)	22/31 (71%)
First incidence (days)	437	502	548	446
Poly-3 test	P=0.273	P=0.552N	P=0.189N	P=0.375
All Organs: Benign or Malignant Neoplasms				
Overall rate	47/50 (94%)	47/50 (94%)	46/50 (92%)	45/50 (90%)
Adjusted rate	95.8%	97.1%	93.4%	96.6%
Terminal rate	36/37 (97%)	35/36 (97%)	31/34 (91%)	31/31 (100%)
First incidence (days)	437	437	531	446
Poly-3 test	P=0.586	P=0.594	P=0.459N	P=0.664

(T) Terminal kill

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, and lung; for other tissues, denominator is number of animals necropsied.

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

[c] Observed incidence at terminal kill

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

[e] Not applicable; no neoplasms in animal group

TABLE C3
Historical Incidence of Squamous Cell Papilloma of the Skin in Control Male B6C3F1/N Mice[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Ethanol Vehicle Dermal Studies	
bis(2-Chloroethoxy)methane (October 2002)	0/50
1,2-Dibromo-2,4-dicyanobutane (June 2002)	0/50
Methyl *trans*-styryl ketone (April 2004)	0/50
Pyrogallol (September 2004)	0/50
Total (%)	0/200
Mean ± standard deviation	
Range	
Overall Historical Incidence: All Routes	
Total (%)	1/1,150 (0.1%)
Mean ± standard deviation	0.1% ± 0.4%
Range	0%-2%

[a] Data as of May 4, 2011

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Pyrogallol[a]

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death				1
Moribund	8	10	10	12
Natural deaths	5	4	6	6
Survivors				
Terminal kill	37	36	34	31
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation				1 (2%)
Epithelium, cyst				1 (2%)
Gallbladder	(49)	(49)	(50)	(46)
Cyst	1 (2%)			
Infiltration cellular, mononuclear cell	4 (8%)	8 (16%)	4 (8%)	3 (7%)
Epithelium, hyperplasia	1 (2%)		1 (2%)	
Intestine large, cecum	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid			1 (2%)	
Inflammation			1 (2%)	
Epithelium, hyperplasia				1 (2%)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Inflammation			1 (2%)	
Epithelium, hyperplasia			2 (4%)	
Muscularis, hypertrophy			1 (2%)	
Intestine small, duodenum	(50)	(50)	(50)	(50)
Inflammation		1 (2%)		
Epithelium, hyperplasia		1 (2%)		
Intestine small, ileum	(50)	(50)	(50)	(50)
Inflammation		2 (4%)	5 (10%)	
Epithelium, hyperplasia		2 (4%)	2 (4%)	
Intestine small, jejunum	(50)	(50)	(50)	(50)
Cyst				1 (2%)
Hyperplasia, lymphoid	9 (18%)	5 (10%)	5 (10%)	
Inflammation	1 (2%)	2 (4%)	3 (6%)	
Inflammation, granulomatous	1 (2%)			
Epithelium, hyperplasia		1 (2%)		1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Atypia cellular				1 (2%)
Basophilic focus	5 (10%)	7 (14%)	7 (14%)	7 (14%)
Clear cell focus	29 (58%)	31 (62%)	29 (58%)	23 (46%)
Cytoplasmic alteration		1 (2%)	1 (2%)	
Degeneration, fatty		1 (2%)		
Eosinophilic focus	31 (62%)	32 (64%)	31 (62%)	26 (52%)
Fibrosis	1 (2%)			
Hematopoietic cell proliferation		4 (8%)	4 (8%)	14 (28%)
Hepatodiaphragmatic nodule	2 (4%)			
Infarct			1 (2%)	
Inflammation	31 (62%)	25 (50%)	29 (58%)	24 (48%)
Metaplasia, osseous	1 (2%)			
Mixed cell focus	5 (10%)	5 (10%)	6 (12%)	

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

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Alimentary System (continued)				
Liver (continued)	(50)	(50)	(50)	(50)
Necrosis	5 (10%)	3 (6%)	1 (2%)	4 (8%)
Pigmentation	2 (4%)		1 (2%)	2 (4%)
Regeneration	1 (2%)			
Tension lipidosis	2 (4%)	3 (6%)	4 (8%)	2 (4%)
Thrombosis		1 (2%)		
Vacuolization cytoplasmic	10 (20%)	23 (46%)	19 (38%)	6 (12%)
Bile duct, cyst				3 (6%)
Bile duct, hyperplasia	2 (4%)	1 (2%)	3 (6%)	2 (4%)
Hepatocyte, hypertrophy	1 (2%)			
Kupffer cell, hyperplasia	1 (2%)			1 (2%)
Kupffer cell, pigmentation	1 (2%)			
Oval cell, hyperplasia	2 (4%)		1 (2%)	1 (2%)
Mesentery	(8)	(6)	(8)	(10)
Inflammation	3 (38%)	1 (17%)	2 (25%)	2 (20%)
Fat, necrosis	4 (50%)	2 (33%)	3 (38%)	3 (30%)
Oral mucosa	(0)	(0)	(0)	(1)
Pancreas	(50)	(50)	(50)	(50)
Cytoplasmic alteration, focal	1 (2%)			
Hemorrhage		1 (2%)		
Infiltration cellular, mononuclear cell	9 (18%)	14 (28%)	15 (30%)	9 (18%)
Inflammation	2 (4%)			1 (2%)
Pigmentation				1 (2%)
Vacuolization cytoplasmic	1 (2%)			
Acinus, atrophy	2 (4%)	2 (4%)	1 (2%)	
Duct, cyst	1 (2%)			1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Atrophy		1 (2%)	1 (2%)	
Infiltration cellular, mononuclear cell	40 (80%)	40 (80%)	41 (82%)	40 (80%)
Inflammation				2 (4%)
Mineralization	1 (2%)	1 (2%)	2 (4%)	
Duct, hyperplasia			1 (2%)	
Submandibular gland, vacuolization Cytoplasmic	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Inflammation	2 (4%)	5 (10%)	3 (6%)	5 (10%)
Ulcer	2 (4%)			2 (4%)
Epithelium, cyst			1 (2%)	
Epithelium, hyperkeratosis	4 (8%)	3 (6%)	6 (12%)	5 (10%)
Epithelium, hyperplasia, squamous	3 (6%)	5 (10%)	6 (12%)	7 (14%)
Stomach, glandular	(50)	(50)	(50)	(50)
Cyst	6 (12%)	19 (38%)	14 (28%)	10 (20%)
Foreign body		1 (2%)		
Hyperplasia	5 (10%)	5 (10%)	1 (2%)	5 (10%)
Infiltration cellular, mononuclear cell		4 (8%)	2 (4%)	
Inflammation		1 (2%)	5 (10%)	
Inflammation, granulomatous			1 (2%)	
Mineralization	1 (2%)	2 (4%)		
Tooth	(36)	(30)	(24)	(22)
Dysplasia	35 (97%)	27 (90%)	21 (88%)	21 (95%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, infiltration cellular, mononuclear cell		1 (2%)		
Aorta, mineralization		1 (2%)		
Carotid artery, inflammation	1 (2%)		3 (6%)	
Carotid artery, thrombosis			1 (2%)	
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	45 (90%)	44 (88%)	46 (92%)	44 (88%)
Infiltration cellular, mononuclear cell	1 (2%)	2 (4%)	1 (2%)	4 (8%)
Inflammation	2 (4%)	5 (10%)	4 (8%)	2 (4%)
Metaplasia, osseous		1 (2%)		
Mineralization	1 (2%)		2 (4%)	2 (4%)
Necrosis	1 (2%)			
Thrombosis	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis		2 (4%)	2 (4%)	1 (2%)
Fibrosis	1 (2%)			
Hematopoietic cell proliferation				2 (4%)
Hyperplasia	1 (2%)			
Hypertrophy	15 (30%)	18 (36%)	13 (26%)	8 (16%)
Inflammation		1 (2%)		
Mineralization			1 (2%)	
Vacuolization cytoplasmic			1 (2%)	
Subcapsular, hyperplasia	40 (80%)	39 (78%)	44 (88%)	44 (88%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia			1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	43 (86%)	43 (86%)	42 (84%)	40 (80%)
Parathyroid gland	(41)	(44)	(42)	(47)
Hyperplasia	1 (2%)			
Pituitary gland	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Pars distalis, hyperplasia	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Pars intermedia, hyperplasia			1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, hyperplasia			1 (2%)	
General Body System				
Peritoneum	(0)	(1)	(1)	(1)
Hyperplasia			1 (100%)	
Inflammation		1 (100%)		1 (100%)
Genital System				
Coagulating gland	(0)	(2)	(1)	(0)
Epididymis	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)		2 (4%)	
Granuloma sperm		2 (4%)		1 (2%)
Infiltration cellular, mononuclear cell	37 (74%)	38 (76%)	39 (78%)	33 (66%)
Inflammation	3 (6%)	4 (8%)	1 (2%)	2 (4%)
Inflammation, granulomatous		1 (2%)		
Mineralization			1 (2%)	
Pigmentation		1 (2%)		
Artery, inflammation		1 (2%)		
Epithelium, hyperplasia				1 (2%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Genital System (continued)				
Penis	(1)	(0)	(0)	(0)
Inflammation	1 (100%)			
Preputial gland	(50)	(50)	(50)	(50)
Cyst				1 (2%)
Ectasia	3 (6%)	3 (6%)	5 (10%)	2 (4%)
Infiltration cellular, mononuclear cell	20 (40%)	22 (44%)	22 (44%)	26 (52%)
Inflammation	12 (24%)	21 (42%)	14 (28%)	11 (22%)
Prostate	(50)	(50)	(50)	(50)
Fibrosis		1 (2%)		
Hyperplasia	12 (24%)	21 (42%)	15 (30%)	19 (38%)
Hypertrophy	1 (2%)			
Infiltration cellular, mononuclear cell	42 (84%)	44 (88%)	43 (86%)	38 (76%)
Inflammation	1 (2%)	4 (8%)	4 (8%)	3 (6%)
Artery, inflammation		1 (2%)		
Seminal vesicle	(50)	(50)	(50)	(50)
Infiltration cellular, mixed cell	1 (2%)			
Infiltration cellular, mononuclear cell	8 (16%)	12 (24%)	19 (38%)	14 (28%)
Inflammation		5 (10%)	1 (2%)	1 (2%)
Bilateral, dilatation	1 (2%)	2 (4%)		
Testes	(50)	(50)	(50)	(50)
Atrophy	1 (2%)		1 (2%)	
Infiltration cellular, mononuclear cell	1 (2%)		2 (4%)	
Mineralization		1 (2%)		1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)	10 (20%)	8 (16%)	15 (30%)
Lymph node	(10)	(12)	(9)	(16)
Axillary, hyperplasia, lymphoid			1 (11%)	3 (19%)
Bronchial, hyperplasia, lymphoid	1 (10%)	2 (17%)	1 (11%)	
Inguinal, hyperplasia, lymphoid	6 (60%)	6 (50%)	8 (89%)	10 (63%)
Inguinal, pigmentation		1 (8%)		
Mediastinal, hyperplasia, lymphoid				3 (19%)
Mediastinal, inflammation				1 (6%)
Renal, necrosis				1 (6%)
Lymph node, mandibular	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation				1 (2%)
Hyperplasia, lymphoid	9 (18%)	16 (32%)	12 (24%)	11 (22%)
Inflammation				1 (2%)
Lymph node, mesenteric	(50)	(49)	(50)	(49)
Angiectasis			1 (2%)	
Hyperplasia, lymphoid	5 (10%)	7 (14%)	10 (20%)	3 (6%)
Inflammation	1 (2%)		1 (2%)	1 (2%)
Pigmentation	1 (2%)			
Spleen	(50)	(49)	(50)	(50)
Angiectasis				1 (2%)
Hematopoietic cell proliferation	37 (74%)	38 (78%)	40 (80%)	42 (84%)
Hyperplasia, lymphoid	5 (10%)	2 (4%)	3 (6%)	5 (10%)
Inflammation				1 (2%)
Pigmentation			1 (2%)	
Thymus	(48)	(50)	(46)	(48)
Hyperplasia, lymphoid	2 (4%)	2 (4%)	1 (2%)	1 (2%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Integumentary System				
Mammary gland	(0)	(0)	(0)	(1)
Hyperplasia				1 (100%)
Skin	(50)	(50)	(50)	(50)
Abscess		1 (2%)		
Fibrosis	1 (2%)	1 (2%)	3 (6%)	10 (20%)
Hyperkeratosis	1 (2%)	1 (2%)	3 (6%)	10 (20%)
Hyperplasia	1 (2%)	1 (2%)	3 (6%)	10 (20%)
Inflammation	1 (2%)	1 (2%)	3 (6%)	10 (20%)
Metaplasia, cartilaginous				1 (2%)
Ulcer		1 (2%)	3 (6%)	10 (20%)
Control, inflammation			1 (2%)	1 (2%)
Hair follicle, site of application, cyst				1 (2%)
Lip, inflammation		1 (2%)		
Sebaceous gland, hyperplasia	1 (2%)	1 (2%)		5 (10%)
Sebaceous gland, site of application, hyperplasia	1 (2%)	6 (12%)	4 (8%)	24 (48%)
Site of application, fibrosis	3 (6%)	6 (12%)	28 (56%)	47 (94%)
Site of application, hyperkeratosis	11 (22%)	43 (86%)	50 (100%)	50 (100%)
Site of application, hyperplasia	8 (16%)	24 (48%)	47 (94%)	50 (100%)
Site of application, inflammation	2 (4%)	6 (12%)	37 (74%)	44 (88%)
Site of application, pigmentation			9 (18%)	39 (78%)
Site of application, ulcer	1 (2%)	1 (2%)	2 (4%)	23 (46%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, hyperostosis			1 (2%)	1 (2%)
Cranium, inflammation	1 (2%)			1 (2%)
Skeletal muscle	(0)	(0)	(1)	(2)
Inflammation			1 (100%)	
Mineralization				1 (50%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Developmental malformation	1 (2%)			
Infiltration cellular, mononuclear cell	4 (8%)	2 (4%)		1 (2%)
Inflammation	1 (2%)			1 (2%)
Cerebellum, gliosis				1 (2%)
Choroid plexus, mineralization	1 (2%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Infiltration cellular, histiocyte	5 (10%)	12 (24%)	7 (14%)	6 (12%)
Inflammation	7 (14%)	3 (6%)	5 (10%)	3 (6%)
Metaplasia, osseous		1 (2%)		2 (4%)
Mineralization		1 (2%)	1 (2%)	
Pigmentation	1 (2%)	3 (6%)	2 (4%)	
Thrombosis			1 (2%)	
Alveolar epithelium, hyperplasia	7 (14%)	3 (6%)	3 (6%)	5 (10%)
Bronchiole, hyperplasia		1 (2%)		1 (2%)
Bronchiole, mineralization	1 (2%)			
Interstitium, fibrosis			1 (2%)	
Mediastinum, necrosis, fatty				1 (2%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Respiratory System (continued)				
Nose	(50)	(50)	(50)	(50)
Foreign body			1 (2%)	
Inflammation	20 (40%)	15 (30%)	16 (32%)	15 (30%)
Necrosis				1 (2%)
Polyp, inflammatory	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Glands, dilatation	2 (4%)		1 (2%)	2 (4%)
Glands, fibrosis	1 (2%)			
Glands, metaplasia	11 (22%)	3 (6%)	1 (2%)	3 (6%)
Goblet cell, hyperplasia				2 (4%)
Olfactory epithelium, degeneration	2 (4%)			3 (6%)
Olfactory epithelium, hyperplasia	1 (2%)	1 (2%)	1 (2%)	
Olfactory epithelium, metaplasia	11 (22%)	8 (16%)	4 (8%)	5 (10%)
Respiratory epithelium, degeneration	2 (4%)		1 (2%)	
Respiratory epithelium, hyperplasia	40 (80%)	45 (90%)	46 (92%)	37 (74%)
Respiratory epithelium, metaplasia	2 (4%)	1 (2%)		1 (2%)
Vomeronasal organ, cyst			1 (2%)	
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract			1 (2%)	1 (2%)
Developmental malformation			1 (2%)	
Infiltration cellular, mononuclear cell			1 (2%)	
Inflammation	4 (8%)	2 (4%)	3 (6%)	1 (2%)
Cornea, hyperplasia	2 (4%)	1 (2%)		1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Hyperplasia	3 (6%)	1 (2%)	1 (2%)	3 (6%)
Infiltration cellular, mononuclear cell	35 (70%)	41 (82%)	41 (82%)	36 (72%)
Inflammation	1 (2%)			1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet				1 (2%)
Cyst		5 (10%)	9 (18%)	4 (8%)
Dilatation		2 (4%)		
Hyperplasia, oncocytic				1 (2%)
Infarct		1 (2%)		
Infiltration cellular, mononuclear cell	2 (4%)		5 (10%)	4 (8%)
Inflammation	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Metaplasia, osseous	6 (12%)	2 (4%)		
Mineralization	39 (78%)	42 (84%)	34 (68%)	38 (76%)
Nephropathy	44 (88%)	46 (92%)	47 (94%)	45 (90%)
Pigmentation	1 (2%)			1 (2%)
Thrombosis	1 (2%)	1 (2%)		
Papilla, necrosis		1 (2%)		
Renal tubule, hyperplasia	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Urethra	(0)	(1)	(1)	(1)
Inflammation		1 (100%)		1 (100%)
Bulbourethral gland, inflammation		1 (100%)	1 (100%)	
Bulbourethral gland, necrosis		1 (100%)		
Transitional epithelium, hyperplasia		1 (100%)		
Urinary bladder	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	20 (40%)	31 (62%)	33 (66%)	23 (46%)
Inflammation		1 (2%)	2 (4%)	

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR DERMAL STUDY OF PYROGALLOL

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of Pyrogallol[a]

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	15	7	29
Natural deaths	8	5	7	4
Survivors				
Died last week of study				1
Terminal kill	33	30	36	16
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(50)	(49)	(49)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Leiomyoma				1 (2%)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)		1 (2%)	
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	4 (8%)	2 (4%)	1 (2%)
Hepatoblastoma	1 (2%)	1 (2%)	1 (2%)	
Hepatocellular adenoma	9 (18%)	12 (24%)	9 (18%)	8 (16%)
Hepatocellular adenoma, multiple	21 (42%)	23 (46%)	25 (50%)	7 (14%)
Hepatocellular carcinoma	12 (24%)	13 (26%)	14 (28%)	7 (14%)
Hepatocellular carcinoma, multiple	5 (10%)	13 (26%)	6 (12%)	1 (2%)
Osteosarcoma, metastatic, uncertain primary site			1 (2%)	
Serosa, sarcoma, metastatic, mesentery		1 (2%)		
Mesentery	(20)	(22)	(14)	(19)
Sarcoma		1 (5%)		
Sarcoma stromal, metastatic, uterus		1 (5%)		
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)	3 (6%)		1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Tooth	(3)	(0)	(4)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	2 (4%)	2 (4%)	1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	3 (6%)		
Parathyroid gland	(45)	(47)	(39)	(38)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Endocrine System (continued)				
Pituitary gland	(50)	(49)	(50)	(50)
Pars distalis, adenoma	4 (8%)	6 (12%)	10 (20%)	2 (4%)
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma		2 (4%)		
General Body System				
Peritoneum	(1)	(0)	(0)	(0)
Tissue NOS	(1)	(0)	(0)	(0)
Mediastinum, hemangiosarcoma	1 (100%)			
Genital System				
Clitoral gland	(49)	(50)	(50)	(49)
Ovary	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)		
Cystadenoma	2 (4%)		1 (2%)	1 (2%)
Granulosa cell tumor benign	1 (2%)			
Hemangioma	1 (2%)		1 (2%)	
Hemangiosarcoma	1 (2%)	4 (8%)	1 (2%)	
Luteoma		1 (2%)		
Oviduct	(1)	(0)	(0)	(0)
Uterus	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)			
Hemangiosarcoma			1 (2%)	
Polyp stromal	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Sarcoma stromal		2 (4%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	3 (6%)		2 (4%)	
Lymph node	(19)	(13)	(18)	(29)
Mediastinal, hemangiosarcoma, metastatic, skin				1 (3%)
Lymph node, mandibular	(50)	(50)	(50)	(50)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)	1 (2%)	
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)		2 (4%)	2 (4%)
Thymus	(50)	(50)	(48)	(49)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)		1 (2%)
Skin	(50)	(50)	(50)	(50)
Sarcoma			1 (2%)	
Squamous cell carcinoma				1 (2%)
Site of application, carcinoma, metastatic, uncertain primary site				1 (2%)
Site of application, hemangioma				1 (2%)
Site of application, squamous cell carcinoma				4 (8%)
Site of application, subcutaneous tissue, lipoma		1 (2%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Integumentary System (continued)				
Skin (continued)	(50)	(50)	(50)	(50)
Subcutaneous tissue, fibrosarcoma	1 (2%)		2 (4%)	
Subcutaneous tissue, hemangiosarcoma	1 (2%)	1 (2%)		1 (2%)
Subcutaneous tissue, sarcoma		1 (2%)		
Subcutaneous tissue, schwannoma benign	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hemangiosarcoma	3 (6%)		3 (6%)	
Osteosarcoma			1 (2%)	
Skeletal muscle	(4)	(1)	(2)	(0)
Hemangiosarcoma	1 (25%)			
Sarcoma stromal, metastatic, uterus		1 (100%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(2)	(0)	(0)	(0)
Spinal cord	(2)	(0)	(0)	(0)
Hemangiosarcoma	1 (50%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Alveolar/bronchiolar carcinoma	2 (4%)	6 (12%)	4 (8%)	3 (6%)
Alveolar/bronchiolar carcinoma, multiple		1 (2%)		
Carcinoma, metastatic, Harderian gland				1 (2%)
Hepatocellular carcinoma, metastatic, liver	4 (8%)	7 (14%)	2 (4%)	2 (4%)
Osteosarcoma, metastatic, uncertain primary site			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Olfactory epithelium, neuroblastoma				1 (2%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	4 (8%)	4 (8%)	5 (10%)
Carcinoma	2 (4%)	4 (8%)	4 (8%)	2 (4%)
Lacrimal gland	(0)	(0)	(1)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)			
Renal tubule, carcinoma			1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Sarcoma stromal, metastatic, uterus		1 (2%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		8 (16%)	2 (4%)	3 (6%)
Lymphoma malignant	13 (26%)	14 (28%)	12 (24%)	5 (10%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	42	48	47	33
Total primary neoplasms	102	136	116	62
Total animals with benign neoplasms	34	38	38	22
Total benign neoplasms	50	60	55	29
Total animals with malignant neoplasms	34	45	38	24
Total malignant neoplasms	52	76	61	33
Total animals with metastatic neoplasms	4	9	3	5
Total metastatic neoplasms	4	11	4	5
Total animals with malignant neoplasms of uncertain primary site			1	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 D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Bone: Hemangiosarcoma				
Overall rate[a]	3/50 (6%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate[b]	6.9%	0.0%	6.5%	0.0%
Terminal rate[c]	1/33 (3%)	0/30 (0%)	2/36 (6%)	0/17 (0%)
First incidence (days)	488	—[e]	573	—
Poly-3 test[d]	P=0.289N	P=0.121N	P=0.632N	P=0.185N
Bone Marrow: Hemangiosarcoma				
Overall rate	3/50 (6%)	0/50 (0%)	2/50 (4%)	0/50 (0%)
Adjusted rate	6.9%	0.0%	4.4%	0.0%
Terminal rate	1/33 (3%)	0/30 (0%)	2/36 (6%)	0/17 (0%)
First incidence (days)	488	—	729 (T)	—
Poly-3 test	P=0.263N	P=0.121N	P=0.476N	P=0.185N
Harderian Gland: Adenoma				
Overall rate	1/50 (2%)	4/50 (8%)	4/50 (8%)	5/50 (10%)
Adjusted rate	2.4%	9.3%	8.7%	15.1%
Terminal rate	1/33 (3%)	3/30 (10%)	4/36 (11%)	1/17 (6%)
First incidence (days)	729 (T)	654	729 (T)	604
Poly-3 test	P=0.088	P=0.181	P=0.201	P=0.053
Harderian Gland: Carcinoma				
Overall rate	2/50 (4%)	4/50 (8%)	4/50 (8%)	2/50 (4%)
Adjusted rate	4.7%	9.3%	8.6%	6.2%
Terminal rate	2/33 (6%)	2/30 (7%)	2/36 (6%)	0/17 (0%)
First incidence (days)	729 (T)	644	597	604
Poly-3 test	P=0.572N	P=0.342	P=0.379	P=0.591
Harderian Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	8/50 (16%)	8/50 (16%)	6/50 (12%)
Adjusted rate	7.0%	18.4%	17.2%	17.9%
Terminal rate	3/33 (9%)	5/30 (17%)	6/36 (17%)	1/17 (6%)
First incidence (days)	729 (T)	644	597	604
Poly-3 test	P=0.282	P=0.102	P=0.128	P=0.137
Liver: Hemangiosarcoma				
Overall rate	1/50 (2%)	4/50 (8%)	2/50 (4%)	1/50 (2%)
Adjusted rate	2.3%	9.2%	4.4%	3.2%
Terminal rate	0/33 (0%)	2/30 (7%)	1/36 (3%)	1/17 (6%)
First incidence (days)	550	611	678	729 (T)
Poly-3 test	P=0.449N	P=0.180	P=0.522	P=0.688
Liver: Hepatocellular Adenoma				
Overall rate	30/50 (60%)	35/50 (70%)	34/50 (68%)	15/50 (30%)
Adjusted rate	67.8%	74.9%	72.6%	43.9%
Terminal rate	25/33 (76%)	24/30 (80%)	28/36 (78%)	8/17 (47%)
First incidence (days)	557	542	597	487
Poly-3 test	P=0.004N	P=0.298	P=0.391	P=0.022N
Liver: Hepatocellular Carcinoma				
Overall rate	17/50 (34%)	26/50 (52%)	20/50 (40%)	8/50 (16%)
Adjusted rate	38.2%	56.3%	42.3%	23.4%
Terminal rate	11/33 (33%)	15/30 (50%)	16/36 (44%)	2/17 (12%)
First incidence (days)	557	582	542	487
Poly-3 test	P=0.020N	P=0.061	P=0.425	P=0.124N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	35/50 (70%)	42/50 (84%)	39/50 (78%)	19/50 (38%)
Adjusted rate	77.5%	86.4%	81.8%	53.8%
Terminal rate	26/33 (79%)	25/30 (83%)	31/36 (86%)	9/17 (53%)
First incidence (days)	557	542	542	487
Poly-3 test	P<0.001N	P=0.188	P=0.395	P=0.015N
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	18/50 (36%)	26/50 (52%)	20/50 (40%)	8/50 (16%)
Adjusted rate	40.4%	56.3%	42.3%	23.4%
Terminal rate	11/33 (33%)	15/30 (50%)	16/36 (44%)	2/17 (12%)
First incidence (days)	557	582	542	487
Poly-3 test	P=0.015N	P=0.093	P=0.512	P=0.087N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	35/50 (70%)	42/50 (84%)	39/50 (78%)	19/50 (38%)
Adjusted rate	77.5%	86.4%	81.8%	53.8%
Terminal rate	26/33 (79%)	25/30 (83%)	31/36 (86%)	9/17 (53%)
First incidence (days)	557	542	542	487
Poly-3 test	P<0.001N	P=0.188	P=0.395	P=0.015N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	2/50 (4%)	7/50 (14%)	4/50 (8%)	3/50 (6%)
Adjusted rate	4.7%	16.1%	8.7%	9.3%
Terminal rate	1/33 (3%)	4/30 (13%)	3/36 (8%)	2/17 (12%)
First incidence (days)	715	634	678	632
Poly-3 test	P=0.569N	P=0.082	P=0.373	P=0.374
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/50 (8%)	7/50 (14%)	6/50 (12%)	5/50 (10%)
Adjusted rate	9.2%	16.1%	13.0%	15.4%
Terminal rate	2/33 (6%)	4/30 (13%)	5/36 (14%)	3/17 (18%)
First incidence (days)	488	634	678	604
Poly-3 test	P=0.410	P=0.260	P=0.407	P=0.326
Ovary: Hemangiosarcoma				
Overall rate	1/50 (2%)	4/50 (8%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.4%	9.2%	2.2%	0.0%
Terminal rate	1/33 (3%)	2/30 (7%)	0/36 (0%)	0/17 (0%)
First incidence (days)	729 (T)	544	710	—
Poly-3 test	P=0.179N	P=0.186	P=0.744N	P=0.557N
Pancreatic Islets: Adenoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	2.4%	7.0%	0.0%	0.0%
Terminal rate	1/33 (3%)	2/30 (7%)	0/36 (0%)	0/17 (0%)
First incidence (days)	729 (T)	718	—	—
Poly-3 test	P=0.198N	P=0.307	P=0.486N	P=0.557N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	4/50 (8%)	6/49 (12%)	10/50 (20%)	2/50 (4%)
Adjusted rate	9.4%	14.2%	21.6%	6.3%
Terminal rate	4/33 (12%)	6/30 (20%)	8/36 (22%)	1/17 (6%)
First incidence (days)	729 (T)	729 (T)	580	682
Poly-3 test	P=0.338N	P=0.363	P=0.099	P=0.476N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Skin: Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	0.0%	0.0%	12.2%
Terminal rate	0/33 (0%)	0/30 (0%)	0/36 (0%)	2/17 (12%)
First incidence (days)	—	—	—	575
Poly-3 test	P<0.001	—[f]	—	P=0.033
Skin: Fibrosarcoma or Sarcoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	2.4%	2.3%	6.5%	0.0%
Terminal rate	1/33 (3%)	1/30 (3%)	1/36 (3%)	0/17 (0%)
First incidence (days)	729 (T)	729 (T)	663	—
Poly-3 test	P=0.437N	P=0.760N	P=0.334	P=0.557N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	2.3%	6.9%	0.0%	3.1%
Terminal rate	0/33 (0%)	2/30 (7%)	0/36 (0%)	0/17 (0%)
First incidence (days)	488	589	—	682
Poly-3 test	P=0.517N	P=0.304	P=0.489N	P=0.688
Uterus: Stromal Polyp				
Overall rate	3/50 (6%)	2/50 (4%)	2/50 (4%)	1/50 (2%)
Adjusted rate	7.0%	4.7%	4.4%	3.1%
Terminal rate	3/33 (9%)	2/30 (7%)	2/36 (6%)	0/17 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	474
Poly-3 test	P=0.380N	P=0.498N	P=0.467N	P=0.407N
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	3/50 (6%)	4/50 (8%)	2/50 (4%)	1/50 (2%)
Adjusted rate	7.0%	9.3%	4.4%	3.1%
Terminal rate	3/33 (9%)	3/30 (10%)	2/36 (6%)	0/17 (0%)
First incidence (days)	729 (T)	590	729 (T)	474
Poly-3 test	P=0.257N	P=0.508	P=0.467N	P=0.407N
All Organs: Hemangiosarcoma				
Overall rate	6/50 (12%)	8/50 (16%)	7/50 (14%)	3/50 (6%)
Adjusted rate	13.6%	18.0%	15.0%	9.4%
Terminal rate	3/33 (9%)	4/30 (13%)	4/36 (11%)	3/17 (18%)
First incidence (days)	488	544	573	729 (T)
Poly-3 test	P=0.287N	P=0.392	P=0.544	P=0.424N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	7/50 (14%)	8/50 (16%)	8/50 (16%)	4/50 (8%)
Adjusted rate	15.8%	18.0%	17.2%	12.2%
Terminal rate	3/33 (9%)	4/30 (13%)	5/36 (14%)	3/17 (18%)
First incidence (days)	488	544	573	347
Poly-3 test	P=0.361N	P=0.498	P=0.540	P=0.459N
All Organs: Histiocytic Sarcoma				
Overall rate	0/50 (0%)	8/50 (16%)	2/50 (4%)	3/50 (6%)
Adjusted rate	0.0%	18.2%	4.3%	9.2%
Terminal rate	0/33 (0%)	5/30 (17%)	0/36 (0%)	1/17 (6%)
First incidence (days)	—	438	580	624
Poly-3 test	P=0.526	P=0.004	P=0.257	P=0.077

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
All Organs: Malignant Lymphoma				
Overall rate	13/50 (26%)	14/50 (28%)	12/50 (24%)	5/50 (10%)
Adjusted rate	29.4%	31.3%	25.7%	15.3%
Terminal rate	8/33 (24%)	8/30 (27%)	8/36 (22%)	2/17 (12%)
First incidence (days)	365	544	529	624
Poly-3 test	P=0.083N	P=0.515	P=0.434N	P=0.122N
All Organs: Benign Neoplasms				
Overall rate	34/50 (68%)	38/50 (76%)	38/50 (76%)	22/50 (44%)
Adjusted rate	75.7%	81.2%	80.3%	60.1%
Terminal rate	28/33 (85%)	26/30 (87%)	31/36 (86%)	11/17 (65%)
First incidence (days)	488	542	580	347
Poly-3 test	P=0.022N	P=0.341	P=0.384	P=0.086N
All Organs: Malignant Neoplasms				
Overall rate	34/50 (68%)	45/50 (90%)	38/50 (76%)	25/50 (50%)
Adjusted rate	71.5%	93.2%	76.8%	66.3%
Terminal rate	20/33 (61%)	28/30 (93%)	26/36 (72%)	10/17 (59%)
First incidence (days)	365	438	529	472
Poly-3 test	P=0.048N	P=0.004	P=0.357	P=0.383N
All Organs: Benign or Malignant Neoplasms				
Overall rate	42/50 (84%)	48/50 (96%)	47/50 (94%)	34/50 (68%)
Adjusted rate	88.4%	97.1%	94.7%	85.9%
Terminal rate	28/33 (85%)	29/30 (97%)	34/36 (94%)	16/17 (94%)
First incidence (days)	365	438	529	347
Poly-3 test	P=0.142N	P=0.093	P=0.214	P=0.490N

(T) Terminal kill

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for bone marrow, liver, lung, ovary, pancreatic islets, 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 vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Pyrogallol[a]

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	15	7	29
Natural deaths	8	5	7	4
Survivors				
Died last week of study				1
Terminal kill	33	30	36	16
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation			1 (2%)	
Epithelium, hyperplasia			1 (2%)	
Gallbladder	(50)	(49)	(49)	(50)
Cyst	1 (2%)			1 (2%)
Infiltration cellular, mononuclear cell	4 (8%)	7 (14%)	6 (12%)	6 (12%)
Inflammation	1 (2%)			
Epithelium, hyperplasia		1 (2%)		
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Epithelium, hyperplasia		1 (2%)		
Intestine large, colon	(50)	(50)	(50)	(50)
Artery, inflammation				1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Artery, inflammation				1 (2%)
Epithelium, hyperplasia			1 (2%)	
Intestine small, duodenum	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Epithelium, hyperplasia			1 (2%)	
Intestine small, ileum	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	1 (2%)		1 (2%)
Epithelium, hyperplasia	1 (2%)	1 (2%)	3 (6%)	
Intestine small, jejunum	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid	2 (4%)	5 (10%)	2 (4%)	2 (4%)
Inflammation			2 (4%)	
Epithelium, hyperplasia			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)		2 (4%)	
Atrophy			1 (2%)	
Basophilic focus	8 (16%)	2 (4%)	3 (6%)	2 (4%)
Clear cell focus	10 (20%)	1 (2%)	3 (6%)	1 (2%)
Eosinophilic focus	26 (52%)	28 (56%)	31 (62%)	12 (24%)
Fibrosis	1 (2%)	1 (2%)		
Hematopoietic cell proliferation	9 (18%)	8 (16%)	9 (18%)	30 (60%)
Hypertrophy, diffuse			1 (2%)	
Inflammation	40 (80%)	32 (64%)	43 (86%)	37 (74%)
Mixed cell focus	6 (12%)	7 (14%)	4 (8%)	1 (2%)
Necrosis	8 (16%)	5 (10%)	2 (4%)	6 (12%)
Pigmentation		1 (2%)		
Tension lipidosis	7 (14%)	6 (12%)	7 (14%)	10 (20%)
Vacuolization cytoplasmic	7 (14%)	5 (10%)	6 (12%)	1 (2%)
Centrilobular, hepatocyte, degeneration	1 (2%)			

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

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Alimentary System (continued)				
Mesentery	(20)	(22)	(14)	(19)
Inflammation	2 (10%)	2 (9%)		1 (5%)
Thrombosis	1 (5%)			
Fat, necrosis	12 (60%)	10 (45%)	11 (79%)	15 (79%)
Fat, thrombosis		1 (5%)		
Pancreas	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Infiltration cellular, mononuclear cell	31 (62%)	25 (50%)	24 (48%)	21 (42%)
Inflammation	3 (6%)			4 (8%)
Acinus, atrophy	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Artery, inflammation		1 (2%)		
Duct, cyst	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Infiltration cellular, mononuclear cell	36 (72%)	37 (74%)	38 (76%)	30 (60%)
Inflammation			1 (2%)	1 (2%)
Mineralization		1 (2%)		2 (4%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Infiltration cellular, mononuclear cell			1 (2%)	2 (4%)
Infiltration cellular, diffuse				1 (2%)
Inflammation		5 (10%)	2 (4%)	
Ulcer		3 (6%)	1 (2%)	
Epithelium, hyperkeratosis	1 (2%)	6 (12%)	2 (4%)	1 (2%)
Epithelium, hyperplasia, squamous	2 (4%)	9 (18%)	3 (6%)	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Cyst	15 (30%)	18 (36%)	15 (30%)	14 (28%)
Hyperplasia	3 (6%)	1 (2%)	1 (2%)	4 (8%)
Infiltration cellular, mononuclear cell	1 (2%)	4 (8%)	5 (10%)	2 (4%)
Infiltration cellular, diffuse				1 (2%)
Inflammation		3 (6%)	1 (2%)	1 (2%)
Mineralization			1 (2%)	2 (4%)
Necrosis		1 (2%)		
Ulcer		1 (2%)	1 (2%)	
Tooth	(3)	(0)	(4)	(0)
Dysplasia	3 (100%)		4 (100%)	
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Carotid artery, mineralization		1 (2%)		
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	42 (84%)	33 (66%)	35 (70%)	38 (76%)
Infiltration cellular, mononuclear cell	1 (2%)	3 (6%)	4 (8%)	3 (6%)
Inflammation	3 (6%)	3 (6%)	1 (2%)	2 (4%)
Mineralization	2 (4%)	4 (8%)	2 (4%)	
Thrombosis	3 (6%)	1 (2%)	1 (2%)	
Myocardium, vacuolization cytoplasmic			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis			2 (4%)	1 (2%)
Degeneration, cystic				1 (2%)
Hematopoietic cell proliferation	3 (6%)	4 (8%)	4 (8%)	14 (28%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Endocrine System (continued)				
Adrenal cortex (continued)	(50)	(50)	(50)	(50)
Hyperplasia			1 (2%)	
Hypertrophy	4 (8%)	2 (4%)	5 (10%)	2 (4%)
Subcapsular, hyperplasia	50 (100%)	49 (98%)	49 (98%)	49 (98%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Hypertrophy		1 (2%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	35 (70%)	15 (30%)	12 (24%)	8 (16%)
Parathyroid gland	(45)	(47)	(39)	(38)
Cyst			2 (5%)	1 (3%)
Hyperplasia			2 (5%)	
Pituitary gland	(50)	(49)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)		
Hemorrhage	1 (2%)		1 (2%)	1 (2%)
Pars distalis, degeneration				1 (2%)
Pars distalis, hyperplasia	17 (34%)	18 (37%)	19 (38%)	17 (34%)
Thyroid gland	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	1 (2%)		2 (4%)	2 (4%)
Follicular cell, hyperplasia		1 (2%)		
General Body System				
Peritoneum	(1)	(0)	(0)	(0)
Inflammation	1 (100%)			
Tissue NOS	(1)	(0)	(0)	(0)
Genital System				
Clitoral gland	(49)	(50)	(50)	(49)
Infiltration cellular, mononuclear cell		2 (4%)	2 (4%)	1 (2%)
Inflammation	10 (20%)	10 (20%)	8 (16%)	9 (18%)
Ovary	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)	2 (4%)	
Cyst	7 (14%)	15 (30%)	13 (26%)	14 (28%)
Infiltration cellular, mononuclear cell		5 (10%)	6 (12%)	
Inflammation	2 (4%)		2 (4%)	1 (2%)
Mineralization	1 (2%)			1 (2%)
Necrosis	1 (2%)			
Pigmentation	1 (2%)			
Thrombosis		1 (2%)		
Germinal epithelium, hyperplasia		1 (2%)	1 (2%)	1 (2%)
Oviduct	(1)	(0)	(0)	(0)
Infiltration cellular, mononuclear cell	1 (100%)			
Uterus	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)		1 (2%)	
Inflammation	7 (14%)	6 (12%)	8 (16%)	4 (8%)
Pigmentation		1 (2%)		
Thrombosis	3 (6%)		1 (2%)	1 (2%)
Ulcer		2 (4%)	1 (2%)	
Cervix, hyperplasia, cystic				1 (2%)
Endometrium, hyperplasia, cystic	46 (92%)	46 (92%)	46 (92%)	43 (86%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	10 (20%)	14 (28%)	10 (20%)	31 (62%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Hematopoietic System (continued)				
Lymph node	(19)	(13)	(18)	(29)
Axillary, hyperplasia, lymphoid				8 (28%)
Axillary, inflammation				1 (3%)
Axillary, pigmentation				1 (3%)
Bronchial, hyperplasia, lymphoid	4 (21%)	1 (8%)	5 (28%)	5 (17%)
Inguinal, hyperplasia, lymphoid	4 (21%)	2 (15%)	7 (39%)	17 (59%)
Inguinal, inflammation, granulomatous	1 (5%)			
Lumbar, angiectasis			1 (6%)	
Lumbar, hyperplasia, lymphoid	2 (11%)		1 (6%)	
Lumbar, inflammation	1 (5%)			
Mediastinal, hyperplasia, lymphoid		2 (15%)	1 (6%)	
Mediastinal, infiltration cellular, histiocyte	1 (5%)			
Pancreatic, hyperplasia, lymphoid			2 (11%)	
Renal, angiectasis			1 (6%)	
Renal, hyperplasia, lymphoid	1 (5%)			1 (3%)
Lymph node, mandibular	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid	14 (28%)	19 (38%)	23 (46%)	27 (54%)
Inflammation				1 (2%)
Pigmentation				1 (2%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy			2 (4%)	
Hematopoietic cell proliferation				1 (2%)
Hyperplasia, lymphoid	13 (26%)	12 (24%)	13 (26%)	4 (8%)
Inflammation	2 (4%)	1 (2%)		1 (2%)
Artery, inflammation				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Hematopoietic cell proliferation	35 (70%)	35 (70%)	30 (60%)	34 (68%)
Hyperplasia, lymphoid	8 (16%)	5 (10%)	11 (22%)	7 (14%)
Inflammation	1 (2%)			
Pigmentation	12 (24%)	8 (16%)	18 (36%)	7 (14%)
Thymus	(50)	(50)	(48)	(49)
Angiectasis		1 (2%)		
Atrophy		1 (2%)	1 (2%)	
Hemorrhage	1 (2%)			
Hyperplasia, lymphoid	18 (36%)	21 (42%)	25 (52%)	22 (45%)
Inflammation			1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia	5 (10%)	9 (18%)	3 (6%)	16 (32%)
Inflammation		2 (4%)		1 (2%)
Duct, dilatation		1 (2%)		2 (4%)
Skin	(50)	(50)	(50)	(50)
Abscess		1 (2%)		
Fibrosis	1 (2%)	2 (4%)	1 (2%)	9 (18%)
Hyperkeratosis	1 (2%)	2 (4%)	1 (2%)	9 (18%)
Hyperplasia	1 (2%)	2 (4%)	1 (2%)	9 (18%)
Inflammation	1 (2%)			9 (18%)
Inflammation, granulomatous		1 (2%)		
Pigmentation			1 (2%)	1 (2%)
Ulcer	1 (2%)	1 (2%)	1 (2%)	9 (18%)
Control, hyperplasia				2 (4%)
Control, inflammation	3 (6%)		1 (2%)	2 (4%)
Sebaceous gland, hyperplasia	1 (2%)		1 (2%)	7 (14%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Integumentary System (continued)				
Skin (continued)	(50)	(50)	(50)	(50)
Sebaceous gland, site of application, hyperplasia	1 (2%)	2 (4%)	6 (12%)	34 (68%)
Site of application, cyst				1 (2%)
Site of application, fibrosis	5 (10%)	6 (12%)	31 (62%)	49 (98%)
Site of application, hyperkeratosis	24 (48%)	38 (76%)	49 (98%)	49 (98%)
Site of application, hyperplasia	20 (40%)	31 (62%)	49 (98%)	49 (98%)
Site of application, infiltration cellular, mast cell			1 (2%)	1 (2%)
Site of application, inflammation	12 (24%)	14 (28%)	42 (84%)	48 (96%)
Site of application, pigmentation			35 (70%)	40 (80%)
Site of application, ulcer	2 (4%)		3 (6%)	33 (66%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibro-osseous lesion	8 (16%)	9 (18%)	9 (18%)	2 (4%)
Skeletal muscle	(4)	(1)	(2)	(0)
Inflammation	1 (25%)		1 (50%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Developmental malformation			1 (2%)	
Infiltration cellular, mononuclear cell	1 (2%)	1 (2%)		
Inflammation	1 (2%)			1 (2%)
Cerebrum, vacuolization cytoplasmic			1 (2%)	
Peripheral nerve	(2)	(0)	(0)	(0)
Sciatic, atrophy	1 (50%)			
Sciatic, infiltration cellular, mononuclear cell	1 (50%)			
Spinal cord	(2)	(0)	(0)	(0)
Atrophy	1 (50%)			
Degeneration	1 (50%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Fibrosis				1 (2%)
Infiltration cellular, histiocyte	2 (4%)	6 (12%)	2 (4%)	2 (4%)
Inflammation	3 (6%)	5 (10%)	1 (2%)	2 (4%)
Metaplasia, osseous	2 (4%)			
Pigmentation	1 (2%)		1 (2%)	
Thrombosis	1 (2%)			
Artery, mineralization	1 (2%)			
Bronchiole, hyperplasia		1 (2%)		1 (2%)
Nose	(50)	(50)	(50)	(50)
Inflammation	13 (26%)	9 (18%)	17 (34%)	9 (18%)
Polyp, inflammatory			1 (2%)	
Glands, dilatation	2 (4%)			
Glands, metaplasia	6 (12%)	7 (14%)	3 (6%)	7 (14%)
Olfactory epithelium, hyperplasia			1 (2%)	
Olfactory epithelium, metaplasia		3 (6%)	2 (4%)	2 (4%)
Respiratory epithelium, foreign body			1 (2%)	
Respiratory epithelium, hemorrhage	1 (2%)		1 (2%)	
Respiratory epithelium, hyperplasia	46 (92%)	48 (96%)	46 (92%)	46 (92%)
Turbinate, thrombosis	1 (2%)			

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Pyrogallol

	Vehicle Control	5 mg/kg	20 mg/kg	75 mg/kg
Respiratory System (continued)				
Trachea	(50)	(50)	(50)	(50)
Inflammation			1 (2%)	
Epithelium, hyperplasia			1 (2%)	
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Infiltration cellular, mononuclear cell	1 (2%)	1 (2%)		1 (2%)
Inflammation	2 (4%)		1 (2%)	3 (6%)
Cornea, hyperplasia	1 (2%)			
Retina, angiectasis			1 (2%)	
Retina, degeneration		1 (2%)		
Harderian gland	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Cyst	1 (2%)			
Hyperplasia	3 (6%)	9 (18%)	6 (12%)	5 (10%)
Infiltration cellular, mononuclear cell	29 (58%)	33 (66%)	31 (62%)	24 (48%)
Inflammation	1 (2%)			
Mineralization				1 (2%)
Lacrimal gland	(0)	(0)	(1)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	2 (4%)	3 (6%)	2 (4%)	
Cyst			1 (2%)	
Dilatation		1 (2%)		2 (4%)
Infarct, chronic			1 (2%)	
Infiltration cellular, mononuclear cell	2 (4%)	5 (10%)	4 (8%)	4 (8%)
Inflammation	1 (2%)			3 (6%)
Metaplasia, osseous	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Mineralization	12 (24%)	7 (14%)	8 (16%)	7 (14%)
Nephropathy	33 (66%)	24 (48%)	28 (56%)	28 (56%)
Pigmentation	3 (6%)	1 (2%)	1 (2%)	
Papilla, necrosis				1 (2%)
Renal tubule, degeneration	1 (2%)	1 (2%)	1 (2%)	
Renal tubule, hyperplasia	1 (2%)			
Renal tubule, vacuolization cytoplasmic			1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	34 (68%)	37 (74%)	37 (74%)	42 (84%)
Inflammation	1 (2%)	1 (2%)	1 (2%)	1 (2%)

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

BACTERIAL MUTAGENICITY TEST PROTOCOL

Two independent bacterial mutagenicity assays were conducted with pyrogallol. The first assay was performed at SRI International (Menlo Park, CA) following protocols reported by Zeiger *et al.* (1992) and used *Salmonella typhimurium* tester strains TA98 and TA100, either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver). The second assay was performed at SITEK Research Laboratories (Rockville, MD); this assay used a slightly modified protocol (activation only with rat liver S9) and the same lot of pyrogallol (010326) that was tested in the 3-month and 2-year studies. In the second assay, *Escherichia coli* strain WP2 *uvrA*/pKM101 was used as a bacterial tester strain in addition to *S. typhimurium* strains TA98 and TA100. In both assays, the test article was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX), and incubated with the bacterial tester strains 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.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of pyrogallol. The high dose was limited by toxicity. All nonnegative trials were repeated.

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, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

MOUSE BONE MARROW MICRONUCLEUS TEST PROTOCOL

Preliminary dose range-finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by pyrogallol exposure. The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Male B6C3F1/N mice were injected intraperitoneally (three times at 24-hour intervals) with pyrogallol dissolved in phosphate-buffered saline. Solvent control animals were injected with phosphate-buffered saline only. The positive control animals received injections of cyclophosphamide. The animals were killed 24 hours after the third injection, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained with acridine orange; 2,000 polychromatic erythrocytes (PCEs, or reticulocytes) were scored for the frequency of micronucleated cells in each of five animals per dose group. In addition, the percentage of PCEs among a population of 200 erythrocytes in the bone marrow was scored for each dose group as a measure of toxicity.

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

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month dermal study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs, or mature erythrocytes) in each of five animals per dose group. In addition, the percentage of PCEs among a population of 1,000 erythrocytes in the peripheral blood was scored for each dose group as a measure of bone marrow toxicity.

The results for NCEs were tabulated and analyzed as described for PCEs in the mouse bone marrow micronucleus test protocol. Results of the 3-month study were accepted without repeat tests because additional test data could not be obtained.

EVALUATION PROTOCOL

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

RESULTS

Pyrogallol was tested in two independent bacterial mutation studies; both studies gave positive results in one or more strains of *S. typhimurium* or *E. coli.* In the first study (concentration range 3 to 333 μg/plate), positive results were seen in *S. typhimurium* strain TA100 with and without 30% S9 derived from hamster or rat liver; negative results were obtained in strain TA98 tested under the same conditions (Table E1). In the second study, which was conducted with the same lot of pyrogallol that was used in the 3-month and 2-year dermal studies, positive results were obtained over a concentration range of 10 to 1,000 μg/plate in *S. typhimurium* strains TA98 and TA100 and in *E. coli* strain WP2 *uvrA*/pKM101 in the absence of S9 (Table E2). With 10% rat liver S9, this sample of pyrogallol was mutagenic in the *E. coli* strain but gave equivocal responses in *S. typhimurium* strains TA98 and TA100 based on small increases in revertants that were not well correlated with dose. Thus, the results of studies in bacteria show that pyrogallol is a direct-acting mutagen.

In vivo, no significant increases in the frequencies of micronucleated polychromatic erythrocytes (reticulocytes) were observed in bone marrow of male B6C3F1/N mice injected intraperitoneally with pyrogallol (39 to 156 mg/kg) once daily for 3 days (Table E3). In a second *in vivo* test, no significant increases in the frequencies of micronucleated erythrocytes, an indicator of chromosomal damage, were observed in peripheral blood of female B6C3F1/N mice treated with pyrogallol (38 to 600 mg/kg) via dermal application for 3 months (Table E4). In male mice, however, results of this assay were judged to be equivocal, based on a significant increase in micronucleated erythrocytes observed at a single dose level (300 mg/kg) at the end of the 3-month study period. No significant alteration in the percentage of PCEs in bone marrow or blood was observed in either study, suggesting that pyrogallol did not induce bone marrow toxicity over the dose ranges tested.

TABLE E1
Mutagenicity of Pyrogallol in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Without S9	Without S9	With 30% hamster S9	With 30% hamster S9	With 30% rat S9	With 30% rat S9
TA100							
	0	124 ± 5	116 ± 7	130 ± 8	109 ± 3	117 ± 4	126 ± 10
	3	111 ± 3	109 ± 5	143 ± 12	108 ± 9	134 ± 3	134 ± 8
	10	156 ± 6	143 ± 8	167 ± 15	155 ± 11	174 ± 10	214 ± 15
	33	184 ± 9	250 ± 7	263 ± 21	342 ± 22	220 ± 7	339 ± 39
	100	284 ± 7	273 ± 20	340 ± 15	438 ± 7	340 ± 28	403 ± 5
	333	206 ± 5[b]	144 ± 19[b]	422 ± 30	461 ± 17	426 ± 17	439 ± 9
Trial summary		Positive	Positive	Positive	Positive	Positive	Positive
Positive control[c]		925 ± 30	845 ± 11	703 ± 6	656 ± 11	642 ± 26	616 ± 4
TA98							
	0	17 ± 3		32 ± 3		19 ± 2	
	3	21 ± 2		30 ± 1		27 ± 5	
	10	24 ± 4		30 ± 2		26 ± 3	
	33	26 ± 7		31 ± 7		31 ± 3	
	100	27 ± 4		37 ± 1		39 ± 3	
	333	5 ± 1[b]		24 ± 2		29 ± 2	
Trial summary		Negative		Negative		Negative	
Positive control		439 ± 18		446 ± 19		411 ± 32	

[a] Study was performed at SRI International. Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol is presented by Zeiger *et al.* (1992). 0 μg/plate was the solvent control.

[b] Slight toxicity

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

TABLE E2
Mutagenicity of Pyrogallol in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100					
	0	73 ± 3	75 ± 1	81 ± 4	83 ± 5
	10	76 ± 6			158 ± 15
	50	98 ± 6	86 ± 3	152 ± 6	142 ± 13
	100	145 ± 20	110 ± 6	138 ± 3	133 ± 15
	250	269 ± 9	243 ± 17	106 ± 4	103 ± 10
	500	260 ± 11[b]	264 ± 4[b]	93 ± 3[b]	95 ± 6[b]
	750		148 ± 6[b]	56 ± 3[b]	
	1,000	38 ± 7[b]	54 ± 7[c]	41 ± 1[b]	31 ± 3[b]
Trial summary		Positive	Positive	Equivocal	Equivocal
Positive control[d]		488 ± 27	635 ± 35	878 ± 19	982 ± 37
TA98					
	0	23 ± 1	23 ± 1	28 ± 1	24 ± 2
	10	35 ± 2		40 ± 2	
	50	38 ± 3	28 ± 2	35 ± 1	44 ± 3
	100	44 ± 1	38 ± 4	39 ± 2	44 ± 6
	250	40 ± 5	45 ± 1	34 ± 1	35 ± 6
	500	22 ± 3[b]	22 ± 1[b]	19 ± 1[b]	32 ± 2[b]
	750		13 ± 1[b]		23 ± 3[b]
	1,000	6 ± 1[b]	6 ± 1[c]	11 ± 0[b]	12 ± 1[b]
Trial summary		Positive	Positive	Equivocal	Equivocal
Positive control		693 ± 26	445 ± 13	1,163 ± 77	742 ± 37
***Escherichia coli* WP2 *uvrA*/pKM101**					
	0	215 ± 8	203 ± 12	246 ± 17	245 ± 12
	10		233 ± 12		375 ± 17
	50	290 ± 9	278 ± 8	515 ± 21	469 ± 19
	100	373 ± 19	376 ± 22	645 ± 28	487 ± 37
	250	455 ± 3	390 ± 4	543 ± 29	480 ± 18
	500	374 ± 8[b]	334 ± 26[b]	454 ± 8[b]	404 ± 12[b]
	750	235 ± 10[b]		241 ± 7[b]	
	1,000	124 ± 4[b]	170 ± 32[b]	215 ± 9[b]	213 ± 7[b]
Trial summary		Positive	Positive	Positive	Positive
Positive control		1,816 ± 74	1,955 ± 18	993 ± 79	1,164 ± 25

[a] Study was performed at SITEK Research Laboratories using the same lot (010326) that was used in the 3-month and 2-year studies. The study used a slight modification of the protocol presented by Zeiger *et al.* (1992). Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.

[b] Precipitate

[c] Slight toxicity and precipitate

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

TABLE E3
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male B6C3F1/N Mice Administered Pyrogallol by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	PCEs[b] (%)
Phosphate-buffered saline[d]	0	5	0.70 ± 0.12		60.7 ± 1.06
Pyrogallol	39	5	1.00 ± 0.32	0.2333	59.5 ± 0.79
	78	5	0.90 ± 0.33	0.3085	60.0 ± 1.17
	156	5	0.80 ± 0.20	0.3981	57.9 ± 1.29
			P=0.477[e]		
Cyclophosphamide[f]	20	5	19.10 ± 1.00	0.0000	47.4 ± 0.29

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by Shelby *et al.* (1993). PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison of each dosed group with the solvent control group is significant at P≤0.008; positive control value is significant at P≤0.05.
[d] Solvent control
[e] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test; significant at P≤0.025
[f] Positive control

TABLE E4
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Dermal Administration of Pyrogallol for 3 Months[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
95% Ethanol[d]	0	5	1.30 ± 0.34		3.62 ± 0.28
Pyrogallol	38	5	2.40 ± 0.83	0.0351	3.72 ± 0.14
	75	5	2.80 ± 0.64	0.0095	3.76 ± 0.36
	150	5	1.80 ± 0.25	0.1844	3.32 ± 0.24
	300	5	3.00 ± 0.42	0.0047	3.94 ± 0.16
	600	5	2.30 ± 0.34	0.0476	4.10 ± 0.27
			P=0.185[e]		
Female					
95% Ethanol	0	5	1.90 ± 0.29		3.84 ± 0.42
Pyrogallol	38	5	1.60 ± 0.10	0.6941	3.90 ± 0.50
	75	5	1.60 ± 0.48	0.6941	3.94 ± 0.32
	150	5	2.50 ± 0.52	0.1826	4.66 ± 0.20
	300	5	2.00 ± 0.42	0.4363	4.04 ± 0.37
	600	5	2.40 ± 0.29	0.2226	4.34 ± 0.21
			P=0.115		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison of each dosed group with the vehicle control group is significant at P≤0.005.
[d] Vehicle control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at P≤0.025

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1
Hematology, Clinical Chemistry, and Thyroid Hormone Data for Rats in the 3-Month Dermal Study of Pyrogallol[a]

	Vehicle Control	9.5 mg/kg	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg
Male						
Hematology						
n						
Day 4	10	10	10	10	9	9
Day 23	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 4	47.8 ± 0.8	45.5 ± 0.7	45.6 ± 0.6	46.8 ± 0.4	46.0 ± 0.7	49.0 ± 1.1
Day 23	50.8 ± 0.4	50.8 ± 0.5	50.7 ± 0.4	50.3 ± 0.4	50.1 ± 0.8	50.9 ± 0.3
Week 14	47.8 ± 0.4	47.7 ± 0.5	46.7 ± 0.5	48.3 ± 0.3	48.1 ± 0.5	48.7 ± 0.5
Hemoglobin (g/dL)						
Day 4	13.9 ± 0.2	13.4 ± 0.2	13.4 ± 0.2	13.6 ± 0.1	13.5 ± 0.2	14.2 ± 0.3
Day 23	15.1 ± 0.1	15.1 ± 0.1	15.1 ± 0.1	15.0 ± 0.1	14.9 ± 0.2	15.1 ± 0.1
Week 14	15.0 ± 0.1	14.8 ± 0.1	14.5 ± 0.1*	15.0 ± 0.1	14.9 ± 0.1	15.1 ± 0.1
Erythrocytes (10^6/μL)						
Day 4	7.50 ± 0.10	7.18 ± 0.12	7.20 ± 0.09	7.30 ± 0.07	7.22 ± 0.13	7.60 ± 0.13
Day 23	8.18 ± 0.06	8.21 ± 0.09	8.23 ± 0.08	8.11 ± 0.06	8.02 ± 0.14	8.19 ± 0.09
Week 14	9.02 ± 0.07	8.94 ± 0.09	8.77 ± 0.08	9.04 ± 0.07	8.95 ± 0.07	9.13 ± 0.07
Reticulocytes (10^6/μL)						
Day 4	544.1 ± 33.2	510.0 ± 29.2	531.0 ± 33.5	545.1 ± 19.0	545.3 ± 25.6	548.7 ± 47.1
Day 23	265.9 ± 10.8	250.1 ± 10.9	258.1 ± 17.5	256.3 ± 8.2	266.7 ± 6.1	270.7 ± 9.4
Week 14	177.8 ± 4.9	181.9 ± 7.1	184.9 ± 7.2	170.0 ± 5.3	187.8 ± 3.0	180.0 ± 6.3
Mean cell volume (fL)						
Day 4	63.8 ± 0.4	63.3 ± 0.4	63.4 ± 0.3	64.2 ± 0.2	63.7 ± 0.5	64.5 ± 0.5
Day 23	62.2 ± 0.3	61.9 ± 0.3	61.6 ± 0.3	62.1 ± 0.2	62.4 ± 0.3	62.3 ± 0.5
Week 14	53.0 ± 0.2	53.3 ± 0.1	53.3 ± 0.2	53.4 ± 0.3	53.7 ± 0.3	53.4 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	18.5 ± 0.1	18.6 ± 0.1	18.6 ± 0.1	18.6 ± 0.1	18.6 ± 0.1	18.7 ± 0.1
Day 23	18.5 ± 0.1	18.4 ± 0.1	18.4 ± 0.1	18.5 ± 0.1	18.6 ± 0.2	18.5 ± 0.1
Week 14	16.6 ± 0.1	16.6 ± 0.1	16.6 ± 0.1	16.5 ± 0.1	16.6 ± 0.0	16.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	29.0 ± 0.1	29.4 ± 0.2	29.4 ± 0.1	29.0 ± 0.1	29.3 ± 0.1	29.1 ± 0.2
Day 23	29.7 ± 0.1	29.7 ± 0.1	29.8 ± 0.1	29.8 ± 0.2	29.8 ± 0.3	29.7 ± 0.1
Week 14	31.3 ± 0.3	31.1 ± 0.1	31.1 ± 0.2	31.0 ± 0.2	30.9 ± 0.2	31.0 ± 0.2
Platelets (10^3/μL)						
Day 4	1,106.9 ± 34.6	1,086.8 ± 36.8	1,158.7 ± 24.0	1,189.3 ± 42.0	1,090.1 ± 47.7	1,122.2 ± 20.7
Day 23	918.4 ± 29.0	920.5 ± 18.4	924.1 ± 23.9	965.9 ± 19.7	951.2 ± 24.2	956.0 ± 23.6
Week 14	673.9 ± 14.8	670.5 ± 13.1	673.8 ± 14.4	643.9 ± 14.2	657.1 ± 17.9	638.4 ± 15.1
Leukocytes (10^3/μL)						
Day 4	8.73 ± 0.27	8.82 ± 0.32	8.68 ± 0.27	8.73 ± 0.20	8.38 ± 0.34	8.71 ± 0.23
Day 23	10.20 ± 0.28	10.92 ± 0.29	11.07 ± 0.47	10.59 ± 0.20	10.19 ± 0.31	10.14 ± 0.47
Week 14	8.77 ± 0.56	9.95 ± 0.52	8.95 ± 0.41	8.35 ± 0.59	8.95 ± 0.65	8.27 ± 0.63
Segmented neutrophils (10^3/μL)						
Day 4	0.92 ± 0.04	0.95 ± 0.06	0.92 ± 0.03	0.89 ± 0.02	0.96 ± 0.06	0.88 ± 0.05
Day 23	0.94 ± 0.05	0.99 ± 0.07	0.99 ± 0.04	1.02 ± 0.05	1.02 ± 0.04	1.15 ± 0.13
Week 14	1.38 ± 0.09	1.45 ± 0.11	1.24 ± 0.05	1.23 ± 0.06	1.52 ± 0.12	1.38 ± 0.13
Lymphocytes (10^3/μL)						
Day 4	7.48 ± 0.25	7.54 ± 0.28	7.41 ± 0.24	7.53 ± 0.19	7.12 ± 0.29	7.50 ± 0.20
Day 23	8.94 ± 0.28	9.58 ± 0.27	9.74 ± 0.43	9.28 ± 0.22	8.85 ± 0.29	8.68 ± 0.47
Week 14	7.02 ± 0.49	8.12 ± 0.45	7.37 ± 0.37	6.77 ± 0.52	7.12 ± 0.55	6.58 ± 0.52
Monocytes (10^3/μL)						
Day 4	0.26 ± 0.02	0.26 ± 0.02	0.27 ± 0.01	0.24 ± 0.02	0.23 ± 0.03	0.25 ± 0.02
Day 23	0.21 ± 0.01	0.23 ± 0.02	0.23 ± 0.02	0.20 ± 0.02	0.19 ± 0.01	0.20 ± 0.02
Week 14	0.23 ± 0.02	0.26 ± 0.02	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.03	0.20 ± 0.03

TABLE F1
Hematology, Clinical Chemistry, and Thyroid Hormone Data for Rats in the 3-Month Dermal Study of Pyrogallol

	Vehicle Control	9.5 mg/kg	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 4	10	10	10	10	9	9
Day 23	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Basophils (10^3/μL)						
Day 4	0.039 ± 0.006	0.034 ± 0.006	0.037 ± 0.005	0.040 ± 0.003	0.030 ± 0.005	0.042 ± 0.006
Day 23	0.047 ± 0.003	0.043 ± 0.003	0.057 ± 0.005	0.049 ± 0.004	0.047 ± 0.004	0.047 ± 0.005
Week 14	0.046 ± 0.007	0.039 ± 0.005	0.038 ± 0.004	0.032 ± 0.005	0.037 ± 0.006	0.034 ± 0.005
Eosinophils (10^3/μL)						
Day 4	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.01	0.03 ± 0.00
Day 23	0.06 ± 0.02	0.08 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.08 ± 0.02	0.06 ± 0.01
Week 14	0.09 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.10 ± 0.02	0.07 ± 0.01	0.07 ± 0.01
Clinical Chemistry and Thyroid Hormones						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	13.0 ± 0.7	13.6 ± 0.4	14.1 ± 0.6	13.5 ± 0.6	12.7 ± 0.6	13.7 ± 0.5
Day 23	15.7 ± 0.5	16.7 ± 0.4	15.6 ± 0.4	15.6 ± 0.3	15.0 ± 0.7	16.1 ± 1.8
Week 14	14.6 ± 0.4	15.1 ± 0.4	14.7 ± 0.4	15.1 ± 0.4	13.9 ± 0.4	14.6 ± 0.5
Creatinine (mg/dL)						
Day 4	0.41 ± 0.01	0.40 ± 0.00	0.40 ± 0.00	0.40 ± 0.00	0.41 ± 0.01	0.41 ± 0.01
Day 23	0.47 ± 0.02	0.48 ± 0.01	0.47 ± 0.02	0.48 ± 0.01	0.47 ± 0.02	0.51 ± 0.01
Week 14	0.60 ± 0.02	0.62 ± 0.01	0.64 ± 0.02	0.62 ± 0.03	0.60 ± 0.02	0.59 ± 0.02
Glucose (mg/dL)						
Week 14	144 ± 2	139 ± 2	146 ± 7	150 ± 6	152 ± 7	143 ± 3
Total protein (g/dL)						
Day 4	5.8 ± 0.1	5.7 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.7 ± 0.1	5.9 ± 0.1
Day 23	6.6 ± 0.0	6.6 ± 0.1	6.7 ± 0.1	6.6 ± 0.0	6.5 ± 0.0	6.5 ± 0.1
Week 14	7.1 ± 0.1	6.9 ± 0.1	7.0 ± 0.1	7.1 ± 0.1	7.0 ± 0.0	7.1 ± 0.1
Albumin (g/dL)						
Day 4	4.1 ± 0.0	4.1 ± 0.0	4.1 ± 0.0	4.2 ± 0.0	4.1 ± 0.0	4.2 ± 0.0
Day 23	4.4 ± 0.0	4.5 ± 0.1	4.5 ± 0.0	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.0
Week 14	4.6 ± 0.0	4.5 ± 0.0	4.5 ± 0.0	4.6 ± 0.0	4.5 ± 0.0	4.6 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	52 ± 2	51 ± 2	51 ± 1	51 ± 1	51 ± 1	52 ± 2
Day 23	45 ± 1	47 ± 1	48 ± 2	51 ± 1*	48 ± 2	49 ± 1
Week 14	67 ± 2	68 ± 4	68 ± 2	76 ± 7	56 ± 3*	64 ± 3
Alkaline phosphatase (IU/L)						
Day 4	618 ± 18	582 ± 12	579 ± 13	598 ± 7	591 ± 12	613 ± 22
Day 23	439 ± 11	421 ± 10	431 ± 13	442 ± 9	439 ± 10	441 ± 16
Week 14	176 ± 5	181 ± 4	188 ± 5	184 ± 5	181 ± 5	185 ± 6
Creatine kinase (IU/L)						
Day 4	252 ± 32	256 ± 34	247 ± 18	297 ± 29	264 ± 32	220 ± 17
Day 23	240 ± 34	241 ± 29	230 ± 29	209 ± 34	205 ± 23	324 ± 72
Week 14	83 ± 9	95 ± 10	104 ± 17	88 ± 18	105 ± 24	84 ± 8
Sorbitol dehydrogenase (IU/L)						
Day 4	11 ± 1	10 ± 0	11 ± 0	11 ± 0	10 ± 1	11 ± 0
Day 23	18 ± 1	19 ± 0	18 ± 1	19 ± 0	18 ± 1	19 ± 1
Week 14	19 ± 1	20 ± 1	19 ± 1	19 ± 1	17 ± 1	19 ± 1

TABLE F1
Hematology, Clinical Chemistry, and Thyroid Hormone Data for Rats in the 3-Month Dermal Study of Pyrogallol

	Vehicle Control	9.5 mg/kg	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg
Male (continued)						
Clinical Chemistry and Thyroid Hormones (continued)						
n	10	10	10	10	10	10
Bile acids (μmol/L)						
Day 4	6.4 ± 1.0	7.7 ± 0.9	7.3 ± 0.8	7.9 ± 1.1	10.0 ± 2.3	7.6 ± 1.2
Day 23	8.7 ± 0.9	8.4 ± 1.2	9.6 ± 1.1	7.9 ± 1.2	8.0 ± 1.3	7.3 ± 1.2
Week 14	4.2 ± 0.9	3.4 ± 0.8	5.6 ± 1.5	6.7 ± 1.4	3.3 ± 0.5	4.3 ± 0.9
Thyroid stimulating hormone (TSH) (ng/mL)						
Day 23	9.84 ± 0.70[b]	10.38 ± 0.64[c]	10.29 ± 0.72[b]	10.65 ± 0.82[b]	12.71 ± 0.70[d]	9.85 ± 0.63[e]
Week 14	8.37 ± 0.42	8.94 ± 0.40	9.85 ± 0.49	8.90 ± 0.40	9.05 ± 0.50	8.33 ± 0.35
Total triiodothyronine (T_3) (ng/dL)						
Day 23	151.6 ± 6.3	160.0 ± 7.5	159.8 ± 7.2	162.5 ± 8.2	144.8 ± 6.7	155.3 ± 8.0
Week 14	187.7 ± 8.6	192.9 ± 6.0	192.7 ± 6.2	189.7 ± 5.3	186.4 ± 6.4	172.3 ± 8.0
Total thyroxine (T_4) (μg/dL)						
Day 23	7.380 ± 0.210	7.420 ± 0.172	7.660 ± 0.279	7.460 ± 0.125	7.240 ± 0.142	7.120 ± 0.245
Week 14	6.540 ± 0.238	6.340 ± 0.212	6.050 ± 0.152	6.320 ± 0.142	6.060 ± 0.300	5.970 ± 0.193
Female						
Hematology						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	9	10	10	9	10	10
Hematocrit (%)						
Day 4	49.6 ± 0.9	48.8 ± 1.1	47.4 ± 0.4	48.1 ± 0.8	47.6 ± 0.5	48.4 ± 1.4
Day 23	48.4 ± 0.3	48.4 ± 0.5	48.3 ± 0.6	48.5 ± 0.5	48.8 ± 0.4	49.4 ± 0.7
Week 14	46.6 ± 0.3	46.7 ± 0.5	46.7 ± 0.5	46.9 ± 0.3	46.1 ± 0.4	45.9 ± 0.4
Hemoglobin (g/dL)						
Day 4	14.9 ± 0.2	14.6 ± 0.3	14.1 ± 0.2	14.4 ± 0.2	14.3 ± 0.1	14.6 ± 0.4
Day 23	15.2 ± 0.1	15.2 ± 0.2	15.1 ± 0.1	15.2 ± 0.1	15.3 ± 0.1	15.4 ± 0.2
Week 14	14.5 ± 0.1	14.7 ± 0.1	14.6 ± 0.1	14.6 ± 0.1	14.4 ± 0.1	14.3 ± 0.1
Erythrocytes (10^6/μL)						
Day 4	8.08 ± 0.14	7.85 ± 0.17	7.61 ± 0.09*	7.80 ± 0.12	7.70 ± 0.06	7.83 ± 0.19
Day 23	8.19 ± 0.06	8.15 ± 0.08	8.13 ± 0.09	8.23 ± 0.06	8.21 ± 0.09	8.27 ± 0.13
Week 14	8.16 ± 0.04	8.20 ± 0.07	8.20 ± 0.08	8.21 ± 0.05	8.08 ± 0.05	8.03 ± 0.08
Reticulocytes (10^6/μL)						
Day 4	303.8 ± 16.1	359.9 ± 16.6	376.6 ± 28.7	381.1 ± 28.5	341.4 ± 33.6	344.8 ± 28.6
Day 23	156.4 ± 5.6	164.1 ± 5.7	153.2 ± 6.9	157.9 ± 4.6	158.3 ± 7.7	163.9 ± 6.5
Week 14	171.0 ± 7.2	168.1 ± 6.9	163.2 ± 4.5	185.4 ± 6.4	180.9 ± 8.3	174.5 ± 7.0
Mean cell volume (fL)						
Day 4	61.3 ± 0.2	62.2 ± 0.3	62.3 ± 0.3	61.7 ± 0.4	61.9 ± 0.4	61.7 ± 0.6
Day 23	59.1 ± 0.2	59.4 ± 0.3	59.4 ± 0.3	58.9 ± 0.4	59.5 ± 0.2	59.7 ± 0.2
Week 14	57.0 ± 0.1	56.9 ± 0.2	57.0 ± 0.2	57.1 ± 0.2	57.0 ± 0.2	57.1 ± 0.1
Mean cell hemoglobin (pg)						
Day 4	18.4 ± 0.1	18.6 ± 0.1	18.6 ± 0.1	18.5 ± 0.1	18.6 ± 0.1	18.6 ± 0.1
Day 23	18.5 ± 0.1	18.6 ± 0.0	18.6 ± 0.1	18.5 ± 0.1	18.6 ± 0.1	18.6 ± 0.1
Week 14	17.8 ± 0.0	17.9 ± 0.1	17.9 ± 0.1	17.8 ± 0.0	17.9 ± 0.1	17.9 ± 0.1

TABLE F1
Hematology, Clinical Chemistry, and Thyroid Hormone Data for Rats in the 3-Month Dermal Study of Pyrogallol

	Vehicle Control	9.5 mg/kg	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	9	10	10	9	10	10
Mean cell hemoglobin concentration (g/dL)						
Day 4	30.0 ± 0.1	30.0 ± 0.2	29.9 ± 0.1	30.0 ± 0.2	30.1 ± 0.2	30.1 ± 0.2
Day 23	31.3 ± 0.2	31.3 ± 0.1	31.4 ± 0.1	31.4 ± 0.2	31.2 ± 0.1	31.2 ± 0.1
Week 14	31.2 ± 0.1	31.4 ± 0.1	31.3 ± 0.1	31.1 ± 0.1	31.4 ± 0.1	31.3 ± 0.1
Platelets (10^3/μL)						
Day 4	945.5 ± 30.9	1,050.6 ± 28.9	1,039.9 ± 35.5	1,000.7 ± 35.2	1,065.0 ± 27.2	1,056.1 ± 33.6
Day 23	872.8 ± 20.6	905.2 ± 30.1	897.4 ± 21.4	814.0 ± 41.9	883.8 ± 18.3	868.0 ± 36.6
Week 14	646.8 ± 14.2	656.8 ± 22.7	647.8 ± 23.7	631.4 ± 32.1	611.3 ± 52.3	684.0 ± 29.5
Leukocytes (10^3/μL)						
Day 4	10.88 ± 0.51	10.89 ± 0.40	9.95 ± 0.26	10.28 ± 0.40	10.26 ± 0.26	10.03 ± 0.30
Day 23	8.53 ± 0.33	8.80 ± 0.38	9.19 ± 0.35	10.06 ± 0.34*	9.19 ± 0.58	9.54 ± 0.37
Week 14	6.16 ± 0.63	5.83 ± 0.55	5.03 ± 0.40	5.64 ± 0.56	5.33 ± 0.49	5.80 ± 0.43
Segmented neutrophils (10^3/μL)						
Day 4	0.97 ± 0.05	1.00 ± 0.08	0.92 ± 0.06	0.95 ± 0.06	1.00 ± 0.06	0.86 ± 0.05
Day 23	0.81 ± 0.06	0.95 ± 0.07	0.91 ± 0.06	1.05 ± 0.10	0.93 ± 0.05	0.97 ± 0.06
Week 14	0.82 ± 0.08	1.02 ± 0.17	0.87 ± 0.11	1.06 ± 0.11	0.96 ± 0.11	1.12 ± 0.10
Lymphocytes (10^3/μL)						
Day 4	9.48 ± 0.44	9.48 ± 0.33	8.68 ± 0.27	8.96 ± 0.37	8.87 ± 0.23	8.82 ± 0.25
Day 23	7.45 ± 0.27	7.55 ± 0.38	8.02 ± 0.33	8.68 ± 0.30*	7.99 ± 0.52	8.25 ± 0.32
Week 14	5.09 ± 0.53	4.53 ± 0.41	3.93 ± 0.27	4.33 ± 0.41	4.12 ± 0.36	4.43 ± 0.34
Monocytes (10^3/μL)						
Day 4	0.30 ± 0.03	0.30 ± 0.01	0.26 ± 0.02	0.28 ± 0.02	0.29 ± 0.02	0.23 ± 0.02
Day 23	0.17 ± 0.02	0.20 ± 0.02	0.17 ± 0.01	0.20 ± 0.02	0.17 ± 0.02	0.21 ± 0.02
Week 14	0.14 ± 0.02	0.18 ± 0.03	0.14 ± 0.03	0.16 ± 0.03	0.15 ± 0.02	0.17 ± 0.03
Basophils (10^3/μL)						
Day 4	0.065 ± 0.012	0.057 ± 0.006	0.056 ± 0.011	0.047 ± 0.008	0.063 ± 0.008	0.063 ± 0.012
Day 23	0.042 ± 0.006	0.044 ± 0.007	0.039 ± 0.003	0.044 ± 0.003	0.044 ± 0.007	0.051 ± 0.006
Week 14	0.040 ± 0.009	0.036 ± 0.009	0.020 ± 0.007*	0.029 ± 0.006	0.038 ± 0.008	0.034 ± 0.007
Eosinophils (10^3/μL)						
Day 4	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.05 ± 0.01
Day 23	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.05 ± 0.00
Week 14	0.06 ± 0.01	0.06 ± 0.01	0.08 ± 0.03	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Clinical Chemistry and Thyroid Hormones						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	9	10	9	9	10	10
Urea nitrogen (mg/dL)						
Day 4	14.5 ± 0.4	14.4 ± 0.3	13.9 ± 0.6	14.2 ± 0.5	15.4 ± 0.3	14.2 ± 0.4
Day 23	16.6 ± 0.6	17.0 ± 0.4	17.3 ± 0.4	16.5 ± 0.5	15.9 ± 0.5	16.6 ± 0.4
Week 14	15.0 ± 0.5	16.3 ± 0.8	17.0 ± 0.8	15.2 ± 0.6	15.7 ± 0.9	15.5 ± 0.5
Creatinine (mg/dL)						
Day 4	0.40 ± 0.00	0.40 ± 0.00	0.39 ± 0.02	0.40 ± 0.00	0.39 ± 0.01	0.42 ± 0.01
Day 23	0.47 ± 0.02	0.45 ± 0.02	0.48 ± 0.01	0.45 ± 0.02	0.43 ± 0.02	0.46 ± 0.02
Week 14	0.52 ± 0.02	0.54 ± 0.02	0.56 ± 0.02	0.56 ± 0.02	0.53 ± 0.02	0.56 ± 0.02

TABLE F1
Hematology, Clinical Chemistry, and Thyroid Hormone Data for Rats in the 3-Month Dermal Study of Pyrogallol

	Vehicle Control	9.5 mg/kg	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg
Female (continued)						
Clinical Chemistry and Thyroid Hormones (continued)						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	9	10	9	9	10	10
Glucose (mg/dL)						
Week 14	139 ± 2	141 ± 3	148 ± 5	139 ± 4	139 ± 3	136 ± 3
Total protein (g/dL)						
Day 4	5.9 ± 0.1	6.0 ± 0.1	5.8 ± 0.0	5.8 ± 0.1	5.8 ± 0.1	5.9 ± 0.1
Day 23	6.2 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.3 ± 0.1
Week 14	6.5 ± 0.1	6.7 ± 0.0	6.6 ± 0.1	6.4 ± 0.1	6.6 ± 0.1	6.6 ± 0.1
Albumin (g/dL)						
Day 4	4.2 ± 0.1	4.2 ± 0.0	4.2 ± 0.0	4.1 ± 0.0	4.2 ± 0.0	4.2 ± 0.1
Day 23	4.3 ± 0.0	4.4 ± 0.0	4.3 ± 0.1	4.3 ± 0.0	4.3 ± 0.0	4.4 ± 0.0
Week 14	4.5 ± 0.1	4.6 ± 0.0	4.6 ± 0.1	4.4 ± 0.0	4.6 ± 0.1	4.6 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	42 ± 2	44 ± 2	41 ± 1	43 ± 2	42 ± 1	44 ± 2
Day 23	34 ± 1	35 ± 1	33 ± 1	34 ± 1	34 ± 1	37 ± 1
Week 14	48 ± 3	62 ± 3*	59 ± 5	55 ± 1	57 ± 5	55 ± 3
Alkaline phosphatase (IU/L)						
Day 4	473 ± 14	496 ± 16	478 ± 11	480 ± 19	465 ± 14	486 ± 21
Day 23	312 ± 8	319 ± 8	313 ± 8	323 ± 10	322 ± 10	332 ± 10
Week 14	159 ± 6	151 ± 4	159 ± 6	158 ± 6	162 ± 4	154 ± 5
Creatine kinase (IU/L)						
Day 4	256 ± 39	268 ± 31	216 ± 12	289 ± 39	232 ± 23	263 ± 32
Day 23	185 ± 40	187 ± 27	143 ± 12	231 ± 29	138 ± 26	163 ± 30
Week 14	83 ± 16	110 ± 15	76 ± 8	96 ± 14	121 ± 27	96 ± 13
Sorbitol dehydrogenase (IU/L)						
Day 4	10 ± 0	12 ± 1	10 ± 0	11 ± 1	11 ± 1	11 ± 1.
Day 23	12 ± 1	12 ± 0	11 ± 1	13 ± 1	12 ± 1	11 ± 0
Week 14	15 ± 0	18 ± 1	17 ± 1	18 ± 2	16 ± 1	16 ± 1
Bile acids (µmol/L)						
Day 4	6.8 ± 1.1	5.9 ± 0.8	6.1 ± 0.8	5.8 ± 0.5	5.1 ± 0.5	6.6 ± 0.8
Day 23	8.2 ± 1.5	7.1 ± 1.4	6.6 ± 0.9	10.8 ± 1.7	5.3 ± 0.5	6.8 ± 1.8
Week 14	8.1 ± 1.2	10.5 ± 1.7	6.7 ± 0.9	6.9 ± 1.2	7.7 ± 0.8	6.0 ± 0.7
Thyroid stimulating hormone (TSH) (ng/mL)						
Day 23	6.31 ± 0.13[b]	6.47 ± 0.16[c]	7.10 ± 0.37[c]	6.28 ± 0.47[f]	6.97 ± 0.36[g]	7.03 ± 0.12[b]
Week 14	8.88 ± 0.34	7.90 ± 0.46	8.31± 0.46	8.06 ± 0.47	8.62 ± 0.35	9.16 ± 0.49
Total triiodothyronine (T_3) (ng/dL)						
Day 23	132.1 ± 2.7	132.2 ± 3.8	135.7 ± 5.2	141.4 ± 4.8	132.8 ± 5.4	127.3 ± 3.8
Week 14	143.8 ± 7.6	157.0 ± 10.5	138.7 ± 9.2	136.8 ± 7.2	149.1 ± 7.3	132.3 ± 10.5
Total thyroxine (T_4) (µg/dL)						
Day 23	4.710 ± 0.259	4.830 ± 0.216	4.870 ± 0.230	5.200 ± 0.219	4.890 ± 0.337	4.680 ± 0.110
Week 14	3.978 ± 0.416	3.660 ± 0.339	3.333 ± 0.317	3.711 ± 0.233	3.930 ± 0.420	3.900 ± 0.297

* Significantly different ($P<0.05$) from the vehicle control group by Dunn's or Shirley's test.
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=6
[c] n=7
[d] n=5
[e] n=4
[f] n=3
[g] n=8

TABLE F2
Hematology Data for Mice in the 3-Month Dermal Study of Pyrogallol[a]

	Vehicle Control	38 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
Male						
n	10	10	10	10	9	10
Hematocrit (%)	45.4 ± 0.6	45.9 ± 0.4	46.3 ± 0.6	45.2 ± 0.6	45.0 ± 0.6	44.5 ± 0.4
Hemoglobin (g/dL)	15.3 ± 0.2	15.5 ± 0.1	15.6 ± 0.2	15.2 ± 0.2	15.0 ± 0.2	14.8 ± 0.2
Erythrocytes (10^6/µL)	10.24 ± 0.15	10.46 ± 0.07	10.47 ± 0.11	10.13 ± 0.14	10.19 ± 0.14	9.96 ± 0.10
Reticulocytes (10^6/µL)	273.2 ± 4.9	266.9 ± 5.8	270.4 ± 6.4	285.1 ± 6.5	300.8 ± 5.4**	351.1 ± 18.4**
Mean cell volume (fL)	44.3 ± 0.2	43.8 ± 0.3	44.3 ± 0.2	44.6 ± 0.2	44.1 ± 0.2	44.7 ± 0.3
Mean cell hemoglobin (pg)	14.9 ± 0.1	14.8 ± 0.1	14.9 ± 0.1	15.0 ± 0.1	14.8 ± 0.0	14.9 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.7 ± 0.1	33.8 ± 0.2	33.6 ± 0.2	33.6 ± 0.2	33.4 ± 0.2	33.3 ± 0.2
Platelets (10^3/µL)	1,032.3 ± 56.0	959.0 ± 49.1	1,021.5 ± 44.3	1,118.6 ± 37.2	1,116.7 ± 50.7	1,148.5 ± 57.3
Leukocytes (10^3/µL)	5.47 ± 0.83	4.62 ± 0.53	6.53 ± 0.51	5.75 ± 0.53	7.35 ± 0.27	6.77 ± 0.57
Segmented neutrophils (10^3/µL)	0.87 ± 0.15	0.75 ± 0.09	1.04 ± 0.08	1.0 ± 0.07	1.32 ± 0.11*	1.03 ± 0.14
Lymphocytes (10^3/µL)	4.40 ± 0.66	3.70 ± 0.44	5.27 ± 0.42	4.53 ± 0.47	5.73 ± 0.21	5.45 ± 0.43
Monocytes (10^3/µL)	0.10 ± 0.02	0.09 ± 0.02	0.10 ± 0.02	0.13 ± 0.02	0.14 ± 0.01	0.17 ± 0.02*
Basophils (10^3/µL)	0.006 ± 0.002	0.009 ± 0.006	0.009 ± 0.002	0.007 ± 0.002	0.013 ± 0.002	0.011 ± 0.003
Eosinophils (10^3/µL)	0.10 ± 0.02	0.07 ± 0.01	0.11 ± 0.02	0.09 ± 0.02	0.15 ± 0.02	0.11 ± 0.02
Female						
n	10	10	10	10	10	10
Hematocrit (%)	47.8 ± 0.6	46.3 ± 0.6	46.4 ± 0.7	46.4 ± 0.6	47.5 ± 0.8	46.8 ± 0.4
Hemoglobin (g/dL)	16.4 ± 0.2	15.8 ± 0.2	16.0 ± 0.2	16.0 ± 0.2	16.1 ± 0.2	15.8 ± 0.1
Erythrocytes (10^6/µL)	10.72 ± 0.13	10.36 ± 0.12	10.36 ± 0.17	10.46 ± 0.15	10.52 ± 0.14	10.43 ± 0.10
Reticulocytes (10^6/µL)	262.2 ± 11.8	278.7 ± 11.0	294.7 ± 18.8	275.0 ± 12.4	295.3 ± 14.3	288.9 ± 14.9
Mean cell volume (fL)	44.6 ± 0.2	44.7 ± 0.3	44.8 ± 0.2	44.4 ± 0.2	45.2 ± 0.2	44.9 ± 0.2
Mean cell hemoglobin (pg)	15.3 ± 0.1	15.3 ± 0.1	15.4 ± 0.1	15.2 ± 0.1	15.3 ± 0.1	15.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)	34.4 ± 0.2	34.2 ± 0.2	34.4 ± 0.2	34.2 ± 0.2	34.0 ± 0.1	33.7 ± 0.2**
Platelets (10^3/µL)	752.0 ± 48.7	837.2 ± 63.1	767.1 ± 44.6	767.9 ± 73.5	787.5 ± 80.3	818.4 ± 48.3
Leukocytes (10^3/µL)	4.40 ± 0.44	5.18 ± 0.62	4.41 ± 0.43	4.98 ± 0.51	5.44 ± 0.97	5.95 ± 0.65
Segmented neutrophils (10^3/µL)	0.60 ± 0.08	0.83 ± 0.13	0.54 ± 0.08	0.65 ± 0.06	0.83 ± 0.12	0.77 ± 0.09
Lymphocytes (10^3/µL)	3.65 ± 0.37	4.13 ± 0.47	3.73 ± 0.35	4.07 ± 0.46	4.37 ± 0.85	4.86 ± 0.55
Monocytes (10^3/µL)	0.10 ± 0.02	0.15 ± 0.04	0.10 ± 0.03	0.22 ± 0.06	0.13 ± 0.03	0.21 ± 0.04
Basophils (10^3/µL)	0.006 ± 0.002	0.003 ± 0.002	0.003 ± 0.003	0.003 ± 0.002	0.015 ± 0.006	0.003 ± 0.002
Eosinophils (10^3/µL)	0.05 ± 0.01	0.07 ± 0.02	0.05 ± 0.02	0.04 ± 0.01	0.10 ± 0.02	0.11 ± 0.03

* Significantly different (P<0.05) from the vehicle control group by Dunn's or Shirley's test.
** P<0.01
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

APPENDIX G
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Dermal Study of Pyrogallol[a]

	Vehicle Control	9.5 mg/kg	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	340 ± 4	340 ± 8	346 ± 4	342 ± 5	330 ± 5	331 ± 4
Heart						
Absolute	0.991 ± 0.014	0.968 ± 0.021	0.982 ± 0.020	0.990 ± 0.022	0.951 ± 0.017	0.995 ± 0.015
Relative	2.918 ± 0.022	2.854 ± 0.045	2.841 ± 0.055	2.896 ± 0.058	2.885 ± 0.045	3.004 ± 0.030
R. Kidney						
Absolute	1.133 ± 0.014	1.097 ± 0.034	1.126 ± 0.018	1.138 ± 0.018	1.101 ± 0.023	1.132 ± 0.017
Relative	3.339 ± 0.036	3.227 ± 0.056	3.258 ± 0.048	3.331 ± 0.036	3.338 ± 0.050	3.421 ± 0.037
Liver						
Absolute	12.89 ± 0.30	13.03 ± 0.53	13.58 ± 0.21	12.94 ± 0.25	12.39 ± 0.24	12.68 ± 0.28
Relative	37.959 ± 0.719	38.233 ± 0.769	39.269 ± 0.390	37.869 ± 0.505	37.566 ± 0.481	38.272 ± 0.558
Lung						
Absolute	1.726 ± 0.060	1.659 ± 0.087	1.840 ± 0.060	1.639 ± 0.043	1.711 ± 0.055	1.641 ± 0.089[b]
Relative	5.077 ± 0.145	4.876 ± 0.205	5.328 ± 0.177	4.802 ± 0.138	5.202 ± 0.191	4.956 ± 0.264[b]
R. Testis						
Absolute	1.490 ± 0.019	1.441 ± 0.028	1.462 ± 0.015	1.479 ± 0.021	1.451 ± 0.018	1.472 ± 0.025
Relative	4.389 ± 0.050	4.256 ± 0.097	4.233 ± 0.045	4.334 ± 0.075	4.407 ± 0.067	4.448 ± 0.057
Thymus						
Absolute	0.304 ± 0.013	0.313 ± 0.013	0.333 ± 0.016	0.306 ± 0.019	0.290 ± 0.017	0.292 ± 0.011
Relative	0.895 ± 0.037	0.923 ± 0.042	0.963 ± 0.042	0.897 ± 0.055	0.879 ± 0.050	0.880 ± 0.030
Thyroid Gland						
Absolute	0.035 ± 0.002	0.033 ± 0.002	0.036 ± 0.003	0.040 ± 0.002	0.035 ± 0.002	0.037 ± 0.003
Relative	0.103 ± 0.005	0.098 ± 0.005	0.103 ± 0.009	0.116 ± 0.005	0.105 ± 0.005	0.112 ± 0.009

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Dermal Study of Pyrogallol

	Vehicle Control	9.5 mg/kg	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg
Female						
n	9	10	10	10	10	10
Necropsy body wt	205 ± 3	208 ± 2	205 ± 5	198 ± 3	201 ± 3	194 ± 3
Heart						
Absolute	0.640 ± 0.014	0.659 ± 0.013	0.645 ± 0.013	0.623 ± 0.016	0.651 ± 0.012	0.626 ± 0.013
Relative	3.124 ± 0.038	3.164 ± 0.064	3.153 ± 0.045	3.145 ± 0.068	3.241 ± 0.065	3.231 ± 0.063
R. Kidney						
Absolute	0.693 ± 0.013	0.693 ± 0.008	0.695 ± 0.015	0.689 ± 0.014	0.698 ± 0.011	0.655 ± 0.011
Relative	3.386 ± 0.052	3.328 ± 0.042	3.395 ± 0.032	3.476 ± 0.041	3.473 ± 0.035	3.381 ± 0.041
Liver						
Absolute	6.579 ± 0.112	6.850 ± 0.062	6.734 ± 0.144	6.284 ± 0.154	6.669 ± 0.170	6.573 ± 0.176
Relative	32.121 ± 0.240	32.898 ± 0.288	32.915 ± 0.396	31.699 ± 0.529	33.200 ± 0.818	33.907 ± 0.638
Lung						
Absolute	1.158 ± 0.034	1.173 ± 0.031	1.297 ± 0.040*	1.098 ± 0.035[b]	1.185 ± 0.040	1.127 ± 0.040
Relative	5.651 ± 0.133	5.641 ± 0.171	6.345 ± 0.177*	5.565 ± 0.154[b]	5.904 ± 0.207	5.804 ± 0.148
Thymus						
Absolute	0.266 ± 0.012	0.262 ± 0.014	0.284 ± 0.016	0.256 ± 0.012	0.276 ± 0.014	0.228 ± 0.013
Relative	1.302 ± 0.068	1.255 ± 0.064	1.378 ± 0.054	1.292 ± 0.054	1.369 ± 0.055	1.173 ± 0.063
Thyroid Gland						
Absolute	0.030 ± 0.001	0.031 ± 0.004	0.032 ± 0.002	0.032 ± 0.002	0.030 ± 0.001	0.029 ± 0.002
Relative	0.146 ± 0.006	0.148 ± 0.017	0.156 ± 0.008	0.161 ± 0.008	0.149 ± 0.008	0.148 ± 0.011
Uterus						
Absolute	0.553 ± 0.062	0.560 ± 0.049	0.607 ± 0.069	0.522 ± 0.037	0.541 ± 0.077	0.638 ± 0.083
Relative	2.710 ± 0.313	2.706 ± 0.262	2.974 ± 0.358	2.632 ± 0.174	2.708 ± 0.399	3.284 ± 0.413

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

[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

[b] n=9

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Dermal Study of Pyrogallol[a]

	Vehicle Control	38 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	34.0 ± 0.7	35.0 ± 0.8	33.5 ± 0.5	34.6 ± 0.8	32.6 ± 1.2	32.5 ± 0.7
Heart						
Absolute	0.187 ± 0.008	0.192 ± 0.009	0.203 ± 0.009	0.194 ± 0.007	0.187 ± 0.008	0.189 ± 0.008
Relative	5.530 ± 0.246	5.487 ± 0.235	6.066 ± 0.252	5.619 ± 0.184	5.759 ± 0.158	5.813 ± 0.200
R. Kidney						
Absolute	0.280 ± 0.006	0.284 ± 0.008	0.291 ± 0.008	0.306 ± 0.009	0.294 ± 0.012	0.278 ± 0.007
Relative	8.239 ± 0.186	8.125 ± 0.203	8.683 ± 0.253	8.838 ± 0.138*	9.028 ± 0.173*	8.575 ± 0.127*
Liver						
Absolute	1.660 ± 0.034	1.740 ± 0.039	1.706 ± 0.045	1.777 ± 0.053	1.641 ± 0.058	1.668 ± 0.027
Relative	48.835 ± 0.575	49.776 ± 0.816	50.881 ± 1.027	51.351 ± 0.768	50.418 ± 0.479	51.456 ± 0.670
Lung						
Absolute	0.252 ± 0.016	0.283 ± 0.013	0.288 ± 0.020	0.262 ± 0.013	0.275 ± 0.019	0.261 ± 0.021
Relative	7.443 ± 0.531	8.125 ± 0.383	8.595 ± 0.598	7.583 ± 0.378	8.426 ± 0.405	8.01 ± 0.625
R. Testis						
Absolute	0.110 ± 0.002	0.119 ± 0.002	0.113 ± 0.002	0.119 ± 0.002	0.116 ± 0.004	0.112 ± 0.003
Relative	3.240 ± 0.077	3.410 ± 0.066	3.361 ± 0.053	3.439 ± 0.059	3.562 ± 0.073*	3.455 ± 0.103
Thymus						
Absolute	0.047 ± 0.002	0.053 ± 0.002	0.044 ± 0.002	0.046 ± 0.002	0.046 ± 0.002	0.047 ± 0.003
Relative	1.373 ± 0.063	1.504 ± 0.048	1.313 ± 0.047	1.324 ± 0.046	1.416 ± 0.068	1.448 ± 0.074
Thyroid Gland						
Absolute	0.007 ± 0.000	0.008 ± 0.001	0.007 ± 0.001	0.008 ± 0.001	0.009 ± 0.001	0.007 ± 0.001
Relative	0.198 ± 0.005	0.220 ± 0.018	0.204 ± 0.018	0.222 ± 0.023	0.266 ± 0.024	0.224 ± 0.023
Female						
Necropsy body wt	30.4 ± 0.7	31.3 ± 1.1	30.9 ± 0.9	29.9 ± 0.7	28.2 ± 0.8	28.6 ± 0.8
Heart						
Absolute	0.178 ± 0.005	0.179 ± .008	0.18 ± 0.005	0.173 ± 0.008	0.183 ± 0.009	0.187 ± 0.008
Relative	5.878 ± 0.230	5.803 ± 0.345	5.852 ± 0.165	5.758 ± 0.193	6.511 ± 0.292	6.558 ± 0.259
R. Kidney						
Absolute	0.196 ± 0.004	0.197 ± 0.005	0.202 ± 0.003	0.191 ± 0.005	0.193 ± 0.004	0.194 ± 0.006
Relative	6.477 ± 0.193	6.362 ± 0.269	6.600 ± 0.220	6.382 ± 0.109	6.867 ± 0.153	6.767 ± 0.109
Liver						
Absolute	1.499 ± 0.031	1.537 ± 0.033	1.550 ± 0.035	1.482 ± 0.041	1.455 ± 0.044	1.541 ± 0.081
Relative	49.306 ± 0.658	49.426 ± 1.252	50.410 ± 1.029	49.544 ± 0.836	51.620 ± 0.952	53.656 ± 1.511**
Lung						
Absolute	0.304 ± 0.010	0.271 ± 0.016	0.274 ± 0.014	0.274 ± 0.009	0.285 ± 0.013	0.307 ± 0.014
Relative	10.057 ± 0.446	8.725 ± 0.561	8.853 ± 0.363	9.169 ± 0.281	10.164 ± 0.534	10.738 ± 0.452
Thymus						
Absolute	0.055 ± 0.002	0.058 ± 0.003	0.062 ± 0.004	0.054 ± 0.003	0.051 ± 0.002	0.049 ± 0.003
Relative	1.808 ± 0.084	1.862 ± 0.120	2.018 ± 0.101	1.812 ± 0.073	1.799 ± 0.058	1.716 ± 0.071
Thyroid Gland						
Absolute	0.007 ± 0.000	0.008 ± 0.000	0.007 ± 0.001	0.007 ± 0.001	0.008 ± 0.000	0.007 ± 0.000
Relative	0.223 ± 0.016	0.248 ± 0.014	0.233 ± 0.019	0.246 ± 0.016	0.277 ± 0.010	0.248 ± 0.014
Uterus						
Absolute	0.146 ± 0.017	0.150 ± 0.016	0.174 ± 0.013	0.145 ± 0.011	0.134 ± 0.012	0.127 ± 0.008
Relative	4.767 ± 0.553	4.818 ± 0.520	5.674 ± 0.455	4.874 ± 0.382	4.711 ± 0.368	4.488 ± 0.327

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

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Dermal Study of Pyrogallol[a]

	Vehicle Control	37.5 mg/kg	75 mg/kg	150 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	340 ± 4	342 ± 5	330 ± 5	331 ± 4
L. Cauda epididymis	0.1547 ± 0.0026	0.1530 ± 0.0022	0.1482 ± 0.0035	0.1577 ± 0.0025
L. Epididymis	0.4334 ± 0.0082	0.4319 ± 0.0070	0.4253 ± 0.0036	0.4355 ± 0.0057
L. Testis	1.5519 ± 0.0224	1.5497 ± 0.0219	1.5104 ± 0.0109	1.5259 ± 0.0218
Spermatid measurements				
Spermatid heads (10^6/testis)	182.6 ± 6.8	188.4 ± 4.1	180.4 ± 4.6	184.9 ± 7.9
Spermatid heads (10^6/g testis)	132.4 ± 4.9	137.6 ± 3.8	135.0 ± 3.9	135.5 ± 5.0
Epididymal spermatozoal measurements				
Sperm motility (%)	80.90 ± 0.43	81.70 ± 0.92	81.80 ± 0.55	82.80 ± 0.65
Sperm (10^6/cauda epididymis)	108.5 ± 4.2	110.0 ± 6.2	122.9 ± 9.3	114.3 ± 7.5
Sperm (10^6/g cauda epididymis)	702.1 ± 27.0	720.1 ± 41.1	828.1 ± 55.1	726.8 ± 50.0

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

TABLE H2
Estrous Cycle Characterization for Female Rats in the 3-Month Dermal Study of Pyrogallol[a]

	Vehicle Control	37.5 mg/kg	75 mg/kg	150 mg/kg
Number weighed at necropsy	9	10	10	10
Necropsy body wt (g)	205 ± 3	198 ± 3	201 ± 3	194 ± 3*
Proportion of regular cycling females[b]	9/9	8/9	9/9	10/10
Estrous cycle length (days)	5.06 ± 0.06	5.00 ± 0.00[c]	5.00 ± 0.00[c]	5.00 ± 0.00
Estrous stages (% of cycle)				
Diestrus	58.3	53.3	47.5	58.3
Proestrus	15.7	16.7	16.7	20.0
Estrus	21.3	20.8	22.5	20.8
Metestrus	2.8	5.0	0.8	0.0
Uncertain diagnoses	1.9	4.2	12.5	0.8

* Significantly different ($P<0.05$) from the vehicle control group by Dunnett' test

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated dosed females did not have extended estrus or diestrus.

[b] Number of females with a regular cycle/number of females cycling

[c] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

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

FIGURE H1
Vaginal Cytology Plots for Female Rats in the 3-Month Dermal Study of Pyrogallol

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

TABLE H3
Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Rats Administered Pyrogallol Dermally for 3 Months

Stage	Comparison[a]	P Value	Trend[b]
Overall Tests	Overall	0.34	
Overall Tests	Low vs. Controls	0.237	N
Overall Tests	Mid vs. Controls	0.435	N
Overall Tests	High vs. Controls	0.325	N
Extended Estrus	Overall	0.792	
Extended Estrus	Low vs. Controls	0.995	
Extended Estrus	Mid vs. Controls	0.348	
Extended Estrus	High vs. Controls	0.604	
Extended Diestrus	Overall	0.162	
Extended Diestrus	Low vs. Controls	0.163	N
Extended Diestrus	Mid vs. Controls	0.186	N
Extended Diestrus	High vs. Controls	0.327	N
Extended Metestrus	Overall	1	
Extended Metestrus	Low vs. Controls	1	
Extended Metestrus	Mid vs. Controls	1	
Extended Metestrus	High vs. Controls	1	
Extended Proestrus	Overall	1	
Extended Proestrus	Low vs. Controls	1	
Extended Proestrus	Mid vs. Controls	1	
Extended Proestrus	High vs. Controls	1	
Skipped Estrus	Overall	1	
Skipped Estrus	Low vs. Controls	1	
Skipped Estrus	Mid vs. Controls	1	
Skipped Estrus	High vs. Controls	1	
Skipped Diestrus	Overall	1	
Skipped Diestrus	Low vs. Controls	1	
Skipped Diestrus	Mid vs. Controls	1	
Skipped Diestrus	High vs. Controls	1	

[a] Controls = Vehicle Control, Low = 37.5 mg/kg, Mid = 75 mg/kg, High = 150 mg/kg
[b] N means that the treated group had a lower probability of transitioning to the relevant abnormal state (extended estrus, extended metestrus, extended proestrus, skipped estrus, or skipped diestrus) than did the vehicle control group.

TABLE H4
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Dermal Study of Pyrogallol[a]

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	34.0 ± 0.7	34.6 ± 0.8	32.6 ± 1.2	32.5 ± 0.7
L. Cauda epididymis	0.0134 ± 0.0003	0.0125 ± 0.0008	0.0139 ± 0.0006	0.0137 ± 0.0007
L. Epididymis	0.0410 ± 0.0006	0.0409 ± 0.0011	0.0424 ± 0.0017	0.0405 ± 0.0010
L. Testis	0.1066 ± 0.0023	0.1109 ± 0.0023	0.1086 ± 0.0038	0.1080 ± 0.0024
Spermatid measurements				
Spermatid heads (10^6/testis)	20.17 ± 0.89	19.84 ± 0.99	19.54 ± 0.75	20.57 ± 1.16
Spermatid heads (10^6/g testis)	202.0 ± 9.5	194.5 ± 8.1	196.0 ± 5.1	209.6 ± 9.4
Epididymal spermatozoal measurements				
Sperm motility (%)	81.00 ± 0.68	80.10 ± 0.66	82.30 ± 0.84	81.60 ± 0.72
Sperm (10^6/cauda epididymis)	14.3 ± 0.9	14.0 ± 0.8	14.6 ± 1.0	13.0 ± 1.4
Sperm (10^6/g cauda epididymis)	1,075 ± 80	1,186 ± 145	1,060 ± 68	961 ± 104

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

TABLE H5
Estrous Cycle Characterization for Female Mice in the 3-Month Dermal Study of Pyrogallol[a]

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	30.4 ± 0.7	29.9 ± 0.7	28.2 ± 0.8	28.6 ± 0.8
Proportion of regular cycling females[b]	10/10	8/9	7/7	9/9
Estrous cycle length (days)	4.28 ± 0.18	4.33 ± 0.19[c]	4.21 ± 0.15[d]	4.22 ± 0.09[c]
Estrous stages (% of cycle)				
Diestrus	40.0	45.0	27.5	25.8
Proestrus	0.0	0.0	0.8	0.8
Estrus	38.3	37.5	37.5	41.7
Metestrus	21.7	17.5	20.8	22.5
Uncertain diagnoses	0.0	0.0	13.3	9.2

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated a significantly ($P<0.001$) higher probability of extended diestrus for female mice in the 150 mg/kg group compared to the vehicle control group.

[b] Number of females with a regular cycle/number of females cycling

[c] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

[d] Estrous cycle was longer than 12 days or unclear in 3 of 10 animals.

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

FIGURE H2
Vaginal Cytology Plots for Female Mice in the 3-Month Dermal Study of Pyrogallol

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

TABLE H6
Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Mice Administered Pyrogallol Dermally for 3 Months

Stage	Comparison[a]	P Value	Trend[b]
Overall Tests	Overall	0.01	
Overall Tests	Low vs. Controls	0.001	
Overall Tests	Mid vs. Controls	0.479	
Overall Tests	High vs. Controls	0.857	N
Extended Estrus	Overall	1	
Extended Estrus	Low vs. Controls	1	
Extended Estrus	Mid vs. Controls	1	
Extended Estrus	High vs. Controls	1	
Extended Diestrus	Overall	0.01	
Extended Diestrus	Low vs. Controls	0.001	
Extended Diestrus	Mid vs. Controls	0.479	
Extended Diestrus	High vs. Controls	0.857	N
Extended Metestrus	Overall	1	
Extended Metestrus	Low vs. Controls	1	
Extended Metestrus	Mid vs. Controls	1	
Extended Metestrus	High vs. Controls	1	
Extended Proestrus	Overall	1	
Extended Proestrus	Low vs. Controls	1	
Extended Proestrus	Mid vs. Controls	1	
Extended Proestrus	High vs. Controls	1	
Skipped Estrus	Overall	1	
Skipped Estrus	Low vs. Controls	1	
Skipped Estrus	Mid vs. Controls	1	
Skipped Estrus	High vs. Controls	1	
Skipped Diestrus	Overall	1	
Skipped Diestrus	Low vs. Controls	1	
Skipped Diestrus	Mid vs. Controls	1	
Skipped Diestrus	High vs. Controls	1	
Summary of Significant Groups			
Overall Tests	Low vs. Controls	0.001	
Extended Diestrus	Low vs. Controls	0.001	

[a] Controls = Vehicle Control, Low = 150 mg/kg, Mid = 300 mg/kg, High = 600 mg/kg

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

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Pyrogallol

Pyrogallol was obtained from Aceto Corporation (Lake Success, NY) in one lot (010326) which was used in the 3-month and 2-year animal studies. Two other lots (A008328201 and 83282/1) were purchased from Acros Organics (Pittsburgh, PA); these two lots were not used in the animal studies but were used in methods development and stability studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Midwest Research Institute (MRI; Kansas City, MO). The study laboratory at Battelle Columbus Operations (Columbus, OH) conducted additional identity analyses, and MRI conducted bulk chemical stability analyses. Reports on analyses performed in support of the pyrogallol studies are on file at the National Institute of Environmental Health Sciences.

Lot 010326 of the chemical, a white crystalline powder, was identified as pyrogallol by the analytical chemistry laboratory using melting point analyses, infrared (IR), ultraviolet (UV), proton nuclear magnetic resonance (NMR), and mass spectroscopy. The study laboratory confirmed the identity of this lot using IR spectroscopy. The melting point range was 131.8° to 135.3° C, consistent with literature values (*Merck*, 1996). All spectra were consistent with the structure and literature spectra (*Aldrich*, 1993, 1997) of pyrogallol. Representative IR and proton NMR spectra are presented in Figures I1 and I2, respectively.

For lot 010326, Karl Fischer titration and weight loss on drying were performed by the analytical chemistry laboratory. The purity of lot 010326 was determined using thin-layer chromatography (TLC) and high-performance liquid chromatography with ultraviolet detection (HPLC/UV) by system A (Table I1). The TLC method included vanillin (Aldrich Chemical Company, Inc., Milwaukee, WI) as a reference standard, ethyl acetate (Burdick and Jackson, Morris Township, NJ):ASTM I water (10:1) as the solvent, and silica gel 60 F_{254} plates (20 cm× 20 cm, 250 µm thickness) (EM Science, Gibbstown, NJ). The dried plates were examined with ultraviolet light at 254 and 366 nm, visible light, and iodine vapor.

Karl Fischer titration results were inconclusive due to a reaction of pyrogallol with the reagent used, and weight loss on drying could not be determined due to the sublimation of the test article. TLC indicated a single major spot and no impurities. HPLC/UV indicated one major peak and no impurities with areas equal to or greater than 0.05% of the total peak areas in four of six containers tested; one container had one impurity, and another had two impurities that had peak areas greater than or equal to 0.05% of the total peak area. The overall purity of lot 010326 was determined to be 99% or greater.

Stability studies of lot A008328201 of the bulk chemical were performed by the analytical chemistry laboratory using HPLC/UV by system B. These studies indicated that pyrogallol was stable as a bulk chemical for at least 2 weeks when stored under an inert headspace, protected from light, at temperatures up to 60° C. To ensure stability, the bulk chemical was stored under an inert headspace, sealed in amber glass bottles kept at room temperature. Periodic reanalyses of the bulk chemical were performed during the 3-month and 2-year studies by the study laboratory using HPLC/UV by system C and no degradation of the bulk chemical was detected.

Ethanol (95%)

USP-grade 95% ethanol was obtained from Spectrum Chemicals and Laboratory Products (Gardena, CA) in multiple lots that were used as the vehicle in the 3-month and 2-year studies. Identity and purity analyses were conducted by the study laboratory. The chemical, a clear liquid, was identified as ethanol containing approximately 5% to 10% water using IR spectroscopy; all spectra were consistent with the structure of ethanol. The purity of each lot was determined using gas chromatography (GC) by system A (Table I2); GC by system B was used to determine if benzene was present in the ethanol. No impurities (including benzene) were detected that exceeded a relative concentration of 0.1% in any lot. Periodic reanalyses of the 95% ethanol vehicle were performed by the study

laboratory at approximately 6-month intervals during the 2-year studies and no degradation of the chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing pyrogallol and 95% ethanol to give the required concentrations (Table I3). The dose formulations were stored at approximately 5° C in amber glass bottles sealed with Teflon®-lined lids inside plastic bags (2-year studies only) for up to 42 days.

Stability studies of a 125 µg/mL formulation made with lot 83282/1 were performed by the analytical chemistry laboratory using HPLC/UV by system D (Table I1). Stability was confirmed for at least 42 days for formulations stored in amber glass containers sealed with Teflon®-lined lids at approximately 5° C and for at least 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of pyrogallol were conducted by the study laboratory using HPLC/UV by system E. During the 3-month studies, the dose formulations were analyzed three times; animal room samples of these dose formulations were also analyzed (Table I4). All 15 dose formulations analyzed for rats and mice were within 10% of the target concentrations; all 15 animal room samples for rats and all 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 3 months; animal room samples were also analyzed (Table I5). Of the dose formulations analyzed, all 27 for rats and all 27 for mice were within 10% of the target concentrations; all nine animal room samples for rats and all nine for mice were within 10% of the target concentrations.

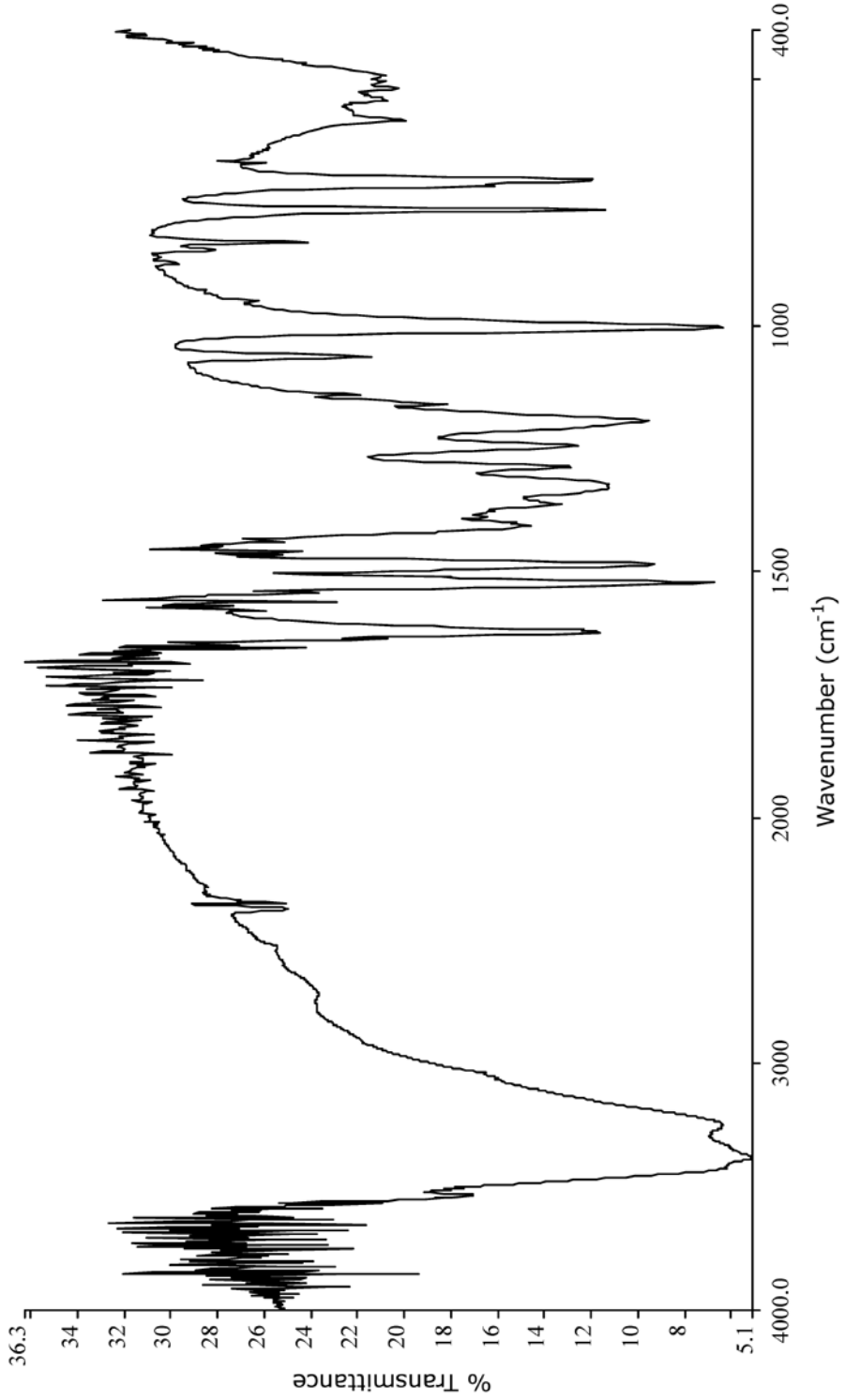

FIGURE I1
Infrared Absorption Spectrum of Pyrogallol

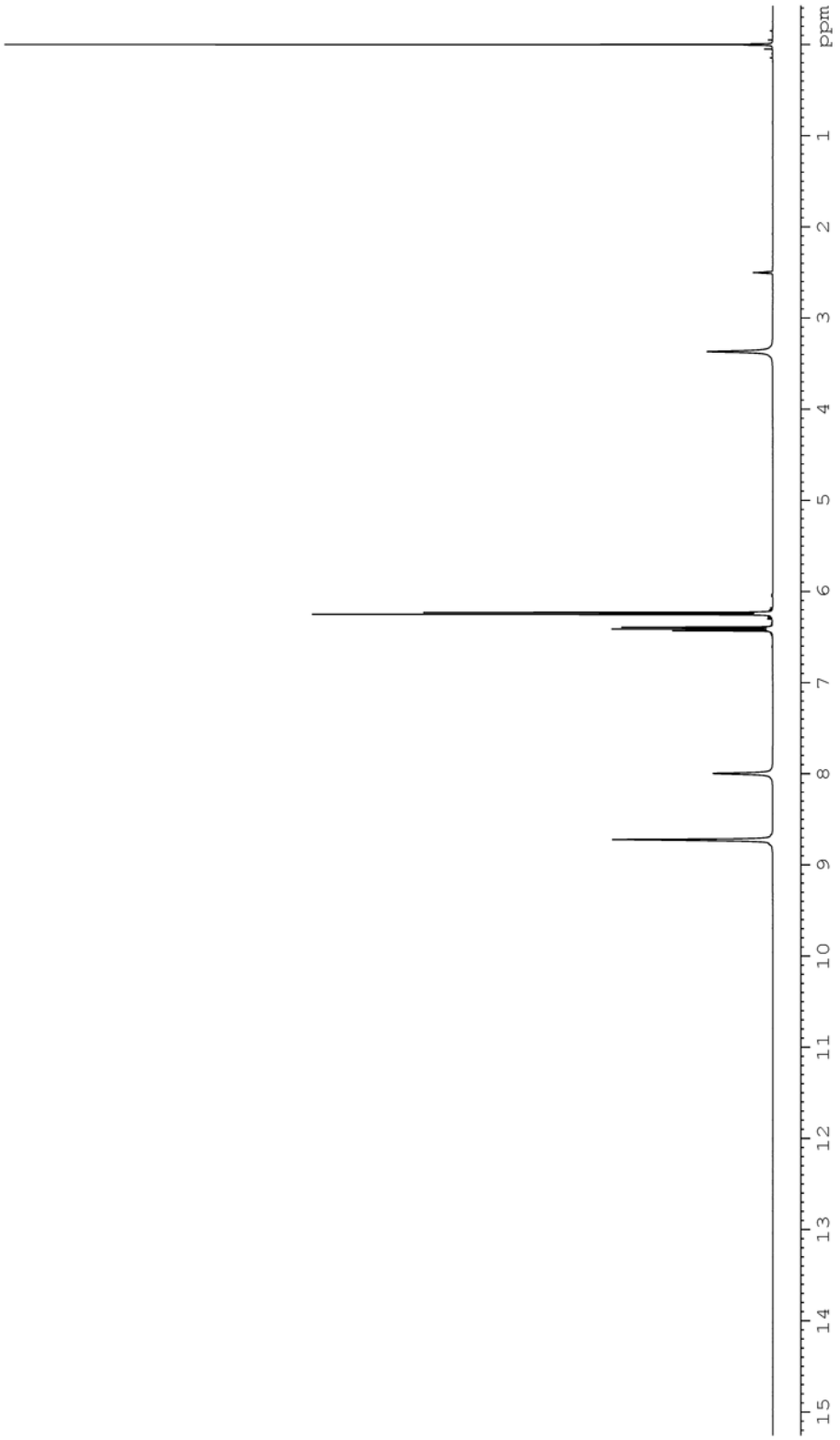

FIGURE I2
Proton Nuclear Magnetic Resonance Spectrum of Pyrogallol

TABLE I1
High-Performance Liquid Chromatography Systems Used in the Dermal Studies of Pyrogallol[a]

Detection System	Column	Solvent System
System A		
Ultraviolet (210 nm) light	Alltima™ C_{18}, 250 mm × 3.2 mm, 5 µm (Alltech Associates, Inc., Deerfield, IL)	A) ASTM Type I reagent grade water with 0.025% phosphoric acid and B) methanol with 0.025% phosphoric acid; 90% A:10% B for 8 minutes; linear gradient to 100% B over 7 minutes, held for 3 minutes; return to 90% A:10% B over 7 minutes; flow rate 0.5 mL/minute
System B		
Ultraviolet (210 nm) light	Alltima™ C_{18}, 250 mm × 3.2 mm, 5 µm (Alltech Associates, Inc.)	A) Acetonitrile with trifluoroacetic and B) ASTM Type I reagent grade water adjusted to pH 3 with trifluoroacetic acid (10% A:90% B), isocratic; flow rate 0.5 mL/minute; *N*,*N*-dimethyl formamide as internal standard
System C		
Ultraviolet (210 nm) light	Prodigy™ ODS 3 C_{18}, 250 mm × 4.6 mm, 5 µm (Phenomenex, Torrance, CA)	A) Milli-Q® water with 0.025% phosphoric acid and B) methanol with 0.025% phosphoric acid (90% A:10% B), isocratic; flow rate 0.9 mL/minute; *N*,*N*-dimethyl formamide as internal standard
System D		
Ultraviolet (230 nm) light	Alltima™ C_{18}, 250 mm × 4.6 mm, 5 µm (Alltech Associates, Inc.)	A) Water with 0.1% phosphoric acid and B) Methanol:acetonitrile (1:1) (85% A:15% B), isocratic; flow rate 1.0 mL/minute
System E		
Ultraviolet (230 nm) light	Prodigy™ ODS 3 C_{18}, 150 mm × 4.6 mm, 5 µm (Phenomenex)	Milli-Q® water:methanol:phosphoric acid (90:10:0.1), isocratic, flow rate 0.5 mL/minute; resorcinol as internal standard

[a] The high-performance liquid chromatographs were manufactured by Waters Corporation (Milford, MA) (systems A, B, and D) or Agilent Technologies, Inc. (Palo Alto, CA) (systems C and E).

TABLE I2
Gas Chromatography Systems Used in the Dermal Studies of Pyrogallol[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	DB-WAX, 30 m × 0.53 mm, 1 µm film (Agilent J&W, Folsom, CA)	Helium at 5 mL/minute	80° C for 5 minutes, then 10° C/minute to 220° C, held for 3 minutes
System B			
Flame ionization	Rtx®-5, 30 m × 0.53 mm, 1.5 µm film (Restek, Bellefonte, PA)	Helium at 10 mL/minute	40° C for 3 minutes, then 10° C/minute to 200° C, held for 3 minutes

[a] The gas chromatographs were manufactured by Agilent Technologies, Inc. (Palo Alto, CA).

TABLE I3
Preparation and Storage of Dose Formulations in the Dermal Studies of Pyrogallol

3-Month Studies	2-Year Studies
Preparation The appropriate amount of pyrogallol was weighed and transferred with three rinses of 95% ethanol to a calibrated glass mixing container. The mixing container was filled to approximately 90% full with 95% ethanol, inverted at least 10 times and then stirred on a stirplate for 15 minutes at a speed that produced a vigorous vortex. The mixing container was filled to final volume with 95% ethanol and the stirring step was repeated. Dose formulations were prepared four times.	Similar to the 3-month studies except that the final solutions were vortexed for 15 minutes (through November 18, 2004) or 5 minutes (after November 18, 2004). Dose formulations were prepared approximately every 4 weeks.
Chemical Lot Number 010326	010326
Maximum Storage Time 42 days	42 days
Storage Conditions Stored in amber glass bottles sealed with Teflon®-lined lids at approximately 5° C.	Stored in amber glass bottles sealed with Teflon®-lined lids inside plastic bags at approximately 5° C.
Study Laboratory Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Dermal Studies of Pyrogallol

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
October 15, 2003	October 15, 2003	19	19.67	+4
		37.5	39.31	+5
		75	78.86	+5
		150	158.6	+6
		300	315.5	+5
	December 17-18, 2003[b]	19	18.99	0
		37.5	39.59	+6
		75	75.59	+1
		150	162.1	+8
		300	309.0 ± 7.2[d]	+3
	December 17-18, 2003[c]	19	19.69	+4
		37.5	40.68	+8
		75	78.23	+4
		150	159.3	+6
		300	302.2	+1
November 12, 2003	November 13, 2003	19	19.67	+4
		37.5	38.59	+3
		75	78.25	+4
		150	158.1	+5
		300	317.6	+6
	January 16-17, 2004[b]	19	19.81	+4
		37.5	39.64	+6
		75	77.41	+3
		150	159.1	+6
		300	315.5	+5
	January 16-17, 2004[c]	19	19.22	+1
		37.5	40.36	+8
		75	77.02	+3
		150	154.4	+3
		300	302.8	+1
January 9, 2004	January 16, 2004	19	19.37	+2
		37.5	38.46	+3
		75	73.75	–2
		150	155.3	+4
		300	296.9	–1
	February 11-12, 2004[b]	19	19.79	+4
		37.5	40.01	+7
		75	78.62	+5
		150	161.8	+8
		300	309.7	+3
	February 11-12, 2004[c]	19	19.75	+4
		37.5	39.36	+5
		75	76.70	+2
		150	158.1	+5
		300	310.3	+3

[a] Results of duplicate analyses. For rats, dosing volume=0.5 mL/kg; 19 mg/mL=9.5 mg/kg, 37.5 mg/mL=18.75 mg/kg, 75 mg/mL=37.5 mg/kg, 150 mg/mL=75 mg/kg, 300 mg/mL=150 mg/kg. For mice, dosing volume=2 mL/kg; 19 mg/mL=38 mg/kg, 37.5 mg/mL=75 mg/kg, 75 mg/mL=150 mg/kg, 150 mg/mL=300 mg/kg, 300 mg/mL=600 mg/kg.

[b] Animal room samples for rats

[c] Animal room samples for mice

[d] Results of triplicate analyses (mean ± standard deviation)

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies of Pyrogallol

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
September 2, 2004	September 3-4, 2004	10	10.30	+3
		40	41.58	+4
		150	153.7	+2
	October 14-15, 2004[b]	10	10.25	+3
		40	41.41	+4
		150	147.7	–2
November 18, 2004	November 19, 2004	10	10.23	+2
		40	40.91	+2
		150	149.8	0
February 8, 2005	February 11-12, 2005	10	10.42	+4
		40	42.86	+7
		150	157.6	+5
May 4, 2005	May 4-5, 2005	10	9.903	–1
		40	40.12	0
		150	148.0	–1
	June 13-14, 2005[b]	10	10.48	+5
		40	41.67	+4
		150	153.4	+2
July 19, 2005	July 21-22, 2005	10	9.574	–4
		40	41.88	+5
		150	143.0	–5
October 11, 2005	October 13-14, 2005	10	10.14 ± 0.07[c]	+1
		40	41.28 ± 0.13[c]	+3
		150	153.2 ± 0.5[c]	+2
January 5, 2006	January 11, 2006	10	9.984	0
		40	39.91	0
		150	152.4	+2
	February 16-18 and 20, 2006[b]	10	10.09	+1
		40	40.77	+2
		150	155.9	+4
March 28, 2006	March 31, 2006	10	9.691	–3
		40	39.57	–1
		150	145.9	–3
June 21, 2006	June 23-24, 2006	10	10.14	+1
		40	41.79	+4
		150	155.7	+4

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies of Pyrogallol

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice				
September 2, 2004	September 3-4, 2004	2.5	2.502	0
		10	10.30	+3
		37.5	38.29	+2
	October 14-15, 2004[b]	2.5	2.433	–3
		10	10.31	+3
		37.5	39.02	+4
November 18, 2004	November 19, 2004	2.5	2.475	–1
		10	10.23	+2
		37.5	37.95	+1
February 8, 2005	February 11-12, 2005	2.5	2.489	0
		10	10.42	+4
		37.5	39.14	+4
May 4, 2005	May 4-5, 2005	2.5	2.390	–4
		10	9.903	–1
		37.5	37.04	–1
	June 13-14, 2005[b]	2.5	2.475	–1
		10	10.74	+7
		37.5	38.52	+3
July 19, 2005	July 21-22, 2005	2.5	2.395	–4
		10	9.574	–4
		37.5	33.81	–10
October 11, 2005	October 13-14, 2005	2.5	2.450 ± 0.014[c]	–2
		10	10.14 ± 0.07[c]	+1
		37.5	37.58 ± 0.04[c]	0
January 5, 2006	January 11, 2006	2.5	2.384	–5
		10	9.984	0
		37.5	36.61	–2
	February 16-18 and 20, 2006[b]	2.5	2.336	–7
		10	10.24	+2
		37.5	36.25	–3
March 28, 2006	March 31, 2006	2.5	2.346	–6
		10	9.691	–3
		37.5	36.02	–4
June 21, 2006	June 23-24, 2006	2.5	2.405	–4
		10	10.14	+1
		37.5	38.03	+1

[a] Results of duplicate analyses. For rats, dosing volume=0.5 mL/kg; 10 mg/mL=5 mg/kg, 40 mg/mL=20 mg/kg, 150 mg/mL=75 mg/kg. For mice, dosing volume=2 mL/kg; 2.5 mg/mL=5 mg/kg, 10 mg/mL=20 mg/kg, 37.5 mg/mL=75 mg/kg.
[b] Animal room samples
[c] Results of triplicate analyses (mean ± standard deviation)

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

TABLE J1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[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 J2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

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

[a] Per kg of finished product

TABLE J3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (%) by weight	14.6 ± 0.69	13.5 – 16.3	25
Crude fat (%) by weight	8.3 ± 0.37	7.6 – 9.3	25
Crude fiber (%) by weight	9.3 ± 0.46	8.4 – 10.0	25
Ash (%) by weight	5.0 ± 0.24	4.6 – 5.4	25
Amino Acids (% of total diet)			
Arginine	0.778 ± 0.068	0.670 – 0.970	21
Cystine	0.220 ± 0.025	0.150 – 0.250	21
Glycine	0.701 ± 0.042	0.620 – 0.800	21
Histidine	0.354 ± 0.079	0.270 – 0.680	21
Isoleucine	0.544 ± 0.045	0.430 – 0.660	21
Leucine	1.092 ± 0.068	0.960 – 1.240	21
Lysine	0.704 ± 0.112	0.310 – 0.840	21
Methionine	0.409 ± 0.047	0.260 – 0.490	21
Phenylalanine	0.626 ± 0.040	0.540 – 0.720	21
Threonine	0.503 ± 0.043	0.430 – 0.610	21
Tryptophan	0.148 ± 0.027	0.110 – 0.200	21
Tyrosine	0.397 ± 0.058	0.280 – 0.540	21
Valine	0.666 ± 0.044	0.550 – 0.730	21
Essential Fatty Acids (% of total diet)			
Linoleic	3.92 ± 0.227	3.49 – 4.54	21
Linolenic	0.30 ± 0.030	0.21 – 0.35	21
Vitamins			
Vitamin A (IU/kg)	4,034 ± 886	2,340 – 6,160	25
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	80.1 ± 22.48	27.0 – 124.0	21
Thiamine (ppm)[b]	7.7 ± 1.16	6.3 – 10.5	25
Riboflavin (ppm)	7.1 ± 1.91	4.20 – 11.20	21
Niacin (ppm)	78.6 ± 9.16	66.4 – 98.2	21
Pantothenic Acid (ppm)	27.1 ± 12.89	17.4 – 81.0	21
Pyridoxine (ppm)[b]	9.47 ± 2.01	6.4 – 13.7	21
Folic Acid (ppm)	1.63 ± 0.49	1.15 – 3.27	21
Biotin (ppm)	0.319 ± 0.10	0.200 – 0.704	21
Vitamin B_{12} (ppb)	53.8 ± 40.6	18.3 – 174.0	21
Choline (ppm)[b]	2,885 ± 459	1,820 – 3,790	21
Minerals			
Calcium (%)	0.978 ± 0.049	0.895 – 1.080	25
Phosphorus (%)	0.568 ± 0.030	0.515 – 0.623	25
Potassium (%)	0.663 ± 0.027	0.626 – 0.732	21
Chloride (%)	0.387 ± 0.039	0.300 – 0.474	21
Sodium (%)	0.190 ± 0.016	0.160 – 0.222	21
Magnesium (%)	0.216 ± 0.063	0.185 – 0.490	21
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	14
Iron (ppm)	185 ± 40.1	135 – 311	21
Manganese (ppm)	51.6 ± 10.49	21.0 – 73.1	21
Zinc (ppm)	53.6 ± 8.62	43.3 – 78.5	21
Copper (ppm)	7.07 ± 2.611	3.21 – 16.30	21
Iodine (ppm)	0.497 ± 0.209	0.158 – 0.972	21
Chromium (ppm)	0.684 ± 0.279	0.330 – 1.380	20
Cobalt (ppm)	0.26 ± 0.164	0.11 – 0.86	19

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

TABLE J4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.23 ± 0.061	0.16 – 0.39	25
Cadmium (ppm)	0.05 ± 0.010	0.04 – 0.09	25
Lead (ppm)	0.09 ± 0.016	0.07 – 0.13	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.28 ± 0.100	0.18 – 0.49	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm)[c]	13.21 ± 5.74	4.76 – 23.7	25
Nitrite nitrogen (ppm)[c]	<0.61		25
BHA (ppm)[d]	<1.0		25
BHT (ppm)[d]	<1.0		25
Aerobic plate count (CFU/g)	10 ± 0.0	10 – 10	25
Coliform (MPN/g)	3.0 ± 0.0	3.0 – 3.0	25
Escherichia coli (MPN/g)	<10		25
Salmonella (MPN/g)	Negative		25
Total nitrosoamines (ppb)[e]	4.9 ± 1.66	2.2 – 9.9	25
N-Nitrosodimethylamine (ppb)[e]	2.7 ± 1.22	1.0 – 6.3	25
N-Nitrosopyrrolidine (ppb)[e]	2.2 ± 0.81	1.1 – 4.1	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCBs	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl chlorpyrifos	0.136 ± 0.128	0.020 – 0.416	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.264 ± 0.248	0.020 – 0.997	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfane sulfate	<0.03		25

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

APPENDIX K
SENTINEL ANIMAL PROGRAM

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 sera or feces 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.

During the 3-month studies, serum samples were collected from five male and five female sentinel rats and mice at 1 month and at the end of the studies. During the 2-year studies, serum samples were collected from five male and five female sentinel rats and mice at 1, 6, 12, and 18 months, and from five randomly selected 75 mg/kg male and female rats and mice at the end of the studies. Fecal samples were also collected at 18 months from sentinel mice and tested for *Helicobacter* species by polymerase chain reaction. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance (Rockville, MD) for determination of antibody titers. The laboratory methods and agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Collection
RATS	
3-Month Study	
ELISA	
PVM (pneumonia virus of mice)	Study start, 1 month, study termination
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	Study start, 1 month, study termination
Sendai	Study start, 1 month, study termination
Immunofluorescence Assay	
Parvovirus	Study start, 1 month, study termination
PVM	Study start
Sendai	Study start
2-Year Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM	Study start, 1, 6, 12, and 18 months, study termination
RCV/SDA	Study start, 1, 6, 12, and 18 months, study termination
Sendai	Study start, 1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	Study start, 1, 6, 12, and 18 months, study termination
Sendai	Study start

Method and Test	Time of Collection
MICE	
3-Month Study	
ELISA	
Ectromelia virus	Study start, 1 month, study termination
EDIM (epizootic diarrhea of infant mice)	Study start, 1 month, study termination
GDVII (mouse encephalomyelitis virus)	Study start, 1 month, study termination
LCM (lymphocytic choriomeningitis virus)	Study start, 1 month, study termination
Mouse adenovirus	Study start, 1 month, study termination
MHV (mouse hepatitis virus)	Study start, 1 month, study termination
PVM	Study start, 1 month, study termination
Reovirus 3	Study start, 1 month, study termination
Sendai	Study start, 1 month, study termination
Immunofluorescence Assay	
Parvovirus	Study start, 1 month, study termination
GDVII	Study termination
2-Year Study	
ELISA	
Ectromelia virus	Study start, 1, 6, 12, and 18 months, study termination
EDIM	Study start, 1, 6, 12, and 18 months, study termination
GDVII	Study start, 1, 6, 12, and 18 months, study termination
LCM	Study start, 1, 6, 12, and 18 months, study termination
Mouse adenoma virus-FL	Study start, 1 and 6 months
Mouse adenoma virus-1	12 and 18 months, study termination
MHV	Study start, 1, 6, 12, and 18 months, study termination
MMV, VP2 (mouse minute virus, viral protein 2)	Study start, 1, 6, 12, and 18 months, study termination
MPV, VP2 (mouse parvovirus, viral protein 2)	Study start, 1, 6, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	Study start, 1, 6, 12, and 18 months, study termination
Reovirus 3	Study start, 1, 6, 12, and 18 months, study termination
Sendai	Study start, 1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
EDIM	Study termination
LCM	1 and 6 months
Mouse adenoma virus-FL	6 months
MCMV (mouse cytomegalovirus)	Study termination
MHV	Study start, 12 months
MPV	Study termination
PVM	18 months
Reovirus 3	6 months
Polymerase Chain Reaction	
Helicobacter species	18 months

RESULTS

All test results were negative.

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