NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF MALACHITE GREEN CHLORIDE AND LEUCOMALACHITE GREEN

(CAS NOS. 569-64-2 AND 129-73-7)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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

February 2005

NTP TR 527

NIH Publication No. 05-4463

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

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and NCTR and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Details about ongoing and completed NTP studies, abstracts of all NTP Technical Reports, and full versions of the completed reports are available at the NTP's World Wide Web site: http://ntp.niehs.nih.gov. In addition, printed copies of these reports are available from NTP as supplies last by contacting (919) 541-1371.

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The studies on malachite green chloride and leucomalachite green were conducted at the FDA's National Center for Toxicological Research under an interagency agreement between the FDA and the NIEHS. The studies were designed and monitored by a Toxicology Study Selection and Review Committee composed of representatives from the NCTR and other FDA product centers, NIEHS, and other *ad hoc* members from other government agencies and academia. The interagency agreement was designed to use the staff and facilities of the NCTR in the testing of FDA priority chemicals and to provide FDA scientists and regulatory policymakers information for hazard identification and risk assessment.

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SUMMARY

Background

Malachite green chloride is a dye used to prevent fungus infections in commercial fisheries. Leucomalachite green is formed from malachite green and remains in the tissues of exposed fish. We studied the effects of malachite green on female rats and female mice, and the effects of leucomalachite green on male and female rats and female mice, to identify potential toxic or cancer-related hazards to humans.

Methods

For each study we mixed the dye into the feed of rats and mice. The doses of malachite green chloride given were 100, 300, or 600 parts per million (ppm) for female rats and 100, 225, or 450 ppm for female mice. Doses of leucomalachite green were 91, 272, or 543 ppm for male and female rats and 91, 204, or 408 ppm for female mice. There were 48 animals in each dose group. Control animals received the same feed with no chemical added. The study lasted for two years. Tissues from more than 40 sites were examined for every animal.

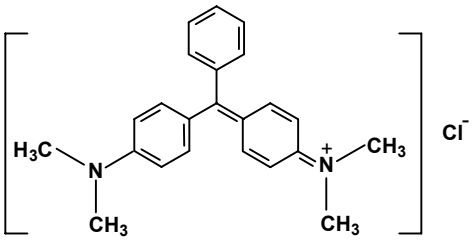
Results

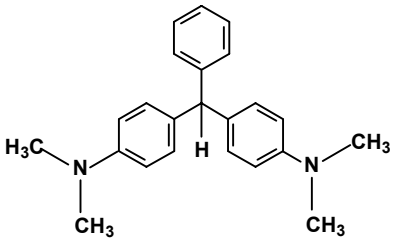
Rats, but not mice, exposed to malachite green chloride or leucomalachite green weighed less on average than the control animals. In rats exposed to the dyes, there were very slight increases in a few types of tumors: cancers of the thyroid gland, liver, and mammary gland in females exposed to malachite green chloride; of the thyroid gland and testes in males exposed to leucomalachite green; and of the thyroid gland and liver of females exposed to leucomalachite green. We saw no increase in cancers in female mice given malachite green chloride, but there was in increase in liver tumors in female mice given leucomalachite green.

Conclusions

We conclude that tumors of the thyroid gland, liver, or mammary gland in female rats might have been caused by malachite green chloride, but that malachite green chloride did not cause cancer in female mice. We conclude that leucomalachite green might have caused cancers of the thyroid gland in male and female rats, and of the testes in male rats and liver in female rats. Leucomalachite green caused an increase in cancer of the liver in female mice.

ABSTRACT





MALACHITE GREEN CHLORIDE

CAS No. 569-64-2

Chemical Formula: $C_{23}H_{25}ClN_2$ Molecular Weight: 364.92

LEUCOMALACHITE GREEN

CAS No. 129-73-7

Chemical Formula: $C_{23}H_{26}N_2$ Molecular Weight: 330.48

Malachite Green Chloride

Synonyms: *bis*[p-(Dimethylamino)phenyl]phenylmethylium chloride; *N*-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-*N*-methylmethanaminium chloride

Trade names: Aniline Green; Benzal Green; Benzaldehyde Green; China Green; C.I. Basic Green 4; C.I. 42000; Diamond Green B; Diamond Green Bx; Diamond Green P Extra; Fast Green; Light Green N; New Victoria Green Extra I; New Victoria Green Extra II; New Victoria Green Extra O; Solid Green O; Victoria Green B; Victoria Green WB

Leucomalachite Green

Synonym: p,p′-Benzylidenebis-*N*,*N*-dimethylaniline

Malachite green chloride is a triphenylmethane dye used in the fish industry as an antifungal agent. Leucomalachite green is formed by the reduction of malachite green chloride and persists in the tissues of exposed fish. These compounds were nominated for study by the United States Food and Drug Administration because of the potential for significant worker and consumer exposure, structural similarity to gentian violet, and lack of carcinogenicity data. After consideration of data obtained from a 28-day feeding study (NTP, 2004), female F344/N Nctr BR rats and B6C3F$_1$/Nctr BR mice were exposed to malachite green chloride (approximately 87% pure) in feed for 2 years. Male and female F344/N Nctr BR rats and female B6C3F$_1$/Nctr BR mice were exposed to leucomalachite green (99% pure) in feed for 2 years.

2-Year Study in Rats

Malachite Green Chloride

Groups of 48 female rats were fed diets containing 0, 100, 300, or 600 ppm malachite green chloride for 2 years (equivalent to average daily doses of approximately 0, 7, 21, and 43 mg malachite green chloride/kg body weight). Survival of exposed groups was similar to that of the control group. Mean body weights of the 300 and 600 ppm females were generally less than those of

the controls. Feed consumption by exposed groups of rats was generally similar to that by the control group. The relative liver weight was significantly increased in the 600 ppm females.

Thyroid follicular cell adenomas and carcinomas occurred in female rats receiving 300 or 600 ppm malachite green chloride, and the combined incidence of adenomas and carcinomas exceeded the historical control range. A low incidence of cystic follicles was observed in the thyroid gland of exposed females. The increases were minimal and not statistically significant; however, due to the rarity of the tumor and related nonneoplastic changes, these increases may be biologically significant. Hepatocellular adenomas were minimally increased (not statistically significant) in the female rats exposed to 300 and 600 ppm malachite green and the incidence in all the exposed and control groups exceeded the historical control range. The incidence of eosinophilic foci in the liver was increased in the exposed groups of rats. The incidence of mononuclear cell leukemia decreased with an exposure-related trend in females; the incidences were significantly decreased in the 300 and 600 ppm females. Mammary gland carcinomas were increased (not statistically significant) in the female rats receiving 600 ppm and the incidence exceeded the historical control range. The reduction in body weight of the treated rats may have reduced the statistical power to demonstrate a more robust effect.

Leucomalachite Green

Groups of 48 male and female rats were fed diets containing 0, 91, 272, or 543 ppm leucomalachite green in feed for 2 years (equivalent to average daily doses of approximately 0, 5, 15, and 30 mg leucomalachite green/kg body weight to males and 0, 6, 17, and 35 mg/kg body weight to females). Survival of 272 ppm males was greater than that of the controls. Mean body weights of 543 ppm males and females and 272 ppm females were less than those of the controls throughout the study; mean body weights of 272 ppm males and 91 ppm females were less than those of the controls during year 2 of the study. Feed consumption by 543 ppm males and females was intermittently less than that by the controls throughout the study; feed consumption by 272 ppm females was intermittently less during year 2 of the study. Liver weights were significantly increased for 272 and 543 ppm males; relative liver weights were significantly increased for 272 and 543 ppm females. Relative thyroid gland weights of 543 ppm males and females were significantly increased.

Hepatocellular adenomas were minimally increased (not statistically significant) in female rats exposed to 91 or 543 ppm leucomalachite green, with the incidence exceeding the historical control range. Nonneoplastic liver lesions including eosinophilic focus, cystic degeneration, and cytoplasmic vacuolization were generally significantly increased in the exposed groups of male and female rats. Thyroid gland follicular cell adenomas or carcinomas (combined) and cysts were observed in exposed males and females. The increases were minimal and not statistically significant; however, the increases may be biologically significant due to the rarity of the neoplasm and related nonneoplastic changes. Testicular interstitial cell adenoma occurred with a positive trend in male rats, and the incidence was significantly increased in the 543 ppm group. The broad range of incidences in the historical control data and the uncertainty of the relationship between pituitary gland neoplasms and testicular adenomas may confound this result. The incidences of mononuclear cell leukemia were significantly decreased in exposed rats. The incidences of pituitary gland adenoma were significantly decreased in exposed male rats.

2-Year Study in Mice

Malachite Green Chloride

Groups of 48 female mice were fed diets containing 0, 100, 225, or 450 ppm malachite green chloride for 2 years (equivalent to average daily doses of approximately 0, 15, 33, and 67 mg malachite green chloride/kg body weight). Survival of exposed groups was similar to that of the controls. Mean body weights of exposed mice were generally similar to the control group throughout most of the study. Feed consumption by exposed groups was generally similar to that by the controls. Relative kidney weights of exposed groups of mice were generally less than those of the controls.

There were no increased incidences of neoplasms in exposed mice. Incidences of intracytoplasmic inclusions of the urinary bladder were significantly increased in exposed mice.

Leucomalachite Green

Groups of 48 female mice were fed diets containing 0, 91, 204, or 408 ppm leucomalachite green for 2 years (equivalent to average daily doses of approximately 0, 13, 31, and 63 mg leucomalachite green/kg body weight). Survival of exposed groups was similar to that of the controls. Mean body weights were generally similar to those of the controls. Feed consumption by exposed groups was generally similar to that by the controls. Relative kidney weights were significantly decreased in all dose groups.

The incidences of hepatocellular adenoma or carcinoma (combined) occurred with a positive trend and the incidence was significantly increased in 408 ppm mice. The incidences of hepatocellular adenoma were increased (not statistically significant) in exposed mice.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity** of malachite green chloride in female F344/N rats based on the occurrence of thyroid gland follicular cell adenoma or carcinoma (combined) and marginal increases in hepatocellular adenoma and mammary gland carcinoma in exposed rats. There was *no evidence of carcinogenic activity* of malachite green chloride in female $B6C3F_1$ mice exposed to 100, 225, or 450 ppm.

Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* of leucomalachite green in male F344/N rats based on an increase in interstitial cell adenoma of the testes and the occurrence of thyroid gland follicular cell adenoma or carcinoma (combined) in exposed rats. There was *equivocal evidence of carcinogenic activity* of leucomalachite green in female F344/N rats based on a marginally increased incidence of hepatocellular adenoma and the occurrence of thyroid gland follicular cell adenoma or carcinoma (combined) in exposed rats. There was *some evidence of carcinogenic activity* of leucomalachite green in female $B6C3F_1$ mice based on an increase in hepatocellular adenoma or carcinoma (combined).

Exposure to malachite green chloride in feed resulted in nonneoplastic lesions in the thyroid gland and liver of female rats and the urinary bladder of female mice. Exposure to leucomalachite green in feed resulted in nonneoplastic lesions in the thyroid gland and liver of male and female rats and the urinary bladder of female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

Summary of the 2-Year Carcinogenesis Studies of Malachite Green Chloride and Leucomalachite Green

	Malachite Green Chloride	
	Female F344/N Rats	**Female $B6C3F_1$ Mice**
Concentrations in feed	0, 100, 300, 600 ppm	0, 100, 225, 450 ppm
Body weights	300 and 600 ppm groups less than the control group	Exposed groups generally similar to the control group
Survival rates	29/48, 23/48, 32/48, 25/48	40/48, 44/48, 40/48, 41/48
Nonneoplastic effects	Thyroid gland: follicle, cyst (0/46, 1/48, 1/47, 3/46) Liver: eosinophilic focus (5/48, 10/48, 13/48, 14/48)	Urinary bladder: inclusion body cytoplasmic (7/47, 15/46, 34/45, 39/48)
Neoplastic effects	None	None
Equivocal findings	Thyroid gland: follicular cell, adenoma or carcinoma (0/46, 0/48, 3/47, 2/46) Liver: hepatocellular adenoma (1/48, 1/48, 3/48, 4/48) Mammary gland: carcinoma (2/48, 2/48, 1/48, 5/48)	None
Decreased incidences	Mononuclear cell leukemia: (19/48, 17/48, 10/48, 1/48)	None
Level of evidence of carcinogenic activity	Equivocal	No evidence

Summary of the 2-Year Carcinogenesis Studies of Malachite Green Chloride and Leucomalachite Green

	Leucomalachite Green		
	Male F344/N Rats	**Female F344/N Rats**	**Female $B6C3F_1$ Mice**
Concentrations in feed	0, 91, 272, or 543 ppm	0, 91, 272, or 543 ppm	0, 91, 204, or 408 ppm
Body weights	272 and 543 ppm groups less than the control group	Exposed groups less than the control group	Exposed groups generally similar to the control group
Survival rates	23/48, 29/47, 34/48, 30/47	33/48, 36/48, 35/48, 33/48	37/48, 41/48, 39/48, 39/48
Nonneoplastic effects	Thyroid gland: follicle cyst (0/47, 0/47, 0/48, 3/46) Liver: eosinophilic focus (3/48, 14/47, 19/48, 33/47); cystic degeneration (4/48, 18/47, 13/48, 19/47)	Thyroid gland: follicle cyst (0/46, 1/46, 0/47, 2/48) Liver: eosinophilic focus (3/48, 12/48, 20/48, 16/48); vacuolization cytoplasmic (5/48, 5/48, 17/48, 22/48)	Urinary bladder: inclusion body intracytoplasmic (14/46, 33/48, 44/47, 44/44)
Neoplastic effects	None	None	Liver: hepatocellular adenoma or carcinoma (3/47, 6/48, 6/47, 11/47)
Equivocal findings	Thyroid gland: follicular cell adenoma or carcinoma (0/47, 2/47, 1/48, 3/46) Testes: interstitial cell, adenoma (37/48, 42/47, 43/48, 45/47); bilateral, interstitial cell adenoma (22/48, 30/47, 38/48, 39/47)	Thyroid gland: follicular cell carcinoma or adenoma (0/46, 1/46, 2/47, 1/48) Liver: hepatocellular adenoma (1/48, 3/48, 0/48, 3/48)	None
Decreased incidences	Mononuclear cell leukemia: (29/48, 16/47, 19/48, 7/47) Pituitary gland: adenoma (30/45, 19/46, 21/48, 13/45)	Mononuclear cell leukemia: (17/48, 8/48, 5/48, 8/48)	None
Level of evidence of carcinogenic activity	Equivocal	Equivocal	Some evidence

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on malachite green chloride and leucomalachite green on February 17, 2004, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

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

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On February 17, 2004, the draft Technical Report on the toxicology and carcinogenesis studies of malachite green chloride and leucomalachite green received public review by the National Toxicology Program's Board of Scientific Counselor's Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. S.J. Culp, NCTR, introduced the toxicology and carcinogenesis studies of malachite green chloride and leucomalachite green by describing the use of the chemicals as an antifungal agent in fisheries, the study design and dose setting, and the data on body weight, blood parameters, and lesions of the liver, mammary gland, thyroid gland, and testes. The proposed conclusions were *equivocal evidence of carcinogenic activity* of malachite green chloride in female F344/N rats, *no evidence of carcinogenic activity* of malachite green chloride in female $B6C3F_1$ mice exposed to 100, 225, or 450 ppm, *equivocal evidence of carcinogenic activity* of leucomalachite green in male and female F344/N rats, and *some evidence of carcinogenic activity* of leucomalachite green in female $B6C3F_1$ mice.

Dr. Storer, the first principal reviewer, felt the genotoxicity, DNA adduct, and transgenic mouse results should be presented more prominently. He also requested more discussion of the alteration in thyroid hormone levels.

Dr. Elwell, the second principal reviewer, thought the dose selection rationale could be clarified. He questioned the justification for combining mammary gland adenomas and carcinomas, while omitting fibroadenomas, to obtain a combination for equivocal evidence. He also thought the lymphocytic infiltrate noted in the malachite green study was a common background lesion. He asked for more explanation of the interpretation of retinal degeneration and also for some discussion comparing these compounds and gentian violet. Regarding the conclusions, he suggested deleting mention of lymphocytic infiltration in mice in the malachite green study and the mammary gland neoplasms in female rats in the leucomalachite green study.

Dr. Roberts, the third principal reviewer, also felt more justification was needed for the dose selection. He thought the retinal degeneration was potentially compound related. He also questioned the study design that focused mainly on female animals, based on 4-week study results.

Dr. Culp agreed to expand the study design and dose selection rationales and the genetic toxicology section.

Dr. P.W. Mellick, Pathology Associates International, agreed that combining fibroadenomas with the other mammary gland tumors would eliminate any effect. Dr. J.R. Bucher, NIEHS, offered that the link between carcinomas and adenomas may be more persuasive than the link between carcinomas and fibroadenomas and noted that these neoplasms occurred despite lower body weights.

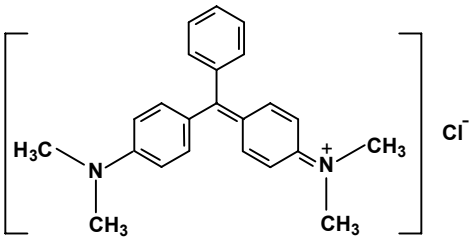
Dr. Culp said the retinal degeneration was central rather than peripheral and was not likely due to fluorescent lighting because the cage placements were rotated. She defended the decision to use primarily female animals, noting the females had more severe effects in the range-finding studies. Dr. F.A. Beland, NCTR, added that the lower background rates for liver tumors in females enhanced the ability to discern small increases.

Dr. Klaunig inquired about the liver adenomas in female rats for both chemicals. Dr. Culp said that while the marginal increases were not statistically significant, the incidences were outside the historical control ranges. Dr. Beland added that the genotoxicity data supported a genotoxic mechanism in the mouse liver while the response in the rat liver was equivocal.

Dr. Storer moved that the conclusions for the malachite green study be accepted as written except that lymphocytic infiltration of the liver be removed from the list of nonneoplastic lesions for female mice. Dr. Elwell seconded the motion. Dr. Piegorsch asked for the reasons supporting the inclusion of thyroid, liver, and mammary gland neoplasms as equivocal responses for female rats. Dr. Elwell explained that for each site there was an occurrence of a few neoplasms; these marginal increases did not achieve statistical significance but did exceed the observed historical control ranges. Dr. Andrews added that noting these equivocal responses was appropriate because the animals might have been able to tolerate even higher doses. The motion was approved with ten members in favor and two opposing.

Dr. Elwell suggested that the conclusions for the leucomalachite green study be accepted as written except that mammary gland neoplasms be removed from the list of lesions supporting equivocal evidence in female rats. There was discussion about which types of mammary gland neoplasms could most appropriately be combined for statistical analyses. Dr. J.R. Hailey, NIEHS, said progression from benign to malignant tumors was less common in the mammary gland than at some other sites. Dr. Storer moved to accept the conclusions as written, including the mammary gland neoplasms. The motion failed for lack of a second. Dr. Storer then moved to accept the conclusion as written with the mammary gland neoplasms for female rats deleted. Dr. Elwell seconded the motion. Dr. Piegorsch inquired whether the thyroid gland neoplasms should also be removed from the conclusion. Dr. Storer replied that these were very uncommon tumors and there was supporting biologic plausibility based on the disturbance of thyroid hormone homeostasis. Dr. Storer also noted that, in support of retaining the liver lesions in the conclusion, only one adenoma had been seen in the control groups for six other studies. The motion was approved with eleven members in favor and one opposing.

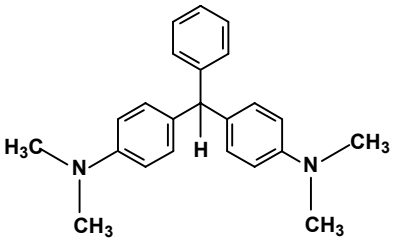
INTRODUCTION



MALACHITE GREEN CHLORIDE

CAS No. 569-64-2

Chemical Formula: $C_{23}H_{25}ClN_2$ Molecular Weight: 364.92



LEUCOMALACHITE GREEN

CAS No. 129-73-7

Chemical Formula: $C_{23}H_{26}N_2$ Molecular Weight: 330.48

Malachite Green Chloride

Synonyms: *bis*[p-(Dimethylamino)phenyl]phenylmethylium chloride; *N*-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-*N*-methylmethanaminium chloride

Trade names: Aniline Green; Benzal Green; Benzaldehyde Green; China Green; C.I. Basic Green 4; C.I. 42000; Diamond Green B; Diamond Green Bx; Diamond Green P Extra; Fast Green; Light Green N; New Victoria Green Extra I; New Victoria Green Extra II; New Victoria Green Extra O; Solid Green O; Victoria Green B; Victoria Green WB

Leucomalachite Green

Synonym: p,p′-Benzylidenebis-*N*,*N*-dimethylaniline

Chemical and Physical Properties

Malachite green chloride is a green crystal with a metallic luster; it is soluble in ethanol, methanol, and amyl alcohol and is very soluble in water. Neutral water solutions are blue-green with an absorption maximum of 616.9 nm; aqueous solutions are yellow below pH 2 (*Merck Index*, 1996). Leucomalachite green is a faint green solid, with an absorption maximum of 266 nm in tetrahydrofuran and an extinction coefficient of 3.34×10^4 M^{-1} (Chemsyn Science Laboratories, unpublished data).

Production, Use, and Human Exposure

Malachite green chloride, a triphenylmethane dye, is prepared as a double salt with zinc chloride for use as a dye. It is synthesized in a stepwise reaction that involves the condensation of benzaldehyde with *N,N*-dimethylaniline and oxidation of the resulting *bis*(p-dimethylaminophenyl)phenylmethane, followed by reaction of the product with hydrochloric acid (Nelson, 1974). Leucomalachite green is prepared by the reduction of malachite green.

The production and uses of malachite green chloride have been reviewed by Culp and Beland (1996). Malachite green is widely used in the dye industry and as an antifungal agent in fish hatcheries. Malachite green is not approved by the FDA or the U.S. Environmental Protection Agency for use on any aquatic species. However, it is relatively inexpensive, readily available, and highly efficacious; therefore, its continued use in some United States fisheries is likely. The chemical has been used routinely in aquaculture since

the early 1930s and is considered by many in the fish industry as the most effective antifungal agent (Schnick, 1988). In a study of over 180 compounds tested for antifungal activity, none equaled malachite green for efficacy and low toxicity (Meyer and Schnick, 1989). A broad range of malachite green concentrations has been used to treat fungal and parasitic infections, with doses of 100 ppm for a few seconds of dip (Nelson, 1974) to a prolonged treatment of 0.1 ppm in ponds (Stoskopf, 1993). Because of its use in commercial fish hatcheries and the dye industry, workers may be exposed to the chemical. The National Occupational Exposure Survey, conducted by the National Institute for Occupational Safety and Health between 1981 and 1983, estimated that more than 180,000 workers are potentially exposed to malachite green annually (NIOSH, 1990). The general public in the United States may become exposed to malachite green through the consumption of treated fish. Additional consumer exposure can occur via fish imported from Europe (Alabaster, 1982; Solbé, 1982; Schlotfeldt, 1992) and Canada (Thorburn and Moccia, 1993), where the use of malachite green has been documented. Random sampling from markets in the United Kingdom indicates the continued use of malachite green in aquaculture (VRC, 2002). Concern about the use of malachite green has also been raised in the United Kingdom. A report prepared by the Water Research Centre for the Department of the Environment, Transport, and the Regions of the United Kingdom recommended an annual average environmental quality standard of 500 ng/L malachite green for the protection of freshwater aquatic life, although no standards were recommended for drinking water due to a lack of data (Burchmore and Wilkinson, 1993).

Human exposure to leucomalachite green has also been documented (Doerge *et al*., 1998a; VRC, 2002). Doerge *et al.* (1998) analyzed edible flesh from trout purchased from retail outlets in the United Kingdom during 1994 and 1995 as part of a nonregulatory food surveillance program. Eight of the 12 samples were positive for malachite green (0.4 to 3.4 ppb) and leucomalachite green (9.0 to 96 ppb). Most noteworthy, the concentration of leucomalachite green in the samples was 12 to 37 times higher than that of malachite green.

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Until the early 1990s little was known about the metabolism of malachite green. Most studies have focused on the metabolism of malachite green in fish. Retention of malachite green in fish was demonstrated by Alderman and Clifton-Hadley (1993) when they exposed trout to a 1.6 ppm malachite green bath treatment for 40 minutes to examine the uptake, distribution, and elimination of the dye. Maximum concentrations of malachite green in serum, liver, and kidney were detected immediately after exposure, and levels ranged from 7.8 to 34.0 ppm; a peak concentration of 10.8 ppm was reached in muscle 90 to 120 minutes after exposure.

The reduction of malachite green to leucomalachite green and the persistence of the dye in this form were demonstrated in the 1960s. Werth and Boiteux (1968) reported the detection of leucomalachite green in liver, kidney, heart, lung, and muscle of rats 2 hours after an intravenous injection of malachite green and in Ehrlich's ascitic tumor cells 3 hours after intraperitoneal injection of malachite green. Evidence of the absorption of malachite green in fish was first reported by Poe and Wilson (1983), who found that frozen fillets of channel catfish treated with malachite green developed a marked green color over a period of weeks after slaughter. They deduced that malachite green was transformed to leucomalachite green upon absorption in fish tissue. Leucomalachite green was oxidized back to malachite green during freezer storage. This was confirmed by the observations of Bauer *et al*. (1988) of the rapid excretion of malachite green in trout, while leucomalachite green was stored in muscle tissue for a relatively long time with a half-life of about 40 days. In another study, Law (1994) demonstrated a rapid absorption of malachite green in fingerling trout exposed to 2 ppm malachite green for 1 hour. The amount of malachite green measured in whole tissue homogenates decreased with time, ranging from approximately 1 ppm 2 hours after treatment to 0.2 ppm 169 hours after treatment. However, the levels of leucomalachite green increased to 3.5 ppm 24 hours after treatment and remained steady

for the remainder of the 7-day study. Analysis of methylene chloride extracts of liver, muscle, and skin 73 hours or more after treatment showed the presence of leucomalachite green but little or no malachite green. Likewise, Plakas *et al*. (1996) measured leucomalachite green levels in the muscle and plasma of channel catfish after exposure to 0.8 ppm malachite green for 1 hour. In the plasma, malachite green levels were less than 0.03 ppm within 10 hours, while leucomalachite green residues were detected for up to 4 weeks. In the muscle, malachite green levels were found for up to 2 weeks and leucomalachite green persisted for 6 weeks. The calculated half-lives in catfish muscle were 2.8 and 10 days for malachite green and leucomalachite green, respectively.

More recently, Doerge *et al*. (1998a), using liquid chromatography/mass spectrometry, identified demethylated derivatives of leucomalachite green and an oxidation product of malachite green in malachite green-treated catfish. The presence of *N*-demethylated and *N*-oxide malachite green and leucomalachite green metabolites, including primary arylamines, was detected in the liver of rats fed 100 or 600 ppm malachite green or similar concentrations of leucomalachite green for 28 days (Culp *et al*., 1999). This may indicate that these compounds are metabolized in a manner similar to carcinogenic aromatic amines.

Humans

No studies on the absorption, distribution, metabolism, or excretion of malachite green chloride or leucomalachite green in humans were found in a review of the literature.

TOXICITY

Experimental Animals

A number of studies on the toxicity of malachite green reported in the early literature did not adequately identify the purity of the malachite green used or the counterion (e.g., malachite green chloride, malachite green oxalate) with which the dye was associated and should be reviewed with caution. Due to extensive use of malachite green in aquaculture, the majority of toxicity studies have been conducted with fish. Results from a number of these studies are summarized below. More extensive listings can be found in Nelson (1974), Bills *et al*. (1977), and Burchmore and Wilkinson (1993).

Bills *et al*. (1977) determined the acute toxicity of malachite green chloride to fingerling fish and nontarget aquatic organisms. After 96 hours of exposure, the LC_{50} values in fish ranged from 30.5 to 383 µg/L, with bluegills being the most sensitive and Coho salmon being the most resistant. Asiatic clams tolerated more than 100 mg/L, with a 96-hour LC_{50} value of 122 mg/L. The toxicity in all species increased with lengthening exposures. With a 3-hour or 6-hour treatment, the toxicity in some of the species was greater in warm water (17° or 22° C) than in cool water (7° or 12° C). Only channel catfish were more sensitive to increased water temperatures during 96 hours of exposure.

Biological effects, rather than mortality, have been the focus of many studies. Gerundo *et al.* (1991) treated rainbow trout with 1.6 ppm malachite green for 40 minutes once every 7 days for 7 weeks. After the third exposure, a fairly consistent pattern of increasing pathological changes was observed in most livers. This included sinusoidal congestion, focal coagulative necrosis, diffuse degenerative changes, and cytoplasmic vacuolation. Similar tissue changes have been reported in fish exposed to other toxins. At the ultrastructural level, mitochondrial damage was evident and was thought to be due to the dye's action as a respiratory enzyme poison. The gills generally demonstrated lesions and necrosis; the latter was more evident following longer periods of exposure.

Physiological changes in fish blood have been reported by a number of investigators. Grizzle (1977) continuously exposed fingerling channel catfish to 100 µg/L malachite green for up to 28 days and assayed blood samples at various times. Compared to the controls, a large increase in neutrophils was measured in exposed catfish 1 and 3 days after treatment and was thought to be indicative of an inflammatory response. Increases were also observed in erythrocyte counts and hemoglobin concentrations. The latter effect was attributed to impairment of gas exchange by the gills due to a thickening of the lamellar epithelium. In an earlier study, Glagoleva and Malikova (1968) found extensive leukopenia and slight erythropenia in Baltic salmon exposed to 1.33 mg/L malachite green for 20 minutes. After 6 days, the number of erythrocytes returned to normal levels, but the number of leukocytes remained low. Hlavek and Bulkley (1980) repeated the experiments and did not observe a difference in the number of leukocytes

in exposed fish when compared to controls. Leukocyte counts in both groups declined during the 24-hour period following treatment and recovered within the next 4 days. These researchers concluded that the leukocyte changes were due to nonspecific vertebrate stress syndrome as opposed to toxicity from exposure to malachite green chloride. Other blood chemistry parameters, including potassium, glucose, sodium, calcium, magnesium, and chloride concentrations, were measured in Coho salmon 28 days after exposure to 100 µg/L malachite green chloride (Bills and Hunn, 1976). An increase was found in potassium concentrations after exposure, while the other constituents remained unchanged.

The therapeutic use of malachite green is not restricted to freshwater species and has been extended to control fungal and epibiotic growth on eggs and larvae of cultured American lobsters. Fisher *et al*. (1976) noted that American lobster larvae exhibited decreased survival when treated with concentrations of malachite green greater than 8 ppm for 16 minutes every other day during their larval rearing period. Brief exposures to 20 ppm resulted in a delay in molting and a decrease in survival, with the majority of the dead animals showing the absence of one or more appendages.

In one of the first mammalian studies with malachite green, Lavender and Pullman (1964) infused malachite green into the renal arteries of dogs and found marked increases in the urinary excretion of water, sodium, potassium, chloride, calcium, and phosphate. The dye was localized primarily in the renal cortex, indicating proximal or distal tubular uptake. In addition, it appeared to cause a direct vasoconstriction of the renal arterioles.

In rabbit studies, Meyer and Jorgenson (1983) reported that nonpregnant New Zealand white rabbits were able to tolerate 13 consecutive daily gavage doses of 50 mg malachite green oxalate/kg body weight. Pregnant rabbits were also dosed with 5, 10, or 20 mg malachite green/kg body weight by gavage on days 6 through 18 of gestation and observed daily for external signs of toxicity. Feed consumption was reduced in treated animals and the average total body weight was consistently lower after 29 days, although there were no overt signs of toxicity. Females in the untreated group gained an average of 230 g. The animals given 5 mg/kg malachite green gained an average of 60 g, while those given 10 or 20 mg/kg lost 30 g and 60 g, respectively.

The instillation of an aqueous solution of 8% malachite green oxalate into the eyes of rabbits resulted in marked edema, substantial discharge, and slight hyperemia of the conjunctiva (Clemmensen *et al*., 1984). Treatment with fine crystals of malachite green oxalate caused total opacification and bright red and edematous conjunctivae that lasted for 2 weeks. Clemmensen *et al*. (1984) also treated the skin of guinea pigs and rats with 400 µL of a 20% suspension of malachite green oxalate and found no visible erythema or edema.

In rodent studies, male and female Wistar rats were administered aqueous solutions of malachite green oxalate by gavage; the animals were observed over a 14-day period (Clemmensen *et al*., 1984). Acute effects included reduced motor activity on the first day and hyperemia and atonia of the intestinal walls where the dye had reached before the death of the animal. Survivors were free of symptoms after 2 days. The oral LD_{50} was calculated to be 275 mg/kg body weight. These investigators also reported an LD_{50} of 50 mg/kg body weight for NMRI mice. The acute oral toxicity of malachite green has also been determined in female Sprague-Dawley rats. Meyer and Jorgenson (1983) administered 300, 450, 600, or 750 mg malachite green oxalate/kg body weight, presumably by gavage. The 24-hour LD_{50} value for malachite green was determined to be 520 mg/kg. Effects observed included depression, prostration, emaciation, coma, and death.

In a 28-day study, Wistar rats were exposed to 0, 10, 100, or 1,000 ppm malachite green oxalate in feed (Clemmensen *et al*., 1984). The animals exposed to 1,000 ppm showed significant decreases in feed consumption and weight gain and increased hyperactivity. In addition, females exposed to 1,000 ppm showed an increase in lymphocytes and decreases in neutrophils and packed cell volume. Males exposed to 1,000 ppm showed a significant increase in plasma urea.

Culp *et al*. (1998a,b; 1999) Fed male and female F344 rats and $B6C3F_1$ mice diets containing 0, 25, 100, 300, 600, or 1,200 ppm malachite green chloride for 28 days. Groups of male F344 rats and female $B6C3F_1$ mice were fed similar levels of leuco-malachite green (0, 290, 580, or 1,160 ppm) for 28 days. In the malachite green chloride study, male and female rats and female mice in the 1,200 ppm groups gained significantly less weight compared to the controls. In the leucomalachite green study,

male rats and female mice in the 580 and 1,160 ppm groups gained significantly less weight. Significant increases in gamma-glutamyltransferase activities were observed in the 600 and 1,200 ppm groups of female rats fed malachite green chloride. Blood hematology measurements in female rats exposed to 1,200 ppm malachite green chloride showed slight (<7%), but significant, decreases in erythrocyte count, hemoglobin, hematocrit, mean erythrocyte hemoglobin, and mean erythrocyte concentration. In male rats, slight (<3%), but significant, decreases in mean erythrocyte hemoglobin occurred in the 300, 600, and 1,200 ppm groups. In male and female mice fed malachite green chloride, there were significant decreases in erythrocyte count, hematocrit values, and hemoglobin concentrations. In the leucomalachite green studies, 1,160 ppm male rats had significantly lower erythrocyte counts, hematocrit values, and hemoglobin concentra-tions compared to the control group. Microscopically, the incidences of hepatocyte cytoplasmic vacuolization were significantly increased in male and female rats exposed to 1,200 ppm malachite green chloride and in male rats exposed to 580 or 1,160 ppm leucomalachite green. The incidence of multifocal apoptosis in transitional epithelium of the urinary bladder was significantly increased in female mice exposed to 1,160 ppm leucomalachite green. Additional groups of rats were fed control diet and the highest doses of malachite green chloride (male and female rats) or leucomalachite green (male rats) for 4 or 21 days and blood was collected for determination of thyroid stimulating hormone, triiodothyronine, and thyroxine concentrations. There were significant decreases in thyroxine concentrations and significant increases in thyroid stimulating hormone concentrations in the male rats fed 1,160 ppm leucomalachite green at both time points.

Humans

Malachite green has been reported to be injurious to the human eye (Grant, 1974). In addition, six of 11 eczema patients were found to be sensitized to patch tests using a 2% aqueous solution of malachite green (Bielicky and Novák, 1969). No toxicity studies or reports of health effects related to exposure to leucomalachite green in humans were found in the literature.

Reproductive and Developmental Toxicity

Experimental Animals

Meyer and Jorgenson (1983) observed significant teratologic effects in New Zealand white rabbits administered 0, 5, 10, or 20 mg malachite green oxalate/kg body weight by gavage on days 6 through 18 of gestation. At all three doses there were significant increases in preimplantation losses, primarily due to early resorption of fetuses and decreases in the number of living fetuses. The body weights of the progeny were less than those in the controls, with the differences being significant in the 5 and 20 mg/kg groups. Developmental anomalies were observed in all treated groups. Skeletal deviations were the most common abnormality and included incomplete ossification of vertebrae and phalanges and malformed skulls. Enlargement of the liver, heart, and abdominal cavity was also observed. The abnormalities did not increase as a function of dose, with 18.5%, 38.0%, 33.9%, and 47.0% of the progeny showing deviations in the 0, 5, 10, and 20 mg treatment groups, respectively. Thalidomide (150 mg/kg body weight), which was used as a positive control, caused similar types of changes in 94% of the progeny.

Damage can also occur to rainbow trout eggs upon exposure to malachite green. Using treatment regimens similar to those used in fisheries, Meyer and Jorgenson (1983) reported a delay in hatching, reduction in the average size of fry, and a significant increase in the percentage of fry with deformities. The abnormalities included head and jaw deformities, curvatures of the spine, missing fins, and a bobtailed condition. On the other hand, the percentage of eggs that hatched was increased in the treated groups compared to the untreated groups.

Humans

No studies of reproductive or developmental effects of malachite green chloride or leucomalachite green in humans were found in a review of the literature.

Carcinogenicity

Experimental Animals

The data relating to the carcinogenicity of malachite green are extremely limited. In a study by Fernandes *et al*. (1991), malachite green enhanced the formation of preneoplastic hepatic lesions in rats initiated with diethylnitrosamine. There is also suggestive evidence of carcinogenicity based on comparisons to gentian violet (reviewed in Culp and Beland, 1996). Gentian violet is a mixture of crystal violet (96%) and methyl violet. Crystal violet differs from malachite green only by the addition of a trimethylamine group. In a 2-year study, B6C3F$_1$ mice were administered 0, 100, 300, or 600 ppm

gentian violet in the diet (Littlefield *et al*., 1985). Dose-related lesions were observed in the liver and harderian gland of both sexes. A dose-related occurrence of reticulum cell sarcoma was also noted in the vagina, uterus, ovaries, and bladder of female mice. A 2-year feed study was also conducted with male and female Fisher 344 rats (Littlefield, 1988). The parents of these rats were exposed to 0, 100, 300, or 600 ppm gentian violet for at least 80 days, including during mating, and the offspring were continued on the same doses for 24 months. A treatment-related increase of follicular cell adenocarcinomas of the thyroid gland was observed in females, resulting in incidences of 1%, 1%, 5%, and 8% after exposure to 0, 100, 300, or 600 ppm gentian violet for 24 months; in the males, the incidences were 1%, 5%, 3%, and 6% in the four dose groups. Adenoma and adenocarcinoma of the clitoral gland were also observed grossly and confirmed microscopically after 24 months, at incidences of 12%, 6%, 18%, and 33% in the 0, 100, 300, and 600 ppm groups.

No data were found in the literature on the carcinogenicity of leucomalachite green.

Humans

No epidemiology studies of malachite green chloride or leucomalachite green have been reported.

Genetic Toxicity, DNA Adduct Formation, and Mutational Analyses

Published data provide little evidence for mutagenicity of malachite green or the metabolite leucomalachite green. Clemmensen *et al*. (1984) reported that malachite green oxalate was mutagenic in *Salmonella typhimurium* strain TA98 in the presence of S9 activation enzymes, but they observed no mutagenicity in TA100, TA1535, or TA1537, with or without S9. Another investigation of malachite green-induced mutagenicity in *Salmonella* found negative results in TA98, TA100, and TA1537, but these investigations were conducted only in the absence of S9 (Ferguson and Baguley, 1988). Fessard *et al*. (1999) reported an absence of mutagenicity for malachite green and leucomalachite green tested in concentrations up to 10 or 2,000 µg/plate, respectively, with and without S9 in *Salmonella* strains TA97a, TA98, TA100, and TA102. Assessment of mutagenicity of malachite green or leucomalachite green in the Chinese hamster ovary (CHO)/HGRT mammalian cell mutagenicity assay yielded negative results, with and without S9 activation (Fessard *et al*., 1999)

An earlier study by Wolfe (1977) reported that malachite green inhibited DNA replication processes in *Escherichia coli* that were catalyzed by polymerase I, and more recently, Panandiker *et al*. (1994) reported induction of DNA single-strand breaks in Syrian hamster embryo cells exposed *in vitro* to 1 µg/mL malachite green.

Au and Hsu (1979) found no evidence of induced chromosomal aberrations in cultured CHO cells incubated for 5 hours with 20 µM malachite green. Furthermore, no increase in micronucleated erythrocytes was observed in bone marrow of mice administered a single dose of 37.5 mg/kg malachite green oxalate by gavage; the frequency of micronucleated erythrocytes was measured 24, 42, or 66 hours posttreatment (Clemmensen *et al*., 1984). The testing protocol used in this *in vivo* assay is not the currently accepted standard; nonetheless, the results of this investigation were clearly negative. Finally, in an abstract, negative results were reported in a mammalian spot test (*in vivo* mammalian mutation assay) conducted in mice treated with 10 to 40 mg/kg malachite green by gavage on days 8, 9, and 10 of pregnancy (Jensen, 1984); no increase in the number of recessive coat color spots was observed in the offspring of treated females.

In more recent studies (NTP, 2004), malachite green chloride was tested at concentrations of 0.1 to 10 µg/plate and was not mutagenic in any of several strains of *Salmonella typhimurium*, with or without S9 metabolic activation. Negative results for malachite green chloride were also obtained in two *in vivo* micronucleus tests; one assessed the induction of micronuclei in male rat bone marrow erythrocytes after three intraperitoneal injections of malachite green chloride and the second determined the level of micronuclei in circulating erythrocytes of male and female $B6C3F_1$ mice following 28-day exposures to malachite green chloride in feed. A weak increase in the frequency of micronucleated normochromatic erythrocytes in peripheral blood was observed in female mice exposed to leucomalachite green in feed for 28 days. Bone marrow was also examined for the induction of micronuclei in female Big Blue rats after 4, 16, and 32 weeks of

exposure to 0, 9, 27, 91, 272, or 543 ppm leucomalachite green in the feed (Manjanatha *et al*., 2004). No significant increase in the frequency of micronuclei was observed for any of the doses or time points assayed.

In a 28-day feeding study, male F344 rats and female $B6C3F_1$ mice were fed 0, 100, or 600 ppm malachite green or similar doses of leucomalachite green (0, 96, or 580 ppm; Culp *et al*., 1999). ^{32}P-Postlabeling analyses of liver DNA indicated a single adduct or co-eluting adducts with both compounds, with the adduct levels increasing significantly ($P<0.05$) as a function of the dose. With the rats, the hepatic DNA adduct levels did not differ between animals administered malachite green or leucomalachite green. In the mice, however, the highest dose of malachite green (600 ppm) gave a significantly ($P<0.001$) higher adduct level than observed with a similar dose (580 ppm) of leucomalachite green. Likewise, ^{32}P-postlabeling analyses of liver DNA from female Big Blue rats fed 0, 9, 27, 91, 272, or 543 ppm leucomalachite green for 4 weeks resulted in a dose-related increase in DNA adduct levels (Culp *et al*., 2002; Manjanatha *et al*., 2004). In these studies, analysis of the livers of female Big Blue rats for *lacI* mutations 4, 16, and 32 weeks after exposure revealed that 21% (17/80) were clonal in origin and that the majority (55/63) of the independent mutations were basepair substitutions involving GC to AT transitions similar to those found for control rats. These data suggest that leucomalachite green is not a mutagen in the livers of female rats and that the DNA adduct formed in the livers of rats fed leucomalachite green does not have a mutagenic consequence.

Study Rationale

The FDA's Center for Veterinary Medicine nominated malachite green for study because of the potential for occupational and consumer exposure, its structural similarity to gentian violet, and a lack of adequate carcinogenicity data. To assist in selecting doses for the 2-year bioassay, a 28-day range-finding study was conducted in which male and female $B6C3F_1$ mice and male and female F344 rats were fed malachite green chloride (0, 25, 100, 300, 600, or 1,200 ppm) in feed. Additional female $B6C3F_1$ mice and male F344 rats were fed leucomalachite green (0, 290, 580, or 1,160 ppm) because it is the major persistent residue of malachite green in edible tissues.

In the range-finding study, the highest doses of malachite green chloride and leucomalachite green caused body weight reductions of approximately 10% to 20% in rats (Culp *et al*., 1998a,b; 1999); thus, these doses were deemed unacceptable for the 2-year bioassay. Liver-to-body weight ratios were significantly elevated in female rats administered 300 ppm or greater malachite green chloride and in male rats fed 600 ppm or greater. Female rats fed malachite green chloride also had significant alterations in T_3 and T_4 levels and in serum gamma-glutamyltransferase, changes that did not occur in male rats fed malachite green. Since limitations were placed on the number of groups that could be included in the study and the data indicated that female rats were more sensitive than male rats to the toxic effects of malachite green chloride, the 2-year bioassay was restricted to female rats, with doses of 100, 300, and 600 ppm. A primary objective of the study was to compare the carcinogenic responses of malachite green chloride and leucomalachite green; thus, equimolar doses were administered in the 2-year bioassay. For rats, the 28-day range-finding study with leucomalachite green was conducted using only males; however, because females appeared to be more susceptible than males to the toxicities of malachite green chloride, both sexes were included in the 2-year bioassay with leucomalachite green. Female mice also appeared to be more sensitive than male mice to malachite green chloride, as indicated by changes in body weight and blood chemistry data. The effects were significantly altered in the 300, 600, and 1,200 ppm groups. Female mice fed leucomalachite green exhibited decreased body weight changes at 580 ppm and above and increased relative liver weight and apoptosis of the bladder at 1,160 ppm. As the number of groups in the study were limited and NTP bioassays have shown female mice to be more predictive of toxicity, the 2-year bioassay in mice was limited to female mice. After reviewing the data, the Toxicology Study Selection and Review Committee, which helped design and monitor the studies, recommended doses of 100, 225,and 450 ppm malachite green chloride and equimolar doses of leucomalachite green.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Malachite green chloride (lot CSL-98-808-08-04) and leucomalachite green (lot CSL-97-718-77-10) were obtained from Chemsyn Science Laboratories (Lenexa, KS). Identity, purity, and stability analyses were conducted by the study laboratory (Appendix E). Reports on analyses performed in support of the malachite green chloride studies are on file at the National Center for Toxicological Research (NCTR).

Malachite Green Chloride

Lot CSL-98-808-08-04, a dark solid, was identified as malachite green chloride by ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectroscopy, direct exposure probe electron impact mass spectrometry (DEP/EI/MS), and high-performance liquid chromatography (HPLC). The purity of malachite green chloride was determined by the study laboratory using HPLC, gas chromatography (GC), and inductively coupled plasma (ICP) spectrometry. HPLC using a post column derivatization with lead dioxide to oxidize leuco-compounds to compounds with spectra similar to that of malachite green chloride, indicated a purity of approximately 88%. Impurities were identified as leucomalachite green (7.5%), *N*-desmethyl malachite green (3.8%), and *N*-desmethyl leucomalachite green (0.5%). GC indicated that malachite green chloride contained 1.4% methanol by weight. ICP spectrometry indicated the presence of less than 4 ppm lead. The overall purity was determined to be approximately 87%.

The bulk chemical was stored in the original container at –70° C. Stability was monitored during the studies with HPLC. Analysis indicated a 3.1% degradation of the compound during the course of the 2-year studies.

Leucomalachite Green

Lot CSL-97-718-77-10, a faint green solid, was identified as leucomalachite green by the supplier using ^{1}H-NMR and infrared spectroscopy and by the study laboratory using DEP/EI/MS and ^{1}H- and ^{13}C-NMR. The purity of leucomalachite green was determined by elemental and heavy metal analysis (performed by Galbraith Laboratories, Inc., Knoxville, TN), HPLC performed by the chemical supplier and the study laboratory, and by the study laboratory using ^{1}H- and ^{13}C-NMR. The overall purity was approximately 99%.

The bulk chemical was stored in the original container at –70° C. Stability was monitored during the studies with HPLC. Analysis indicated a 0.6% degradation of the compound during the course of the 2-year studies. The impurity was identified as malachite green.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

For malachite green chloride, the dose formulations were prepared approximately every 2 months by dissolving the chemical in water and then mixing it with feed (Table E2). The solution was blended with feed in a Patterson-Kelly V-shell blender using an intensifier bar and a heater under a vacuum of at least 15 mm mercury for approximately 20 minutes. For leucomalachite green, dose formulations were prepared approximately every 2 months by mixing the chemical with feed (Table E2). A premix was prepared by hand, then blended with additional feed in a Patterson-Kelly V-shell blender using an intensifier bar for approximately 20 minutes. Dose formulations were stored in stainless steel feed cans at 4° ± 2° C for up to 92 days (malachite green chloride) or 95 days (leucomalachite green).

For malachite green chloride, a homogeneity study of a 100 ppm dose formulation and a stability study of a 25 ppm dose formulation were performed by the study laboratory using HPLC. Homogeneity was confirmed, and stability was confirmed for at least 10 days for dose formulations stored at room temperature exposed to light and for at least 92 days for formulations stored at up to 6° C protected from light. For leucomalachite green, homogeneity studies of 96 and 91 ppm dose formulations were performed by the study laboratory using HPLC. Stability studies of a 96 ppm dose formulation were also performed by the study laboratory with HPLC. Homogeneity was confirmed, and stability was confirmed for at least 32 days for dose formulations stored

at room temperature exposed to light and for at least 95 days for formulations stored at up to 6° C protected from light.

For both chemicals, periodic analyses of the dose formulations were conducted by the study laboratory using HPLC. The dose formulations were analyzed approximately every 7 weeks (Tables E3 and E4). Of the malachite green chloride dose formulations analyzed and used, 96% (65/68) of the dose formulations for rats and 97% (33/34) of the dose formulations for mice were within 10% of the target concentrations. All of the leucomalachite green dose formulations analyzed and used for rats and mice were within 10% of the target concentrations.

2-Year Studies

Study Design

Groups of 48 female mice were fed diets containing 0, 100, 225, or 450 ppm malachite green chloride or 0, 91, 204, or 408 ppm leucomalachite green for 104 weeks. Groups of 48 female rats were fed diets containing 0, 100, 300, or 600 ppm malachite green chloride for 104 weeks. Groups of 48 male and 48 female rats were fed diets containing 0, 91, 272, or 543 ppm leucomalachite green for 104 weeks.

Source and Specification of Animals

Male and female F344/N Nctr BR rats and female B6C3F$_1$/Nctr BR (C57BL/6N × C3H/HeN MTV$^-$) mice were obtained from the National Center for Toxicological Research (NCTR) breeding colony for use in the 2-year studies. Rats and mice were acclimated in the study rooms for 2 weeks and were approximately 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 Study Laboratory's Sentinel Animal Program (Appendix H).

Animal Maintenance

Rats were housed two per cage and mice were housed four per cage. Feed and water were available *ad libitum* until the day before sacrifice when feed was withheld overnight. Feed consumption was measured weekly for the first 12 weeks and approximately every 4 weeks thereafter. Cages were changed once a week and rotated every 3 weeks. Further details of animal maintenance are given in Table 1. Information on food composition and contaminants is provided in Appendix G.

Clinical Examinations and Pathology

All animals were observed twice daily and clinical findings were recorded weekly. Body weights were recorded initially, weekly for the first 12 weeks, approximately every 4 weeks until week 92, weekly for the last 12 weeks, and at the end of the studies.

Complete necropsies and microscopic evaluations were performed on all rats and mice. The kidneys and liver of rats and mice and the thyroid gland of rats were weighed. 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 Tissue Prep II, sectioned to a thickness of approximately 5 µm, and stained with hematoxylin and eosin for microscopic examination. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist and the pathology data for the malachite green chloride studies were entered into the NTP's Toxicology Data Management System (TDMS); the pathology data for the leucomalachite green studies were entered into the study laboratory's Micropath Data Management System, extracted and transferred to the TDMS. The slides, paraffin blocks, and residual wet tissues were sent to the study laboratory's Block and Slide Laboratory for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment group. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. A quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the liver of rats and mice, the thyroid gland of rats, the urinary bladder of mice, the lungs of rats exposed to leucomalachite green, and the forestomach of mice exposed to leucomalachite green.

Differences of opinion were reconciled between the study and quality assessment pathologists. The quality assessment pathologist served as chairperson of the Pathology Working Group (PWG) and presented histopathology slides containing the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing

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

TABLE 1
Experimental Design and Materials and Methods in the 2-Year Feed Studies of Malachite Green Chloride and Leucomalachite Green

Malachite Green Chloride	Leucomalachite Green
Study Laboratory National Center for Toxicological Research (Jefferson, AR)	Same as malachite green chloride study.
Strain and Species F344/N Nctr BR rats $B6C3F_1$/F344/Nctr BR (C57BL/6N × C3H/HeN MTV^-) mice	Same as malachite green chloride study
Animal Source National Center for Toxicological Research (Jefferson, AR)	Same as malachite green chloride study.
Acclimation Time 2 weeks	Same as malachite green chloride study.
Average Age When Studies Began 6 weeks	Same as malachite green chloride study.
Dates of First Exposure April 13-May 11, 1999	Rats: November 26-December 24, 1998 Mice: October 19-November 16, 1998
Duration of Exposure 104 weeks	Same as malachite green chloride study.
Dates of Last Exposure Rats: April 9-May 7, 2001 Mice: April 8-May 6, 2001	Rats: November 19-December 18, 2000 Mice: October 15-November 12, 2000
Necropsy Dates Rats: April 10-May 8, 2001 Mice: April 9-May 7, 2001	Rats: November 20-December 19, 2000 Mice: October 16-November 13, 2000
Average Age at Necropsy 109 weeks	Same as malachite green chloride study.
Size of Study Groups 48 females	Rats: 48 males and 48 females Mice: 48 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as malachite green chloride study.
Animals per Cage Rats: 2 Mice: 4	Same as malachite green chloride study.
Method of Animal Identification Ear clip	Ear clip

Table 1
Experimental Design and Materials and Methods in the 2-Year Feed Studies of Malachite Green Chloride and Leucomalachite Green

Malachite Green Chloride	Leucomalachite Green
Diet	
NIH-31 open formula meal pellets were autoclaved then ground to powder (Purina Mills, Richmond, IN), available *ad libitum* until the day before sacrifice	Same as malachite green chloride study.
Water	
Millipore-filtered water (Jefferson municipal supply) via 480 mL water bottles, available *ad libitum*	Same as malachite green chloride study.
Cages	
Polycarbonate cages (Allentown Caging Equipment Co., Allentown, NJ), changed weekly and rotated every 3 weeks	Same as malachite green chloride study.
Bedding	
Hardwood chips (Northeastern Products, Inc., Warrensburg, NY), changed weekly	Same as malachite green chloride study.
Cage Bonnets	
Microisolator tops (Lab Products, Inc., Maywood, NJ)	Same as malachite green chloride study.
Racks	
Metal animal cage racks (Allentown Caging Equipment Co., Allentown, NJ), changed weekly	Same as malachite green chloride study.
Animal Room Environment	
Average temperature: Rats: 73.3° F Mice: 72.9° F Average relative humidity: Rats 49.4% Mice: 51.5% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Average temperature: Rats: 73.8° F Mice: 73.5° F Average relative humidity: Rats 50.9% Mice: 50.8% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour
Exposure Concentrations	
Rats: 0, 100, 300, or 600 ppm in feed, available *ad libitum* Mice: 0, 100, 225, or 450 ppm in feed, available *ad libitum*	Rats: 0, 91, 272, or 543 ppm in feed, available *ad libitum* Mice: 0, 91, 204, or 408 ppm in feed, available *ad libitum*
Type and Frequency of Observation	
Observed twice daily and clinical findings recorded weekly. Animals were weighed initially, weekly for the first 12 weeks, approximately every 4 weeks until week 92, weekly for the last 12 weeks, and at the end of the studies. Feed consumption was recorded weekly for the first 12 weeks and approximately every 4 weeks thereafter.	Same as malachite green chloride study
Method of Sacrifice	
Carbon dioxide asphyxiation	Same as malachite green chloride study
Necropsy	
Necropsies were performed on all rats and mice. Organs weighed were the kidneys and liver (rats and mice) and the thyroid gland (rats).	Same as malachite green chloride study

TABLE 1
Experimental Design and Materials and Methods in the 2-Year Feed Studies of Malachite Green Chloride and Leucomalachite Green

Malachite Green Chloride	Leucomalachite Green
Histopathology	
Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone marrow (femur, sternum), brain (cerebellum, cerebrum, stem), clitoral gland, esophagus, eye, gallbladder (mice), harderian gland, heart with aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, muscle (thigh), nerve (sciatic), nose, ovaries, pancreas, parathyroid gland, pituitary gland, salivary gland, skin, spinal cord (thoracic), spleen, stomach (forestomach and glandular), thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, vagina, and Zymbal's gland.	Same as malachite green chloride study. In addition, the coagulating gland, epididymis, preputial gland, prostate gland, seminal vesicle, and testes were examined in male rats.

STATISTICAL METHODS

Survival Analyses

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

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A5, B1, B5, C1, and C5 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 A3, B3, and C3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, and C3 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, to animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the

animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). 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 $B6C3F_1$ mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

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

Analysis of Continuous Variables

For both body weights and food consumption, the mixed models approach to repeated measures ANOVA was used. Testing for linear and quadratic dose trends was conducted at each time interval. Organ weights, terminal body weights, and the ratios of organ weight to terminal body weight for terminally sacrificed animals were analyzed using ANOVA procedures. Terminal body weights were also used as a covariant in an ANACOVA procedure. For each end point analyzed, Dunnett's two-sided test (Dunnett, 1955) was used to compare the control group mean to each treatment group mean, either overall or at each point of time, whichever was appropriate.

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. The historical database for these studies included studies conducted by the NCTR using dietary or drinking water exposure.

Quality Assurance Methods

The studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). The Quality Assurance Unit of the NCTR performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report were conducted. Audit procedures and findings are presented in the reports and are on file at the NCTR. The audit findings were reviewed and assessed by NCTR staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

RESULTS

RATS

2-YEAR STUDIES

Survival

Malachite Green Chloride

Estimates of 2-year survival probabilities for female rats exposed to malachite green chloride are shown in Table 2 and in the Kaplan-Meier survival curves (Figure 1). Survival of all exposed groups was similar to that of the control group.

Leucomalachite Green

Estimates of 2-year survival probabilities for male and female rats exposed to leucomalachite green are shown in Table 3 and in the Kaplan-Meier survival curves (Figure 2). Survival of males in the 272 ppm group was greater than that of the control group; survival of all other exposed groups of males and all exposed groups of females was similar to that of the control group.

TABLE 2
Survival of Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
Animals initially in study	48	48	48	48
Moribund	18	22	15	17
Natural deaths	1	3	1	6
Animals surviving to study termination	29	23	32	25
Percent probability of survival at end of study[a]	60	48	67	52
Mean survival (days)[b]	675	665	700	647
Survival analysis[c]	P=0.739N	P=0.252	P=0.399N	P=0.369

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

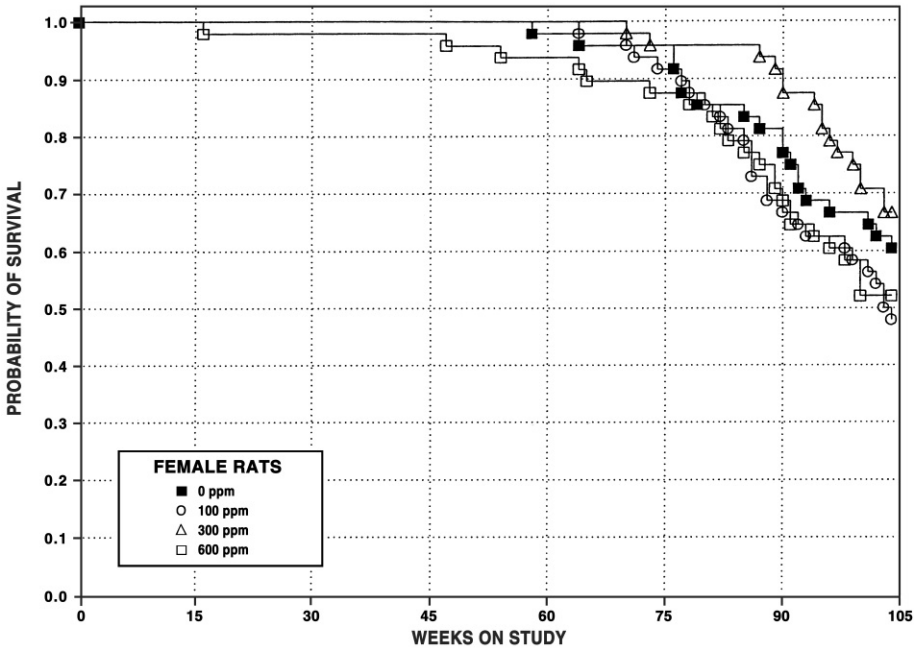
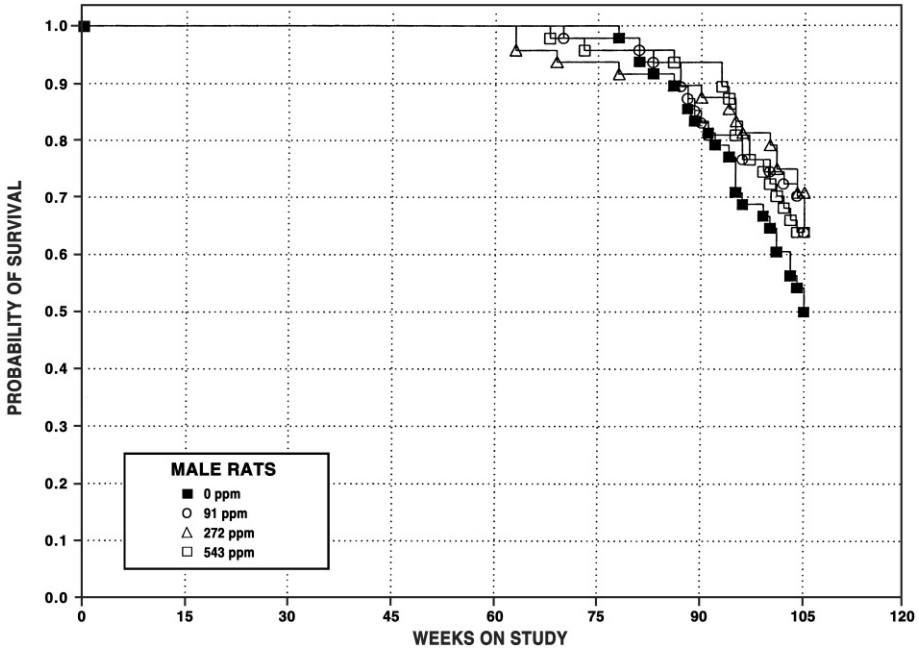


FIGURE 1
Kaplan-Meier Survival Curves for Female Rats Exposed to Malachite Green Chloride in Feed for 2 Years

TABLE 3
Survival of Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Male				
Animals initially in study	48	48	48	48
Missexed[a]	0	1	0	1
Moribund	20	12	11	10
Natural deaths	5	6	3	7
Animals surviving to study termination	23	29	34	30
Percent probability of survival at end of study[b]	48	62	71	64
Mean survival (days)[c]	683	692	690	695
Survival analysis[d]	P=0.163	P=0.185N	P=0.037N	P=0.133N
Female				
Animals initially in study	48	48	48	48
Moribund	12	11	12	11
Natural deaths	3	1	1	4
Animals surviving to study termination	33	36	35	33
Percent probability of survival at end of study	69	75	73	69
Mean survival (days)	698	697	690	689
Survival analysis	P=0.749N	P=0.462N	P=0.646N	P=0.946

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



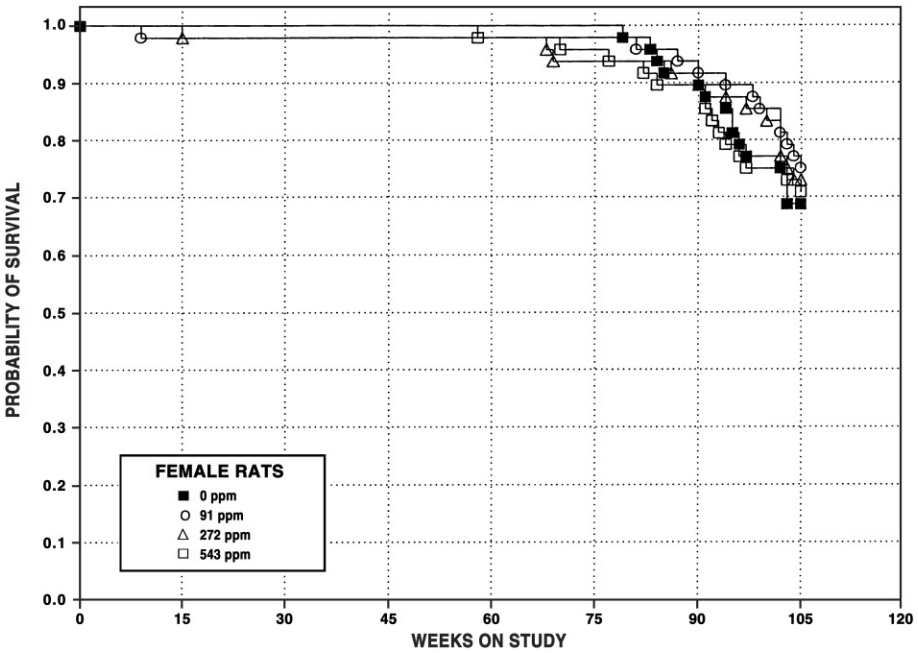


FIGURE 2
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to Leucomalachite Green in Feed for 2 Years

Body Weights, Feed and Compound Consumption, and Clinical Findings

Malachite Green Chloride

Mean body weights of female rats exposed to 300 or 600 ppm malachite green chloride were generally less than those of the controls during most of the study (Figure 3 and Table 4). Feed consumption by exposed rats was generally similar to that by controls throughout the study (Table F1). Dietary concentrations of 100, 300, or 600 ppm resulted in average daily doses of approximately 7, 21, or 43 mg malachite green chloride/kg body weight. No clinical findings were attributed to malachite green chloride exposure.

Leucomalachite Green

Mean body weights of 543 ppm males and females and 272 ppm females were less than those of the controls throughout the study (Figure 4; Tables 5 and 6). Mean body weights of 272 ppm males and 91 ppm females were less than those of the controls during year 2 of the study (Figure 4;Tables 5 and 6). Feed consumption by males and females exposed to 543 ppm leucomalachite green was intermittently less than that by the controls throughout the study; feed consumption by 272 ppm females was intermittently less than that by controls during year 2 of the study (Tables F2 and F3). Dietary concentrations of 91, 272, or 543 ppm resulted in average daily doses of approximately 5, 15, or 30 mg leucomalachite green/kg body weight to males and 6, 17, or 35 mg/kg body weight to females. No clinical findings were attributed to leucomalachite green exposure.

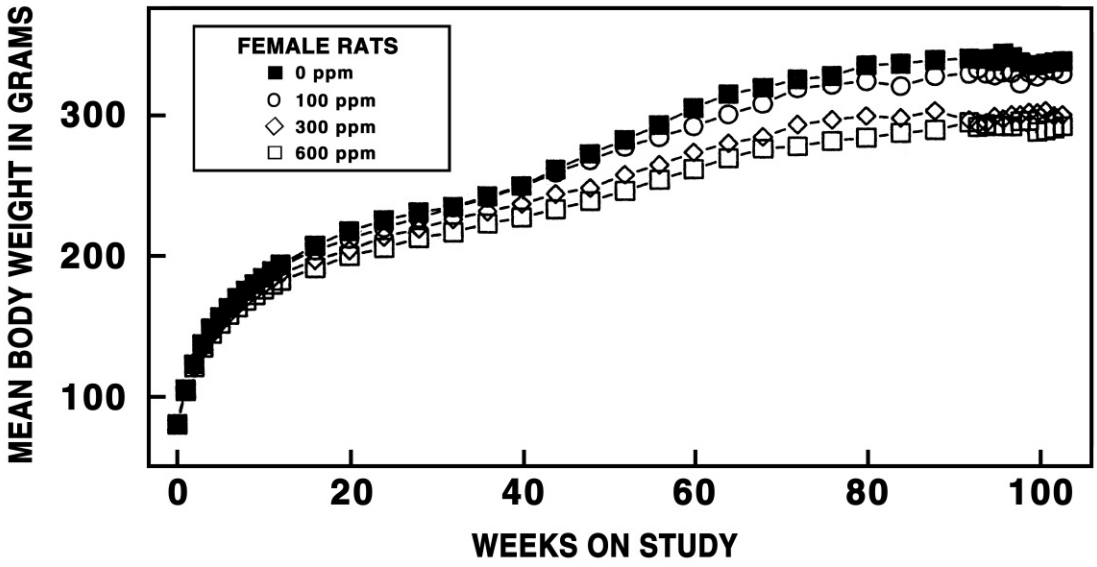


FIGURE 3
Growth Curves for Female Rats Exposed to Malachite Green Chloride in Feed for 2 Years

TABLE 4
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of Malachite Green Chloride

Weeks on Study	0 ppm		100 ppm			300 ppm			600 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	105	48	105	100	48	103	98	48	103	98	48
2	123	48	122	99	48	121	98	48	121	98	48
3	138	48	137	99	48	135	98	48	135	98	48
4	149	48	148	99	48	144	97	48	145	97	48
5	157	48	154	99	48	152	97	48	152	97	48
6	163	48	163	100	48	159	97	48	158	97	48
7	170	48	170	100	48	165	97	48	163	96	48
8	175	48	175	100	48	170	97	48	168	96	48
9	180	48	179	100	48	175	97	48	172	96	48
10	184	48	184	100	48	179	97	48	176	95	48
11	189	48	188	100	48	183	97	48	179	95	48
12	193	48	192	99	48	187	97	48	182	94	48
16	206	48	203	98	48	196	95	48	191	92	47
20	217	48	211	97	48	203	94	48	199	92	47
24	224	48	219	98	48	213	95	48	205	91	47
28	230	48	225	98	48	218	95	48	211	92	47
32	233	48	233	100	48	225	96	48	216	92	47
36	241	48	240	99	48	230	95	48	222	92	47
40	249	48	248	100	48	237	95	48	226	91	47
44	261	48	258	99	48	244	94	48	231	89	47
48	272	48	269	99	48	250	92	48	238	87	46
52	282	48	277	98	48	260	92	48	244	86	46
56	292	48	284	97	48	267	91	48	251	86	45
60	305	47	292	96	48	276	91	48	259	85	45
64	315	46	302	96	47	283	90	48	266	84	44
68	320	46	310	97	47	288	90	48	276	86	43
72	325	46	315	97	45	295	91	47	279	86	43
76	330	44	317	96	44	301	91	46	281	85	42
80	335	41	326	97	41	303	90	46	281	84	41
84	337	41	322	95	39	301	89	46	288	85	38
88	335	39	328	98	33	302	90	45	291	87	36
92	340	34	326	96	31	298	88	42	295	87	31
96	343	32	327	95	30	297	86	38	298	87	29
100	335	32	322	96	29	298	89	36	295	88	25
103	332	30	326	98	24	300	90	32	293	88	25
Mean for weeks											
1-13	160		160	100		156	97		155	96	
14-52	242		238	99		228	94		218	90	
53-103	325		313	96		293	90		279	86	

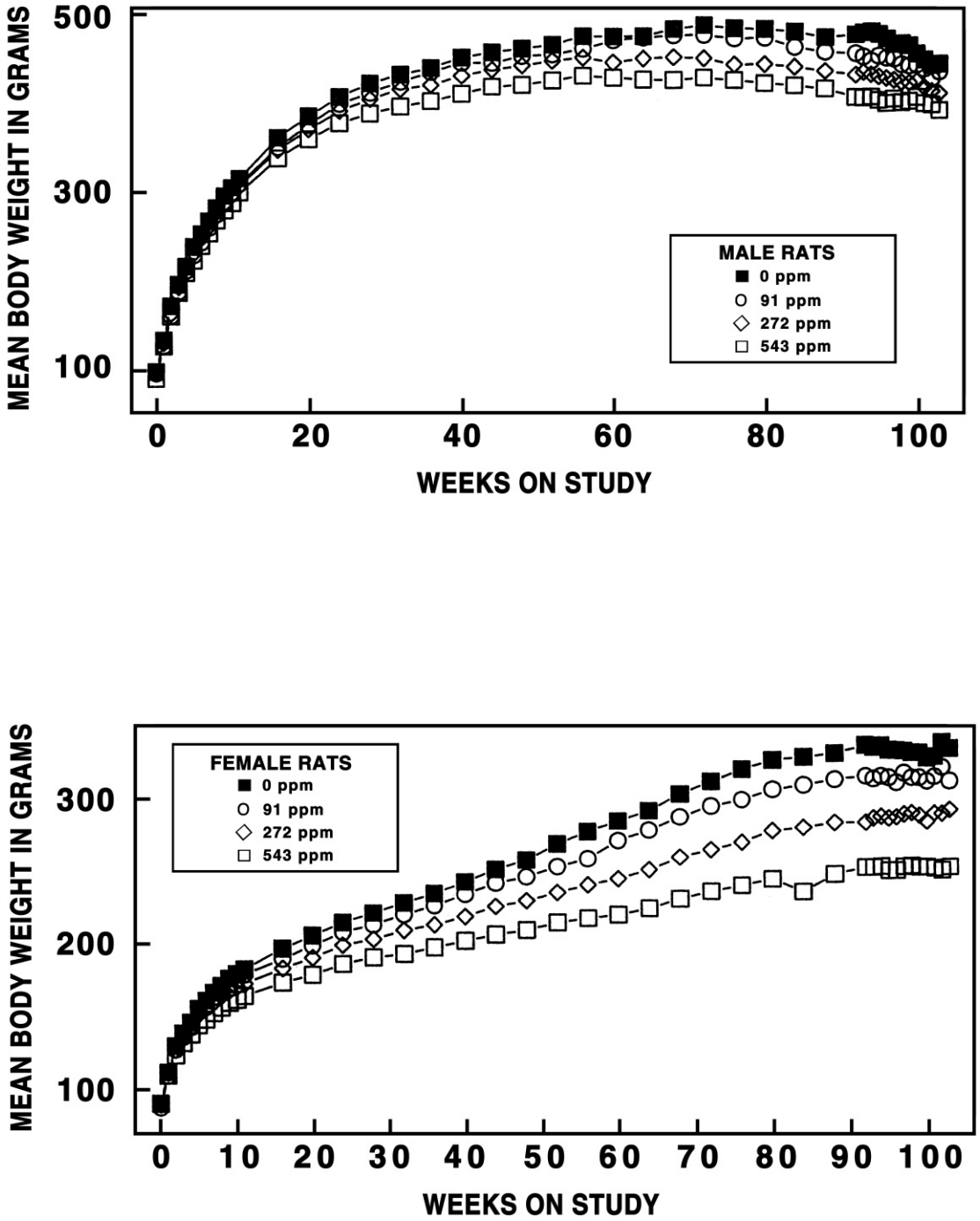


FIGURE 4
Growth Curves for Male and Female Rats Exposed to Leucomalachite Green in Feed for 2 Years

TABLE 5
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of Leucomalachite Green

Weeks on Study	0 ppm		91 ppm			272 ppm			543 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	131	48	127	97	47	129	99	48	126	96	47
2	162	48	153	95	47	156	97	48	152	94	47
3	190	48	182	96	47	186	97	48	180	95	47
4	214	48	208	97	47	211	98	48	205	96	47
5	235	48	228	97	47	231	98	48	223	95	47
6	253	48	246	97	47	248	98	48	241	95	47
7	268	48	260	97	47	261	98	48	254	95	47
8	282	48	275	97	47	273	97	48	267	94	47
9	296	48	287	97	47	287	97	48	280	95	47
10	305	48	298	98	47	297	97	48	288	94	47
11	316	48	309	98	47	307	97	48	299	95	47
12	343	48	333	97	47	312	91	48	316	92	47
16	362	48	351	97	47	348	96	48	339	94	47
20	386	48	376	97	47	371	96	48	360	93	47
24	408	48	400	98	47	392	96	48	378	93	47
28	423	48	413	97	47	405	96	48	389	92	47
32	433	48	425	98	47	417	96	48	397	92	47
36	440	48	436	99	47	422	96	48	404	92	47
40	452	48	446	99	47	432	96	48	412	91	47
44	458	48	446	97	47	439	96	48	417	91	47
48	462	48	454	98	47	443	96	48	422	91	47
52	467	48	456	98	47	449	96	48	424	91	47
56	476	48	462	97	47	452	95	48	432	91	47
60	476	48	472	99	47	449	94	48	430	90	47
64	476	48	475	100	47	453	95	46	428	90	47
68	485	48	477	98	47	453	94	45	428	88	46
72	489	48	478	98	46	452	92	45	431	88	45
76	486	47	474	98	46	447	92	45	428	88	45
80	488	45	475	97	45	446	91	44	425	87	45
84	479	44	465	97	44	440	92	44	422	88	45
88	479	40	462	97	40	436	91	43	419	87	44
92	475	37	458	96	38	434	91	41	411	86	42
96	475	33	453	96	36	431	91	39	403	85	36
100	459	29	445	97	34	427	93	36	399	87	33
103	446	26	440	99	31	416	93	34	395	89	30
Mean for weeks											
1-13	243		235	97		236	97		229	94	
14-52	429		420	98		412	96		394	92	
53-103	478		466	97		443	93		421	88	

TABLE 6
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of Leucomalachite Green

Weeks on Study	0 ppm		91 ppm			272 ppm			543 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	110	48	107	98	48	106	96	48	107	98	48
2	124	48	121	97	48	122	98	48	119	96	48
3	136	48	133	98	48	134	98	48	130	95	48
4	145	48	142	98	48	142	98	48	137	94	48
5	155	48	151	98	48	151	97	48	143	93	48
6	161	48	156	97	48	156	97	48	148	92	48
7	167	48	162	97	48	160	96	48	152	92	48
8	171	48	168	98	47	164	96	48	156	91	48
9	176	48	172	98	47	167	95	48	159	90	48
10	179	48	175	98	47	170	95	48	161	90	48
11	182	48	179	98	47	172	95	48	164	90	48
12	186	48	178	95	47	173	93	48	164	88	48
16	196	48	189	96	47	183	93	47	173	88	48
20	205	48	198	97	47	190	93	47	178	87	48
24	214	48	207	97	47	198	93	47	185	87	48
28	220	48	212	96	47	202	92	47	190	86	48
32	227	48	219	96	47	208	92	47	192	85	48
36	234	48	225	96	47	212	91	47	197	84	48
40	242	48	233	96	47	218	90	47	201	83	48
44	250	48	241	96	47	223	89	47	205	82	48
48	257	48	245	95	47	229	89	47	208	81	48
52	268	48	253	94	47	234	87	47	214	80	48
56	277	48	258	93	47	240	87	47	217	78	48
60	284	48	271	95	47	244	86	47	219	77	47
64	291	48	278	95	47	250	86	47	223	77	47
68	302	48	287	95	47	259	86	45	230	76	46
72	311	48	294	95	47	264	85	45	235	76	46
76	319	48	299	93	47	269	84	45	239	75	45
80	326	47	306	94	46	276	85	45	243	75	45
84	328	44	308	94	46	279	85	45	244	74	43
88	327	44	312	95	45	281	86	44	247	76	43
92	334	41	314	94	44	284	85	43	252	75	39
96	332	37	312	94	43	286	86	41	249	75	36
100	326	37	312	96	41	288	88	40	251	77	36
103	326	33	310	95	37	294	90	35	251	77	35
Mean for weeks											
1-13	156		152	97		150	96		144	92	
14-52	231		222	96		210	91		194	84	
53-103	313		296	95		269	86		237	76	

Organ Weights

Malachite Green Chloride

The relative liver weight was significantly increased in females exposed to 600 ppm malachite green chloride (Table D1).

Leucomalachite Green

Relative liver weights were significantly increased in females exposed to 272 or 543 ppm leucomalachite green (Tables 7 and D2). Absolute and relative liver weights were significantly increased in male rats exposed to 272 or 543 ppm leucomalachite green. Relative thyroid gland weights were significantly increased in males and females exposed to 543 ppm leucomalachite green.

TABLE 7
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Year Feed Study of Leucomalachite Green[a]

	0 ppm	91 ppm	272 ppm	543 ppm
Male				
n	23	29	34	29
Necropsy body wt	432 ± 8	424 ± 10	397 ± 10**	377 ± 10***
Liver				
Absolute	14.7 ± 0.523	14.8 ± 0.691	17.2 ± 0.668**	19.3 ± 0.691***
Relative	34.3 ± 1.38	35.0 ± 1.86	43.6 ± 1.80***	51.7 ± 1.86***
Thyroid gland				
Absolute	0.038 ± 0.002	0.042 ± 0.003	0.036 ± 0.002[b]	0.042 ± 0.003[c]
Relative	0.09 ± 0.00	0.10 ± 0.01	0.09 ± 0.01[b]	0.11 ± 0.01**[c]
Female				
n	33	36	35	33
Necropsy body wt	312 ± 7	299 ± 7	277 ± 7***	240 ± 7***
Liver				
Absolute	10.3 ± 0.368	9.87 ± 0.37	10.4 ± 0.367	11.2 ± 0.373
Relative	33.8 ± 1.58	33.1 ± 1.45	37.9 ± 1.46*	46.6 ± 1.48***
Thyroid gland				
Absolute	0.033 ± 0.002	0.032 ± 0.002[d]	0.032 ± 0.002[e]	0.033 ± 0.002[e]
Relative	0.11 ± 0.01	0.11 ± 0.01[d]	0.12 ± 0.01[e]	0.14 ± 0.01***[e]

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
*** P≤0.001
[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). Statistical tests were performed on unrounded data.
[b] n=33
[c] n=28
[d] n=35
[e] n=32

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the thyroid gland, liver, and other organs. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and NCTR historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Malachite Green Chloride

Thyroid Gland: Follicular cell adenomas and carcinomas were observed in female rats exposed to 300 or 600 ppm malachite green chloride, and the incidences of adenoma or carcinoma (combined) in these groups exceeded the historical control range (Tables 8, B1a, B3a, and B4a). A dose-related increasing trend (P=0.049) in the incidence of cystic follicles was observed in exposed rats (Tables 8 and B5a). Cystic follicles consisted of very large thyroid follicles that were distended with colloid and lined by flattened follicular epithelial cells. Lesions diagnosed as follicular cell hyperplasia were also cystic, but there were small fronds and foci of follicular epithelial cells protruding into distended follicles. Although the increases were not statistically significant, thyroid follicular cell hyperplasia was only observed in rats exposed to malachite green chloride.

TABLE 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
Number Examined Microscopically	46	48	47	46
Follicular Cell Cyst[a]	0	1 (3.0)[b]	1 (3.0)	3 (2.0)
Follicular Cell Hyperplasia	0	0	1 (1.0)	2 (1.0)
Follicular Cell Adenoma	0	0	1	1
Follicular Cell Carcinoma	0	0	2	1
Follicular Cell Adenoma or Carcinoma[c]				
Overall rate[d]	0/46 (0%)	0/48 (0%)	3/47 (6%)	2/46 (4%)
Adjusted rate[e]	0.0%	0.0%	7.1%	5.7%
Terminal rate[f]	0/29 (0%)	0/23 (0%)	3/31 (10%)	2/24 (8%)
First incidence (days)	—[h]	—[i]	728 (T)	728 (T)
Poly-3 test[g]	P=0.064	—	P=0.137	P=0.218

(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year studies with controls given NIH-31 diet: 7/517 (1.4%), range 0%-3%
[d] Number of animals with neoplasm per number of animals with thyroid gland examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice.
[h] Not applicable; no neoplasms in animal group
[i] Value of statistic cannot be computed.

Liver: There were modest, but not statistically significant, increases in the incidences of hepatocellular adenoma in female rats exposed to malachite green chloride (Tables 9, B1a, and B3a). However, the incidences in all groups, including the controls, exceeded the historical control range (Tables 9 and B4b). Hepatocellular adenomas consisted of well-demarcated lesions that occupied an area greater in size than one hepatic lobule with distinct compression of adjacent parenchyma. A single hepatocellular carcinoma was found in one 300 ppm female and was a large well-demarcated lesion that consisted of anaplastic hepatocytes arranged in a trabecular pattern in some areas. Corresponding increases in the incidences of eosinophilic foci and centrilobular necrosis were observed in female rats exposed to malachite green chloride (Tables 9 and B5a). Eosinophilic foci were characterized by distinct, variably sized foci in which hepatocytes were larger than normal due to increased amounts of eosinophilic cytoplasm. The incidence of centrilobular necrosis, in which the necrotic cells were oriented around central veins in the hepatic lobule, exhibited an increasing trend (P=0.002).

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
Number Examined Microscopically	48	48	48	48
Eosinophilic Focus (includes multiple)[a]	5	10	13	14*
Centrilobular, Necrosis	0	0	0	4 (2.5)[b]
Hepatocellular Adenoma, Multiple	1	0	0	1
Hepatocellular Adenoma (includes multiple)[c]				
Overall rate[d]	1/48 (2%)	1/48 (2%)	3/48 (6%)[h]	4/48 (8%)
Adjusted rate[e]	2.5%	2.6%	6.9%	10.8%
Terminal rate[f]	1/29 (3%)	1/23 (4%)	2/32 (6%)	4/25 (16%)
First incidence (days)	728 (T)	728 (T)	652	728 (T)
Poly-3 test[g]	P=0.059	P=0.751	P=0.336	P=0.155

* Significantly different (P≤0.05) from the control group by the Poly-3 test
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year studies with controls given NIH-31 diet: 1/541 (0.2%), range 0%-0.6%
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice.
[h] A single incidence of carcinoma occurred in an animal that also had an adenoma.

Mammary Gland: The incidence of mammary gland carcinoma in female rats exposed to 600 ppm malachite green chloride exceeded the historical control range (Tables 10, B3a, and B4c).

Pituitary Gland: There was a statistically significant increase in the incidence of adenoma of the pituitary gland (pars distalis) in female rats exposed to 100 ppm malachite green chloride (Tables 11 and B3a). Incidences of pars distalis adenoma in 100 and 300 ppm females exceeded the historical control range for pars distalis adenoma or carcinoma (combined) (Tables 11 and B4d).

Mononuclear Cell Leukemia: A dose-related decreasing trend in the incidences of mononuclear cell leukemia occurred in female rats exposed to malachite green chloride, with statistically significant decreased incidences in the 300 and 600 ppm groups (Tables 12 and B3a).

TABLE 10
Incidences of Mammary Gland Carcinoma in Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
Carcinoma[a]				
Overall rate[b]	2/48 (4%)	2/48 (4%)	1/48 (2%)	5/48 (10%)
Adjusted rate[c]	5.0%	5.2%	2.3%	13.0%
Terminal rate[d]	1/29 (3%)	1/23 (4%)	1/32 (3%)	2/25 (8%)
First incidence (days)	704	686	728 (T)	508
Poly-3 test[e]	P=0.113	P=0.679	P=0.473N	P=0.197

(T)Terminal sacrifice
[a] Historical incidence for 2-year studies with controls given NIH-31 diet: 4/534 (0.7%), range 0%-4%
[b] Number of animals with neoplasm per number of animals necropsied.
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposure group is indicated by **N**.

TABLE 11
Incidences of Pituitary Gland (Pars Distalis) Adenoma in Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate[a]	26/48 (54%)	36/47 (77%)	32/46 (70%)	29/45 (64%)
Adjusted rate[b]	60.7%	82.7%	71.6%	76.2%
Terminal rate[c]	16/29 (55%)	21/23 (91%)	22/32 (69%)	21/25 (84%)
First incidence (days)	552	485	603	570
Poly-3 test[d]	P=0.233	P=0.014	P=0.193	P=0.092
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma[e]				
Overall rate	26/48 (54%)	36/47 (77%)	32/46 (70%)	30/45 (67%)
Adjusted rate	60.7%	82.7%	71.6%	78.5%
Terminal rate	16/29 (55%)	21/23 (91%)	22/32 (69%)	21/25 (84%)
First incidence (days)	552	485	603	570
Poly-3 test	P=0.162	P=0.014	P=0.193	P=0.056

[a] Number of animals with neoplasm per number of animals with pituitary gland examined microscopically.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice.
[e] Historical incidence for 2-year studies with controls given NIH-31 diet: 306/528 (58.0%), range 51%-68%

TABLE 12
Incidences of Mononuclear Cell Leukemia in Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
Mononuclear Cell Leukemia[a]				
Overall rate[b]	19/48 (40%)	17/48 (35%)	10/48 (21%)	1/48 (2%)
Adjusted rate[c]	43.7%	40.0%	22.5%	2.7%
Terminal rate[d]	9/29 (31%)	4/23 (17%)	4/32 (13%)	0/25 (0%)
First incidence (days)	526	443	619	546
Poly-3 test[e]	P<0.001N	P=0.447N	P=0.026N	P<0.001N

[a] Historical incidence for 2-year studies with controls given NIH-31 diet: 188/542 (34.7%), range 13%-45%; includes lymphocytic, monocytic, mononuclear, or undifferentiated leukemia
[b] Number of animals with neoplasm per number of animals necropsied
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by **N**.

Leucomalachite Green

Thyroid Gland: Follicular cell adenomas and carcinomas and follicle cell cysts were observed in male and female rats exposed to leucomalachite green (Tables 13, A1, A3, B1b, and B3b). These lesions were not observed in control rats. The incidences of follicular cell adenoma or carcinoma (combined) in 543 ppm males and in 272 ppm females exceeded the historical control range.

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Male				
Number Examined Microscopically	47	47	48	46
Follicle Cyst[a]	0	0	0	3 (1.3)[b]
Follicular Cell Hyperplasia	2 (1.0)	1 (4.0)	3 (2.3)	3 (2.0)
Follicular Cell Adenoma	0	2	0	1
Follicular Cell Carcinoma (includes bilateral)	0	0	1	2
Follicular Cell Adenoma or Carcinoma[c]				
Overall rate[d]	0/47 (0%)	2/47 (4%)	1/48 (2%)	3/46 (7%)
Adjusted rate[e]	0.0%	4.8%	2.3%	7.2%
Terminal rate[f]	0/24 (0%)	2/30 (7%)	0/34 (0%)	3/30 (10%)
First incidence (days)	—[h]	733 (T)	662	733 (T)
Poly-3 test[g]	P=0.122	P=0.244	P=0.512	P=0.121
Female				
Number Examined Microscopically	46	46	47	48
Follicle Cyst	0	1 (2.0)	0	2 (1.0)
Follicular Cell Hyperplasia	1 (2.0)	0	0	3 (1.3)
Follicular Cell Adenoma	0	0	0	1
Follicular Cell Carcinoma	0	1	2	0
Follicular Cell Adenoma or Carcinoma[i]				
Overall rate	0/46 (0%)	1/46 (2%)	2/47 (4%)	1/48 (2%)
Adjusted rate	0.0%	2.3%	4.7%	2.4%
Terminal rate	0/33 (0%)	0/35 (0%)	2/34 (6%)	1/34 (3%)
First incidence (days)	—	732	734 (T)	734 (T)
Poly-3 test	P=0.367	P=0.506	P=0.241	P=0.503

(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year studies with controls given NIH-31 diet: 2/511 (0.4%), range 0%-2%
[d] Number of animals with neoplasm per number of animals with thyroid gland examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice.
[h] Not applicable; no neoplasms in animal group
[i] Historical incidence: 7/517 (1.4%), range 0%-3%

Mammary Gland: A dose-related increase in the combined incidences of adenoma and carcinoma occurred in the mammary gland of female rats exposed to leucomalachite green (Tables 14, B1b, and B3b). The incidence in the 543 ppm group exceeded the historical control incidence (Tables 14 and B4c). These neoplasms were not observed in the control group.

Liver: Hepatocellular adenomas were observed in all groups of male rats exposed to leucomalachite green and in female rats exposed to 91 and 543 ppm leucomalachite green. The incidence of adenoma in the leucomalachite green-exposed rats and in the control rats exceeded the historical control range (Tables 15, A1, A3, B1b, B3b, and B4b). Nonneoplastic lesions in the liver of rats exposed to leucomalachite green included eosinophilic foci, cystic degeneration, and cytoplasmic vacuolization of hepatocytes (Tables 15, A5, and B5b). The incidences of eosinophilic foci were significantly increased in all groups of rats exposed to leucomalachite green. Eosinophilic foci were characterized by distinct, variably sized foci in which hepatocytes were larger than normal due to increased amounts of eosinophilic cytoplasm. The incidences of cystic degeneration of the liver were treatment-related in male rats, but not in female rats. Cystic degeneration was a multilocular cystic lesion containing finely granular or flocculent eosinophilic material. The cyst-like structures were not lined by epithelial or endothelial cells. Occasionally, cells at the periphery of the cystic spaces contained clear cytoplasmic vacuoles. Severity grades assigned were based on the number and size of the cystic lesions. The incidences of cytoplasmic vacuolization of hepatocytes were increased in female rats exposed to 272 or 543 ppm leucomalachite green. The clear cytoplasmic vacuoles (probably intracellular lipid accumulation) were of variable size and the location of vacuolated cells within the liver lobules varied considerably.

TABLE 14
Incidences of Mammary Gland Neoplasms in Female Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Number Necropsied	48	48	48	48
Adenoma[a]	0	1	1	2
Carcinoma	0	1	2	2
Adenoma or Carcinoma[b]				
Overall rate[c]	0/48 (0%)	2/48 (4%)	3/48 (6%)	4/48 (8%)
Adjusted rate[d]	0.0%	4.5%	6.8%	9.3%
Terminal rate[e]	0/33 (0%)	0/36 (0%)	1/35 (3%)	3/34 (9%)
First incidence (days)	—[g]	690	657	585
Poly-3 test[f]	P=0.047	P=0.244	P=0.120	P=0.058

[a] Number of animals with lesion
[b] Historical incidence for 2-year studies with controls given NIH-31 diet: 9/534 (1.7%), range 0%-6%
[c] Number of animals with neoplasm per number of animals necropsied
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice.
[g] Not applicable; no neoplasms in animal group

TABLE 15
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Male				
Number Examined Microscopically	48	47	48	47
Eosinophilic Focus (includes multiple)[a]	3	14**	19**	33**
Degeneration, Cystic	4 (2.0)[b]	18**(1.3)	13* (1.5)	19**(1.4)
Vacuolization Cytoplasmic	9 (2.0)	21**(1.9)	10 (1.9)	13 (1.8)
Hepatocellular Adenoma[c]				
Overall rate[d]	2/48 (4%)	2/47 (4%)	3/48 (6%)	2/47 (4%)
Adjusted rate[e]	4.8%	4.8%	7.0%	4.7%
Terminal rate[f]	0/24 (0%)	2/30 (7%)	3/34 (9%)	2/30 (7%)
First incidence (days)	639	733 (T)	733 (T)	733 (T)
Poly-3 test[g]	P=0.568	P=0.692N	P=0.512	P=0.690N
Female				
Number Examined Microscopically	48	48	48	48
Eosinophilic Focus (includes multiple)	3	12*	20**	16**
Degeneration, Cystic	3 (2.0)	2 (1.0)	5 (1.6)	3 (2.0)
Vacuolization Cytoplasmic	5 (2.0)	5 (1.4)	17**(1.7)	22**(1.8)
Hepatocellular Adenoma[h]				
Overall rate	1/48 (2%)	3/48 (6%)	0/48 (0%)	3/48 (6%)
Adjusted rate	2.3%	6.7%	0.0%	7.0%
Terminal rate	1/33 (3%)	3/36 (8%)	0/35 (0%)	3/34 (9%)
First incidence (days)	734 (T)	734 (T)	—[i]	734 (T)
Poly-3 test	P=0.360	P=0.314	P=0.500N	P=0.297

* Significantly different (P≤0.05) from the control group by the Poly-3 test
** P≤0.01
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year studies with controls given NIH-31 diet: 4/548 (0.7%), range 0%-2%
[d] Number of animals with neoplasms per number of animals with liver examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposed group is indicated by **N**.
[h] Historical incidence: 1/541 (0.2%), range 0%-1%
[i] Not applicable; no neoplasms in animal group

Testis: A positive trend was observed in the incidences of testicular interstitial cell adenoma in male rats exposed to leucomalachite green, and the incidence in the 543 ppm group was significantly increased and exceeded the historical control range (Tables 16, A1, A3, and A4b). High incidences of bilateral interstitial cell adenoma were observed in male rats examined at study termination and in male rats that were removed early from study due to death or morbidity (Table 17).

Eye: Female rats administered leucomalachite green exhibited an increasing trend (P<0.001) in the incidence of central retinal degeneration and the incidence was significantly (P=0.002) increased in the 543 ppm group (0 ppm, 4/48; 91 ppm, 7/48; 272 ppm, 11/48; 543 ppm, 16/48; Table B5b). A corresponding increase in central retinal degeneration was not observed in male rats (Table A5).

TABLE 16
Incidences of Neoplasms of the Testis in Male Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Number Examined Microscopically	48	47	48	47
Interstitial Cell Adenoma, Bilateral[a]	22	30	38	39*
Interstitial Cell Adenoma (includes bilateral)[b]				
Overall rate[c]	37/48 (77%)	42/47 (89%)	43/48 (90%)	45/47 (96%)
Adjusted rate[d]	82.5%	93.3%	92.2%	95.7%
Terminal rate[e]	23/24 (96%)	29/30 (97%)	32/34 (94%)	28/30 (93%)
First incidence (days)	564	579	435	475
Poly-3 test[f]	P=0.036	P=0.078	P=0.115	P=0.029

* Significantly different (P≤0.05) from the control group by the Poly-3 test
[a] Number of animals with lesion
[b] Historical incidence for 2-year studies with controls given NIH-31 diet: 469/547 (85.7%), range 69%-90%
[c] Number of animals with neoplasm per number of animals with testis examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice.

TABLE 17
Incidences of Unilateral and Bilateral Interstitial Cell Adenoma of the Testis in Male Rats in the 2-Year Feed Study of Leucomalachite Green[a]

	0 ppm	91 ppm	272 ppm	543 ppm
Days 401-600[b]				
Unilateral	0/5 (0%)	1/5 (20%)	1/5 (20%)	1/3 (33%)
Bilateral	2/5 (40%)	2/5 (40%)	2/5 (40%)	2/3 (67%)
Combined	2/5 (40%)	3/5 (60%)	3/5 (60%)	3/3 (100%)
Days 601-723				
Unilateral	9/20 (45%)	4/13 (31%)	0/9 (0%)	3/14 (21%)
Bilateral	4/20 (20%)	7/13 (54%)	8/9 (89%)	11/14 (79%)
Combined	13/20 (65%)	11/13 (85%)	8/9 (89%)	14/14 (100%)
Terminal Sacrifice				
Unilateral	6/23 (26%)	7/29 (24%)	4/34 (12%)	2/30 (7%)
Bilateral	16/23 (70%)	21/29 (72%)	28/34 (82%)	26/30 (87%)
Combined	22/23 (96%)	28/29 (97%)	32/34 (94%)	28/30 (93%)
Total				
Unilateral	15/48 (31%)	12/47 (26%)	5/48 (10%)	6/47 (13%)
Bilateral	22/48 (46%)	30/47 (64%)	38/48 (79%)	39/47 (83%)
Combined	37/48 (77%)	42/47 (89%)	43/48 (90%)	45/47 (96%)

[a] Number of animals with neoplasm per number of animals removed during the indicated study interval
[b] Study interval (no rats were removed before day 400).

Mononuclear Cell Leukemia: The incidences of mononuclear cell leukemia were significantly decreased in all groups of rats exposed to leucomalachite green (Tables 18, A3, and B3b).

Pituitary Gland (Pars Distalis): The incidences of pituitary gland pars distalis adenoma were significantly decreased in all groups of male rats exposed to leucomalachite green; there were no significant changes in this lesion in female rats exposed to leucomalachite green (Tables 19, A3, and B3b).

TABLE 18
Incidences of Mononuclear Cell Leukemia in Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Male				
Mononuclear Cell Leukemia[a]				
Overall rate[b]	29/48 (60%)	16/47 (34%)	19/48 (40%)	7/47 (15%)
Adjusted rate[c]	63.6%	36.6%	41.6%	16.0%
Terminal rate[d]	12/24 (50%)	9/30 (30%)	9/34 (27%)	2/30 (7%)
First incidence (days)	564	579	435	601
Poly-3 test[e]	P<0.001N	P=0.007N	P=0.026N	P<0.001N
Female				
Mononuclear Cell Leukemia[f]				
Overall rate	17/48 (35%)	8/48 (17%)	5/48 (10%)	8/48 (17%)
Adjusted rate	37.0%	17.8%	11.0%	18.2%
Terminal rate	8/33 (24%)	6/36 (17%)	1/35 (3%)	3/34 (9%)
First incidence (days)	552	683	482	484
Poly-3 test	P=0.038N	P=0.033N	P=0.003N	P=0.037N

[a] Historical incidence for 2-year studies with controls given NIH-31 diet: 240/550 (43.6%), range 31%-58%; includes lymphocytic, monocytic, mononuclear, or undifferentiated leukemia
[b] Number of animals with neoplasm-per number of animals necropsied
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by **N**.
[f] Historical incidence: 188/543 (34.6%), range 13%-45%; includes lymphocytic, monocytic, mononuclear, or undifferentiated leukemia

TABLE 19
Incidences of Pituitary Gland (Pars Distalis) Adenoma in Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Male				
Adenoma				
Overall rate[a]	30/45 (67%)	19/46 (41%)	21/48 (44%)	13/45 (29%)
Adjusted rate[b]	69.6%	43.0%	47.5%	31.3%
Terminal rate[c]	15/23 (65%)	10/30 (33%)	16/34 (47%)	11/30 (37%)
First incidence (days)	541	487	599	645
Poly-3 test[d]	P<0.001N	P=0.008N	P=0.026N	P<0.001N
Female				
Adenoma				
Overall rate	26/47 (55%)	23/47 (49%)	17/45 (38%)	20/46 (43%)
Adjusted rate	58.6%	51.1%	39.7%	48.1%
Terminal rate	19/33 (58%)	18/35 (51%)	12/35 (34%)	17/32 (53%)
First incidence (days)	627	561	599	632
Poly-3 test	P=0.170N	P=0.306N	P=0.056N	P=0.218N

[a] Number of animals with neoplasm per number of animals with pituitary gland (pars distalis) examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by **N**.

MICE

2-YEAR STUDIES

Survival

Estimates of 2-year survival probabilities for female mice exposed to malachite green chloride and leucomalachite green are shown in Tables 20 and 21 and in the Kaplan-Meier survival curves (Figures 5 and 6). Survival of the exposed and control groups was similar in both studies.

TABLE 20
Survival of Female Mice in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	225 ppm	450 ppm
Animals initially in study	48	48	48	48
Accidental Death[a]	0	0	1	0
Moribund	4	3	3	4
Natural deaths	4	1	4	3
Animals surviving to study termination	40	44	40	41
Percent probability of survival at end of study[b]	83	92	85	85
Mean survival (days)[c]	706	720	698	700
Survival analysis[d]	P=0.900N	P=0.229N	P=0.834N	P=0.788N

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

TABLE 21
Survival of Female Mice in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	204 ppm	408 ppm
Animals initially in study	48	48	48	48
Moribund	4	5	7	4
Natural deaths	7	2	2	5
Animals surviving to study termination	37	41	39	39
Percent probability of survival at end of study[a]	77	85	81	81
Mean survival (days)[b]	688	716	699	708
Survival analysis[c]	P=0.803	P=0.279N	P=0.627N	P=0.584N

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

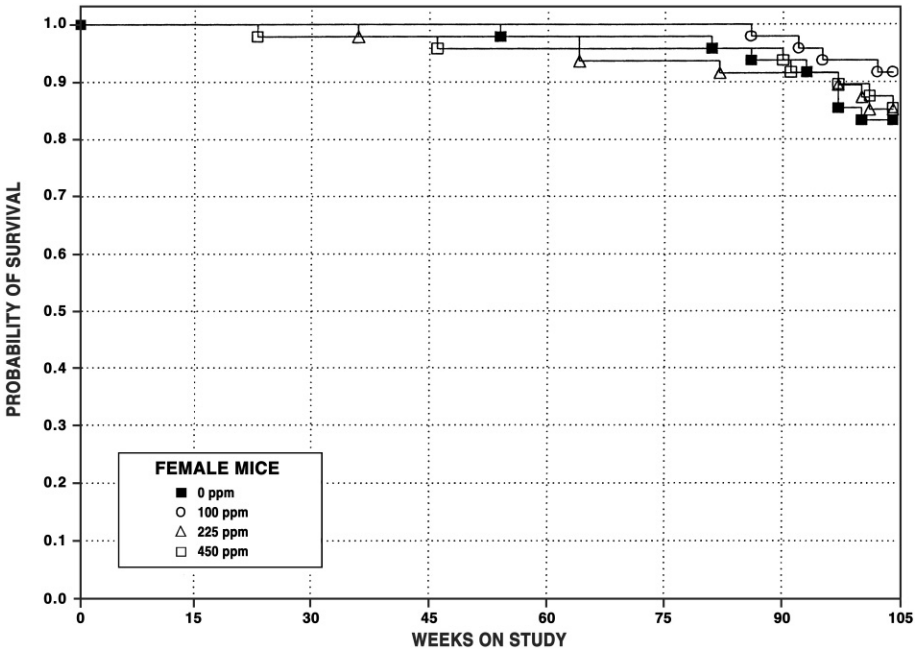


FIGURE 5
Kaplan-Meier Survival Curves for Female Mice Exposed to Malachite Green Chloride in Feed for 2 Years

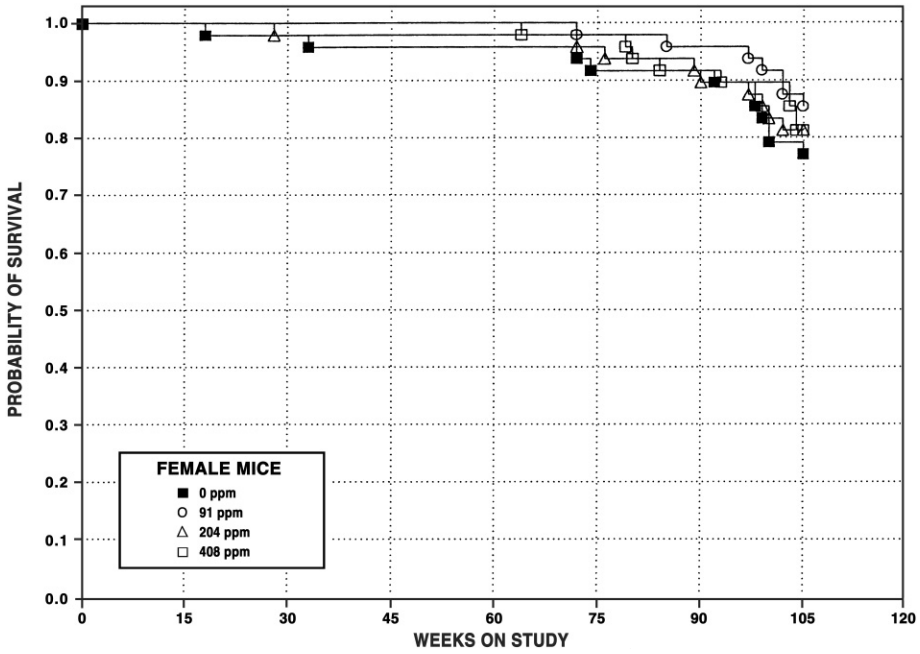


FIGURE 6
Kaplan-Meier Survival Curves for Female Mice Exposed to Leucomalachite Green in Feed for 2 Years

Body Weights, Feed and Compound Consumption, and Clinical Findings

Malachite Green Chloride

Mean body weights of exposed female mice were generally similar to those of the controls throughout most of the study (Figure 7 and Table 22). Feed consumption by exposed groups of females was similar to that by the controls (Table F4). Dietary concentrations of 100, 225, or 450 ppm malachite green chloride resulted in average daily doses of approximately 15, 33, or 67 mg malachite green chloride/kg body weight. No clinical findings were attributed to malachite green chloride exposure.

Leucomalachite Green

Mean body weights of female mice exposed to leucomalachite green were generally similar to controls throughout the study (Figure 8 and Table 23). Feed consumption by female mice exposed to leucomalachite green was similar to that by the controls (Table F5). Dietary concentrations of 91, 204, or 408 ppm leucomalachite green resulted in average daily doses of approximately 15, 31, or 63 mg leucomalachite green/kg body weight. No clinical findings were attributed to leucomalachite green exposure.

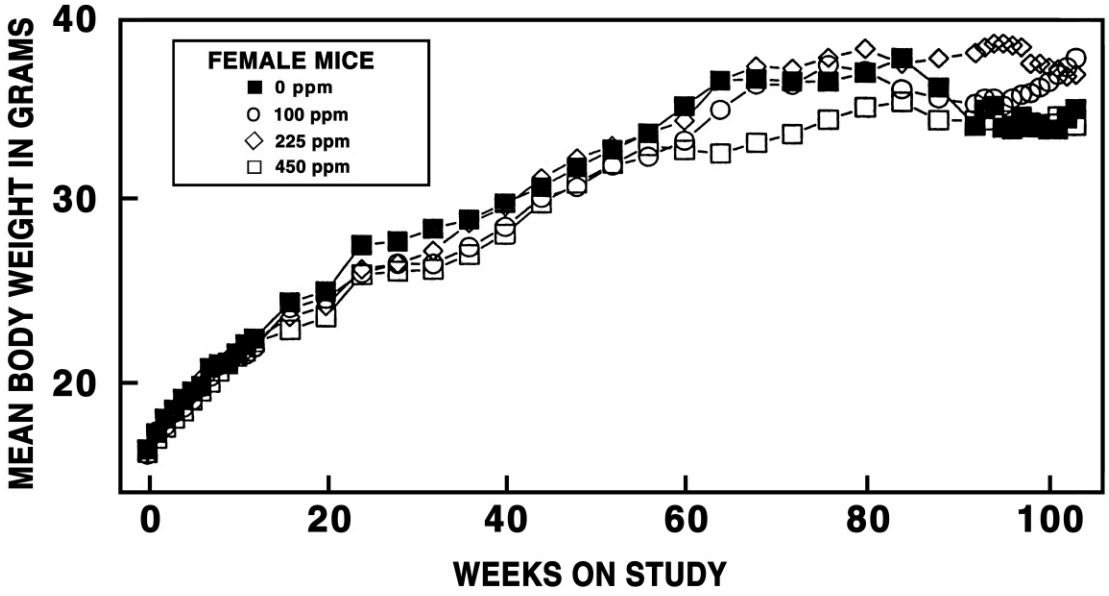


FIGURE 7
Growth Curves for Female Mice Exposed to Malachite Green Chloride in Feed for 2 Years

TABLE 22
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of Malachite Green Chloride

Weeks on Study	0 ppm		100 ppm			225 ppm			450 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	16.3	48	16.0	99	48	16.2	100	48	16.1	99	48
2	17.2	48	17.3	101	48	17.1	100	48	16.9	98	48
3	18.0	48	17.6	98	48	17.8	99	48	17.5	97	48
4	18.5	48	18.3	99	48	18.3	99	48	18.0	97	48
5	19.1	48	18.6	97	48	19.0	99	48	18.4	96	48
6	19.5	48	19.0	97	48	19.3	99	48	19.0	97	48
7	19.8	48	19.7	100	48	20.2	102	48	19.5	98	48
8	20.8	48	20.3	98	48	20.5	99	48	20.0	96	48
9	20.9	48	20.9	100	48	21.0	101	48	20.6	98	48
10	21.1	48	21.1	100	48	21.3	101	48	21.0	100	48
11	21.6	48	21.5	100	48	21.6	100	48	21.4	99	48
12	22.1	48	21.5	97	48	21.7	98	48	21.7	98	48
13	22.4	48	21.9	98	48	22.3	100	48	22.2	99	48
17	24.6	48	24.1	98	48	23.6	96	48	23.2	94	48
21	25.4	48	24.6	97	48	24.4	96	48	23.5	93	48
25	27.6	48	26.0	94	48	26.2	95	48	25.9	94	47
29	28.0	48	26.6	95	48	26.5	95	48	26.1	93	47
33	28.4	48	26.5	93	48	27.3	96	47	26.4	93	47
37	29.0	48	27.7	96	48	28.7	99	46	27.3	94	47
41	29.9	48	28.8	96	48	29.8	100	46	28.5	95	47
45	31.2	48	30.3	97	48	31.2	100	46	30.0	96	47
49	32.2	48	31.0	96	48	32.3	100	46	31.1	97	46
53	33.5	48	32.1	96	48	32.8	98	46	32.0	96	46
57	34.3	47	32.6	95	48	33.7	98	46	33.3	97	46
61	35.7	47	33.5	94	48	34.4	97	46	33.2	93	46
65	37.1	47	35.2	95	48	36.6	99	44	32.8	88	46
69	37.0	47	36.4	99	48	37.4	101	44	33.4	90	46
73	36.5	47	36.6	100	48	37.3	102	44	33.7	92	46
77	37.0	46	37.7	102	48	37.9	102	44	34.7	94	46
81	37.7	46	37.4	99	48	38.2	101	43	35.0	93	46
85	38.2	46	36.4	95	47	37.6	98	43	35.5	93	46
89	37.1	45	35.7	96	47	37.9	102	43	34.9	94	46
93	35.7	44	35.4	99	46	38.2	107	43	34.3	96	44
97	35.3	41	35.7	101	45	38.8	110	42	34.1	96	43
101	35.4	40	36.6	103	45	37.5	106	40	34.5	98	42
104	36.1	—[a]	37.7	104	—	37.1	103	—	34.7	96	—
Mean for weeks											
1-13	19.8		19.5	99		19.7	100		19.4	98	
14-52	28.5		27.3	96		27.8	98		26.9	94	
53-104	36.2		35.6	98		36.8	102		34.0	94	

[a] Terminal sacrifice occurred during week 104 after animals were weighed.

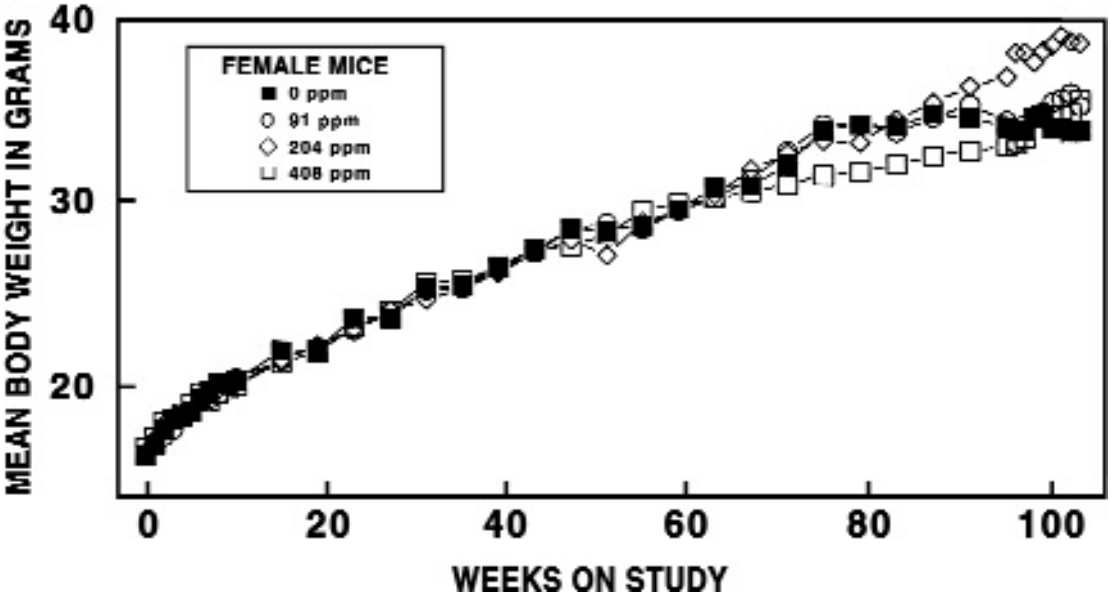


FIGURE 8
Growth Curves for Female Mice Exposed to Leucomalachite Green in Feed for 2 Years

TABLE 23
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of Leucomalachite Green

Weeks on Study	0 ppm		91 ppm			204 ppm			408 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	16.5	48	16.5	100	48	16.8	102	48	17.0	103	48
2	16.9	48	17.0	101	48	17.3	102	48	17.3	102	48
3	17.7	48	17.3	98	48	18.0	102	48	18.1	102	48
4	18.2	48	17.7	97	48	18.3	101	48	18.4	101	48
5	18.5	48	18.3	99	48	18.6	101	48	18.5	100	48
6	18.7	48	18.7	101	48	18.8	101	48	19.1	103	48
7	19.4	48	19.2	99	48	19.4	100	48	19.6	101	48
8	19.8	48	19.4	98	48	19.7	99	48	19.4	98	48
9	20.2	48	19.6	97	48	19.9	98	48	19.7	97	48
10	20.2	48	20.1	100	48	20.0	99	48	19.9	99	48
11	20.4	48	20.5	100	48	20.2	99	48	20.2	99	48
16	22.0	48	21.5	98	48	21.5	98	48	21.4	97	48
20	22.1	47	22.2	101	48	22.3	101	48	21.9	99	48
24	23.8	47	23.1	97	48	23.2	98	48	23.3	98	48
28	23.8	47	24.1	101	48	24.2	101	47	24.2	102	48
32	25.4	46	25.4	100	48	25.0	98	47	25.7	101	48
36	25.6	46	25.6	100	48	25.4	99	47	25.8	101	48
40	26.5	46	26.5	100	48	26.2	99	47	26.6	100	48
44	27.7	46	27.5	99	48	27.3	98	47	27.5	99	48
48	28.6	46	28.8	101	48	28.0	98	47	27.8	97	48
52	28.5	46	29.3	103	48	27.2	96	47	28.3	100	48
56	28.8	46	28.6	99	48	29.0	101	47	29.6	103	48
60	29.8	46	29.8	100	48	29.8	100	47	30.6	103	48
64	30.9	46	30.6	99	48	30.7	99	47	30.6	99	47
68	31.0	46	31.9	103	48	32.1	104	47	30.9	100	47
72	32.2	45	33.4	104	47	33.6	104	46	31.5	98	47
76	34.1	44	34.7	102	47	35.0	103	45	31.7	93	47
80	34.4	44	34.7	101	47	34.9	101	45	32.2	94	45
84	34.5	44	34.7	101	46	35.2	102	45	32.8	95	44
88	35.2	44	35.4	101	46	35.9	102	44	33.0	94	44
92	35.1	43	36.2	103	46	37.1	106	43	33.7	96	43
96	34.6	43	35.3	102	45	37.5	109	42	34.2	99	43
100	35.1	38	35.5	101	44	39.3	112	40	35.5	101	43
104	34.3	—[a]	35.6	104	—	39.4	115	—	36.2	106	—
Mean for weeks											
1-13	18.8		18.6	99		18.8	100		18.8	100	
14-52	25.4		25.4	100		25.0	99		25.3	100	
53-104	33.1		33.6	101		34.6	104		32.5	98	

[a] Terminal sacrifice occurred during week 104 after animals were weighed.

Kidney Weights

Malachite Green Chloride

Kidney weights of female mice exposed to malachite green chloride were generally less than those of the controls (Tables 24 and D3). The absolute right kidney weights were significantly decreased in the 225 and 450 ppm groups as were the relative weights in the 100 and 225 ppm groups. The absolute left kidney weight was significantly decreased in the 450 ppm group, and the relative weight was decreased in the 225 ppm group.

Leucomalachite Green

Relative kidney weights were significantly decreased in all leucomalachite green exposure groups compared to the control group (Tables 25 and D4).

TABLE 24
Kidney Weights of Female Mice in the 2-Year Feed Study of Malachite Green Chloride[a]

	0 ppm	100 ppm	225 ppm	450 ppm
n	40	44	40	41
Necropsy body wt	33.8 ± 0.9	35.8 ± 1.0	34.7 ± 1.0	32.4 ± 1.0
R. Kidney				
Absolute	0.224 ± 0.004	0.217 ± 0.005	0.210 ± 0.005**	0.206 ± 0.005***
Relative	6.74 ± 0.14	6.12 ± 0.18**	6.08 ± 0.18**	6.42 ± 0.18
L. Kidney				
Absolute	0.209 ± 0.003[b]	0.208 ± 0.004	0.201 ± 0.005	0.188 ± 0.005***
Relative	6.26 ± 0.15[b]	5.87 ± 0.17	5.83 ± 0.18*	5.86 ± 0.18

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
*** P≤0.001
[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). Statistical tests were performed on unrounded data.
[b] n=39

TABLE 25
Kidney Weights of Female Mice in the 2-Year Feed Study of Leucomalachite Green[a]

	0 ppm	91 ppm	204 ppm	408 ppm
n	37	41	39	39
Necropsy body wt	32.7 ± 0.6	34.0 ± 1.0	37.6 ± 1.0***	34.5 ± 1.0
R. Kidney				
Absolute	0.227 ± 0.005	0.216 ± 0.005	0.227 ± 0.005	0.217 ± 0.005
Relative	7.03 ± 0.21	6.43 ± 0.20*	6.12 ± 0.21***	6.34 ± 0.21**
L. Kidney				
Absolute	0.216 ± 0.004	0.202 ± 0.005*	0.216 ± 0.005	0.204 ± 0.005
Relative	6.69 ± 0.20	6.00 ± 0.20**	5.83 ± 0.20***	5.96 ± 0.20**

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
*** P≤0.001
[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). Statistical tests were performed on unrounded data.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the liver and urinary bladder for female mice exposed to malachite green chloride and in the incidences of neoplasms and/or nonneoplastic lesions of the liver and urinary bladder for female mice exposed to leucomalachite green. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and NCTR historical incidences for the neoplasms mentioned in this section are presented in Appendix C.

Malachite Green Chloride

Urinary Bladder: Significantly (P<0.05) increased incidences of intracytoplasmic inclusions were observed in all groups of female mice exposed to malachite green chloride (0 ppm, 7/47; 100 ppm, 15/46; 225 ppm, 34/45; 450 ppm, 39/48; Table C5a). Intracytoplasmic inclusions were found in the transitional epithelium of the urinary bladder. The inclusions were variable in size, yellow-orange, slightly refractile and generally found in the superficial epithelial cells. The inclusions were found in many mice exposed to malachite green chloride and the number of inclusions (severity) increased with increasing exposure concentration (1.1, 1.1, 1.4, 1.4). The inclusions were much less prominent than those observed in female mice exposed to leucomalachite green. The pathogenesis of the intracytoplasmic inclusions is unknown, but the inclusions are thought to represent degradation products. The presence of the inclusions did not have an apparent effect on the general health or mortality of the affected mice.

Leucomalachite Green

Liver: An increasing trend in the combined incidences of hepatocellular adenoma or carcinoma was observed in female mice exposed to leucomalachite green, and the incidence was significantly increased in the 408 ppm group; the historical control range was exceeded by all exposed groups (Tables 26, C3b, and C4). Female mice exposed to leucomalachite green also exhibited an increase in the incidences of hepatocellular adenoma. Although the increase was not statistically significant, the historical range in control animals was exceeded by the incidence in the 91 and 408 ppm groups. Histologically, the hepatocellular neoplasms were morphologically similar to those that occur spontaneously in the liver.

Urinary Bladder: Increased incidences (P<0.001) of intracytoplasmic inclusions occurred in all groups of female mice exposed to leucomalachite green [0 ppm, 14/46; 91 ppm, 33/48, 204 ppm, 44/47; 408 ppm, 44/44; Table C5b). Intracytoplasmic inclusions were found in the transitional epithelium of the urinary bladder. The inclusions were variable in size, yellow-orange, slightly refractile and generally found in the superficial epithelial cells. In control mice, the cytoplasm tended to be granular with only rare inclusions noted. Inclusions were more apparent with the periodic acid Schiff (PAS) reaction and were more prominent than those observed in mice fed malachite green. The inclusions were found in most mice exposed to leucomalachite green, and the number of inclusions (severity) increased with dose (1.0, 1.1, 1.9, 2.9). As observed in mice exposed to malachite green chloride, the pathogenesis of the intracytoplasmic inclusions is unknown, but the inclusions are thought to represent degradation products. The presence of the inclusions did not have an apparent effect on the general health or mortality of the affected mice.

TABLE 26
Incidences of Neoplasms of the Liver in Female Mice in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	204 ppm	408 ppm
Number Examined Microscopically	47	48	47	47
Hepatocellular Adenoma, Multiple[a]	0	0	1	1
Hepatocellular Adenoma (includes multiple)[b]				
Overall rate[c]	3/47 (6%)	6/48 (13%)	5/47 (11%)	9/47 (19%)
Adjusted rate[d]	6.9%	12.8%	11.4%	20.2%
Terminal rate[e]	2/37 (5%)	5/41 (12%)	5/39 (13%)	9/39 (23%)
First incidence (days)	682	498	733 (T)	733 (T)
Poly-3 test[f]	P=0.055	P=0.282	P=0.363	P=0.065
Hepatocellular Carcinoma	0	0	1	2
Hepatocellular Adenoma or Carcinoma[g]				
Overall rate	3/47 (6%)	6/48 (13%)	6/47 (13%)	11/47 (23%)
Adjusted rate	6.9%	12.8%	13.6%	24.6%
Terminal rate	2/37 (5%)	5/41 (12%)	6/39 (15%)	11/39 (28%)
First incidence (days)	682	498	733 (T)	733 (T)
Poly-3 test	P=0.013	P=0.282	P=0.248	P=0.022

(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Historical incidence for 2-year studies with controls given NIH-31 diet: 26/563 (4.6%), range 0%-11%
[c] Number of animals with neoplasm per number of animals with liver examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice.
[g] Historical incidence: 34/563 (6.0%), range 0%-11%

DISCUSSION AND CONCLUSIONS

Malachite green is a triphenylmethane dye that has been used widely as an antifungal agent in the aquaculture industry. The compound was nominated by the FDA Center for Veterinary Medicine for toxicity and carcinogenicity studies due to the potential for consumer exposure through the consumption of treated fish, its structural similarity to the carcinogenic dye gentian violet, and a lack of carcinogenicity data in the literature. Malachite green is reduced to leucomalachite green in the tissues of treated fish and persists in this form. Accordingly, the toxicity and carcinogenicity of leucomalachite green was also assessed.

In the 2-year studies of malachite green chloride and leucomalachite green, survival of control and exposed groups of rats and mice was similar. Mean body weights of female rats exposed to 300 or 600 ppm malachite green chloride or equimolar doses of leucomalachite green (272 and 543 ppm) were less than the controls during most of the study, while females exposed to 91 ppm leucomalachite green weighed less than the controls during the second year of the study. Mean body weights of 543 ppm leucomalachite green-exposed male rats were less than those of the controls throughout the study, while the 272 ppm males were less than the controls during year 2 of the study. Mean body weights of mice exposed to malachite green chloride or leucomalachite green were generally similar to the control groups throughout most of the study.

In the 2-year study in mice, the incidence of combined hepatocellular adenomas and carcinomas in female mice fed 408 ppm leucomalachite green was significantly greater than in the controls. In addition, the increases observed in leucomalachite green-exposed female mice exceeded the historical range for NCTR historical control data. In an earlier 2-year feed study, Littlefield *et al.* (1985) observed increases in the incidence of hepatocellular carcinomas in both sexes of B6C3F$_1$ mice fed gentian violet, a structurally similar triphenylmethane dye [females: 0 ppm, 7/185 (4%); 100 ppm, 5/93 (5%); 300 ppm, 30/93 (32%); 600 ppm, 73/95 (77%); males: 0 ppm, 27/183 (15%); 100 ppm, 15/92 (17%); 300 ppm, 17/93 (18%); 600 ppm, 33/95 (35%)]. In contrast to leucomalachite green and gentian violet, no increase in the incidence of liver neoplasms was found in female mice fed malachite green. Analyses of gene mutations in transgenic mice provided mechanistic support for the increased incidences of liver neoplasms in leucomalachite green-exposed mice compared to those treated with malachite green in the 2-year studies. In the transgenic mouse study, female Big Blue mice were fed up to 408 ppm leucomalachite green or 450 ppm malachite green for 16 weeks and the livers were analyzed for mutations in the lambda *cII* gene (Appendix I). The *cII* mutant frequency was significantly increased in the livers of Big Blue mice exposed to leucomalachite green, but not malachite green, as compared to the controls. Furthermore, the spectrum of *cII* mutations in the mice fed leucomalachite green was significantly different from the control group, with a notable increase in G:C to T:A transversions. Mutagenic aromatic amines are known to produce predominantly G:C to T:A transversions in Big Blue rodents (Cunningham *et al.*, 1996; Suter *et al.*, 1996; Staedtler *et al.*, 1999). *N*-Demethylated metabolites, including primary arylamines, have also been detected in liver extracts from mice exposed to leucomalachite green (Culp *et al.* 1999). Furthermore, B6C3F$_1$ mice treated with leucomalachite green, but not malachite green chloride, for 28 days exhibited an increase in the frequency of micronucleated erythrocytes in peripheral blood (NTP, 2004). An increase in the peripheral blood micronucleus frequency was not observed in transgenic mice fed malachite or leucomalachite green (Appendix I). These results suggest that the arylamine derivatives from leucomalachite green could undergo subsequent activation similar to carcinogenic arylamine metabolites that react with DNA either directly or after esterification. Misreplication of these lesions may result in mutations that lead to liver tumors. Indeed, ^{32}P-postlabeling analyses indicated a single DNA adduct or co-eluting adducts in the liver DNA of leucomalachite green-treated mice, with the adduct levels increasing as a function of dose (Culp *et al.*, 1999); although adducts were not observed in the transgenic mice exposed to leucomalachite green

(Appendix I). Nevertheless, these data suggest that leucomalachite green can act as a genotoxic liver carcinogen in the female mouse. *N*-Demethylated metabolites and DNA adducts were also detected in malachite green-exposed mice, suggesting that DNA adducts formed in the livers of mice fed malachite green have little mutagenic or carcinogenic consequence.

In the 2-year study in rats, thyroid follicular cell adenomas or carcinomas were observed in malachite green chloride and leucomalachite green-treatment groups. Although there was not a statistically significant trend or increase in the incidence, the occurrence may be significant considering the low prevalence of these tumors in control groups in NCTR studies [female F344 rats: 1.4% (7/517), range 0%-3%; male F344 rats: 0.4% (2/511), range 0%-2%] and NTP oral studies [female F344 rats: 0.9% (8/898), range 0%-4%; male F344 rats: 2.0% (18/902), range 0%-8%, NIEHS, 1998]. Nonneoplastic lesions involving thyroid follicular cells, which included cystic follicles and thyroid follicular cell hyperplasia, were observed only in malachite green chloride or leucomalachite green-exposed rats. These findings support the possibility that thyroid follicular cells are a target for malachite green toxicity. In a 2-year feed study with the structurally related dye gentian violet, Littlefield (1988) administered 0, 100, 300, or 600 ppm gentian violet to male and female F344 rats in the diet for at least 80 days, including during mating, and the offspring were continued on the same doses for 24 months. Treatment-related increases in the incidences of follicular cell adenocarcinoma of the thyroid gland were observed in females, at incidences of 1%, 1%, 5%, and 8% for the four respective gentian violet dose groups after being fed for 24 months; in males, the incidences were 1%, 5%, 3%, and 6% for the four respective dose groups. Additionally, in short-term studies with leucomalachite green, male rats exhibited increased thyroid stimulating hormone (TSH) levels and decreased thyroxine (T_4) levels, indicative of hypothyroidism. The effects were less robust with malachite green in short-term studies, although significant increases in TSH were observed in male rats and decreases in T_4 were found in female rats. Doerge *et al*. (1998b) have demonstrated that leucomalachite green inhibits thyroid peroxidase-catalyzed formation of T_4 and tyrosine *in vitro*, which also results in the oxidative *N*-demethylation of leucomalachite green to a primary arylamine. These findings suggest that the anti-thyroid effects observed in rats treated with leucomalachite green result from blockade of hormone synthesis through alternate substrate inhibition and that chronic exposure could cause thyroid follicular cell tumors through a hormonal mechanism. Moreover, the formation of a primary arylamine, that may be metabolically activated and bind to DNA, raises the possibility of a genotoxic mechanism for tumor formation.

In the female rats fed malachite green there was a small increase in the incidence of hepatocellular adenoma. Although the increase was not statistically significant, hepatocellular adenomas are very rare in female F344 rats at NCTR. The incidences of hepatocellular adenoma in female rats fed 91 or 543 ppm leucomalachite green also exceeded the NCTR historical control range. It is not clear if the increase is of biological significance, as the mechanism behind the induction of these tumors is uncertain. Dose-dependent formation of a DNA adduct has been demonstrated in the livers of F344 and Big Blue rats administered malachite green and leucomalachite green in the diet (Culp *et al*., 2002; Manjanatha *et al*., 2004). Nonetheless, analyses of liver *lacI* and *cII* mutants revealed that mutation frequencies in treated rats were similar to those of control rats, and that the majority of the independent mutations in treated Big Blue rats were base-pair substitutions, with mutation spectra similar to that found for control rats (R.H. Heflich, NCTR, unpublished observation). These data suggest that malachite green and leucomalachite green might be promoting spontaneous lesions, in a manner similar to that reported by Fernandes *et al*. (1991), who conducted an initiation/promotion experiment in Wistar rats, using diethylnitrosamine as an initiator and malachite green as a promoter.

A significant increasing trend was observed in the incidence of testicular interstitial cell adenoma in male rats exposed to leucomalachite green, and the incidence was increased in the 543 ppm group compared to the control. Examination of the data divided into unilateral and bilateral incidences of adenoma and segregated based on the number of days the rats were on study revealed a high incidence of bilateral tumors, even for the rats on study for a shorter period of time. The cause of death of all of the control male rats removed before terminal sacrifice (25 of 48) in the leucomalachite green study was attributed to mononuclear cell leukemia or pituitary gland adenoma. Significantly lower incidences of both mononuclear cell leukemia and pituitary gland adenoma were observed in leucomalachite green-exposed male rats compared to the control group. One may speculate that the decreased incidences of these lethal tumors in treated rats would result in increased survival compared to the control group. Since testes adenomas are common lesions in aged male F344 rats, this could enhance the

statistical significance of the testicular cancer in the treated rats. However, with the exception of the 272 ppm group, survival of the leucomalachite green-exposed male rats was not statistically increased compared to the control group. Haseman *et al*. (1997) reported that decreases in interstitial cell tumors of the testes have been associated with elevated rates of pituitary gland tumors in male F344 rats. It is not clear if the opposite association observed in our study, of an increased incidence of testes adenoma and a decreased incidence of pituitary gland adenoma (or mononuclear cell leukemia), applies. Due to the high spontaneous incidence of testicular tumors in male F344 rats, it is not a target for statistical correlation studies (Haseman *et al*., 1997). The NCTR historical control range is 69%-90%, encompassing the data reported in this study. The NTP historical control incidence (oral studies) for testicular adenoma in control rats is 89% (802/903) with a range of 74%-96% (NIEHS, 1998). Based on the broad range of incidences in the historical control data and the uncertainty of the relationship between incidence of pituitary gland tumors and testes adenomas, it is not clear if the increased incidence of testicular adenomas in male rats exposed to leucomalachite green is treatment related.

The incidence of mammary gland carcinoma in female rats fed malachite green chloride was more than double the historical control range in F344 rats at NCTR, although the change was not statistically significant. In female rats exposed to leucomalachite green, a significant increasing trend in the combined incidence of mammary gland adenoma or carcinoma was observed. The incidences of adenoma or carcinoma in the highest exposure group slightly exceeded the historical range observed for control F344 female rats in NCTR 2-year studies. The effects of other aminotriphenylmethane dyes upon mammary gland tumorigenesis have varied, with increased incidences of mammary gland carcinomas in female Sprague-Dawley rats fed Benzyl Violet 4B for 1 year (Ikeda *et al.*, 1974; IARC, 1978), but no effect in female F344 rats fed gentian violet for 2 years (Littlefield, 1988). The mammary gland tumor data are confounded by decreases in body weight associated with the administration of both malachite green chloride and leucomalachite green. Seilkop (1995) performed an analysis of 53 F344 rat studies conducted by the NTP and found that the statistical power to detect a treatment-related increase in the incidence of mammary gland tumors can be seriously diminished by a mean body weight depression of as little as 10%. In an evaluation of 84 2-year NTP studies, Haseman *et al.* (1997) showed a strong positive correlation between the incidence of mammary gland tumors and 12-month body weight in female F344 rats. Body weight decreases in the female rats fed malachite green chloride for 52 weeks were approximately 2%, 8%, and 14% for the 100, 300, and 600 ppm groups, respectively, compared to the control group. Female rats fed leucomalachite green for 52 weeks experienced body weight decreases of approximately 6%, 13%, and 20%, respectively, for the 91, 272, and 543 ppm groups compared to the control group. (Body weight reduction of this magnitude would be associated with approximately 15% decreases in the mammary gland tumor incidence.) Thus, the slight increase in mammary gland carcinomas in female rats exposed to malachite green chloride is probably biologically significant and the effects from exposure to malachite green chloride or leucomalachite green might have been more substantial without the decrease in body weight.

In the 2-year rat studies, the incidence of mononuclear cell leukemia was significantly decreased in female rats exposed to malachite green chloride and in male and female rats exposed to leucomalachite green, with decreases in all dose groups compared to the control groups. Mononuclear cell leukemia is one of the most common neoplasms in the F344 rat. Decreases in leukemia incidences have been associated with chemicals that cause splenic toxicity (Elwell *et al*., 1996; Haseman and Johnson, 1996). Spleen toxicity was not observed in the current studies. Higami *et al*. (1994, 1995) reported that dietary restriction reduces the incidence and delays the onset, but not the progression, of leukemia in male F344 rats and suggested that the incidence and age of onset of leukemia relate to the total cumulative energy intake of the rat (i.e., age multiplied by mean daily energy intake). During the last year of the 2-year study, body weight decreases of approximately 3%, 7%, and 12% were measured in male rats fed 91, 272, or 543 ppm leucomalachite green, respectively, compared to the control group. As reported earlier, body weight decreases were also observed in female rats exposed to malachite green chloride or leucomalachite green. However, there were no consistent trends in food consumption of female rats fed malachite green chloride and statistical decreases in food consumption in male and female rats fed leucomalachite green was intermittent throughout the study. The significance and mechanism of the relationship between the decreased incidence of mononuclear cell leukemia and the administration of malachite green chloride or leucomalachite green are not clear.

The incidences of adenoma of the pituitary gland pars distalis were significantly decreased in male rats exposed to leucomalachite green with decreases in all exposed groups compared to the control group. A number of studies have shown that decreased rates of pituitary gland adenoma and carcinoma in F344 rats are associated with reduced body weight often found in treated rats (Haseman and Johnson, 1996; Haseman *et al*., 1997; Seilkop, 1995). As mentioned earlier, female rats fed leucomalachite green for 52 weeks experienced considerable weight decreases compared to the control group. However, changes were less pronounced in the leucomalachite green-exposed male rats, with decreases of approximately 3%, 7%, and 12% for the 91, 272, and 543 ppm leucomalachite green dose groups, respectively, compared to the control group during the last year of the study. The incidences of pituitary gland pars distalis adenoma or carcinoma for control groups of F344 male rats in NCTR studies range from 31% to 70% and in NTP studies (all routes) ranged from 18% to 78% (NTP unpublished data). These data suggest that the decrease is treatment-related, although a mechanism for this effect is not readily apparent. This was not the case with female rats exposed to malachite green chloride. There was a statistically significant increase in the incidence of pituitary gland pars distalis adenoma in the female rats fed 100 ppm malachite green chloride compared to the control group.

Central retinal degeneration was observed in both malachite green- and leucomalachite green-exposed rats. Female rats administered leucomalachite green exhibited an increasing trend in central retinal degeneration and an increase in the 543 ppm group compared to the control group. Female rats administered malachite green did not exhibit statistical increases in central retinal degeneration, although the incidence in the control female rats in the malachite green study was five times greater than the incidence in the control female rats in the leucomalachite green study. F344 rats have a genetic predisposition to peripheral retinal degeneration, but central retinal degeneration is most likely due to exposure to fluorescent light of high intensity or exposure to test article (Yoshitomi and Boorman, 1990). In the malachite green and leucomalachite green studies, it was ascertained that light intensity in the animal cages on the top shelf did exceed the recommended 30 ft-candles (NRC, 1996). However, cages were rotated on a regular basis such that all cages received similar amounts of light intensity during the study period. Since previous NTP and NCTR studies do not report separately the incidences of peripheral and central retinal degeneration, information on control incidences is not available. As a result, the effect of malachite green and leucomalachite green exposure on the retina remains uncertain.

During the conduct of these studies, the FDA was contacted by the United States Fish and Wildlife Service (USFWS) and the Centers for Disease Control and Prevention regarding a possible correlation between the formation of acoustic neuromas and malachite green exposure in USFWS hatchery workers (K. Rosa, USFWS; E. Page, CDC; personal communication). Acoustic neuromas are histologically benign tumors derived from the Schwann cells of the 8th cranial nerve, which can lead to hearing loss, imbalance, and brainstem compression (Kumazawa *et al.*, 1994). Acoustic neuromas are rarely found in rodents (Fujii and Nomoto, 1983; Viala *et al*., 1986), although nude and severe combined immune deficiency mice have been used as hosts (i.e., tumors were grafted from humans to mice; Charabi *et al*., 1994; Kumazawa *et al*., 1994). Special consideration was taken in the 2-year chronic studies to allow for the examination of the 8th cranial nerve and the surrounding area. No acoustic neuromas were observed in any of the animals on the study.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity** of malachite green chloride in female F344/N rats based on the occurrence of thyroid gland follicular cell adenoma or carcinoma (combined) and marginal increases in hepatocellular adenoma and mammary gland carcinoma in exposed rats. There was *no evidence of carcinogenic activity* of malachite green chloride in female $B6C3F_1$ mice exposed to 100, 225, or 450 ppm.

Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* of leucomalachite green in male F344/N rats based on an increase in interstitial cell adenoma of the testes and the occurrence of thyroid gland follicular cell adenoma or carcinoma (combined) in exposed rats. There was *equivocal evidence of carcinogenic activity* of leucomalachite green in female F344/N rats based on a marginally increased incidence of hepatocellular adenoma and the occurrence of thyroid gland follicular cell adenoma or carcinoma (combined) in exposed rats. There was *some evidence of carcinogenic activity* of leucomalachite green in female $B6C3F_1$ mice based on an increase in hepatocellular adenoma or carcinoma (combined).

Exposure to malachite green chloride in feed resulted in nonneoplastic lesions in the thyroid gland and liver of female rats and the urinary bladder of female mice. Exposure to leucomalachite green in feed resulted in nonneoplastic lesions in the thyroid gland and liver of male and female rats and the urinary bladder of female mice.

Decreased incidences of mononuclear cell leukemia in female F344/N rats were attributed to malachite green chloride exposure. Decreased incidences of mononuclear cell leukemia in male and female F344/N rats and pituitary gland adenomas in male rats were attributed to leucomalachite green exposure.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

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

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Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Leucomalachite Green[a]

	0 ppm	91 ppm	272 ppm	543 ppm
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund	20	12	11	10
Natural deaths	4	5	3	7
Survivors				
Died last week of study	1	1		
Terminal sacrifice	23	29	34	30
Missexed		1		1
Animals examined microscopically	48	47	48	47
Alimentary System				
Intestine large	(48)	(47)	(48)	(47)
Colon, adenoma			1 (2%)	
Rectum, sarcoma, metastatic, skin		1 (2%)		
Intestine small	(47)	(47)	(48)	(47)
Ileum, leiomyoma	1 (2%)	1 (2%)		
Jejunum, leiomyoma				1 (2%)
Liver	(48)	(47)	(48)	(47)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Hepatocellular adenoma	2 (4%)	2 (4%)	3 (6%)	2 (4%)
Histiocytic sarcoma	1 (2%)			
Sarcoma, metastatic, skin		1 (2%)		
Mesentery	(5)	(3)	(6)	(3)
Pancreas	(48)	(47)	(48)	(47)
Sarcoma, metastatic, skin		1 (2%)		
Acinar cell, adenocarcinoma				1 (2%)
Salivary glands	(48)	(47)	(48)	(47)
Fibrous histiocytoma				1 (2%)
Histiocytic sarcoma		1 (2%)		
Stomach	(48)	(47)	(47)	(47)
Cardiovascular System				
Blood vessel	(48)	(47)	(48)	(47)
Heart	(48)	(47)	(48)	(47)
Histiocytic sarcoma		1 (2%)		
Sarcoma, metastatic, skin				1 (2%)
Endocrine System				
Adrenal gland	(48)	(47)	(48)	(47)
Bilateral, medulla, pheochromocytoma benign		1 (2%)		
Cortex, adenoma	1 (2%)	1 (2%)	1 (2%)	
Medulla, pheochromocytoma malignant		2 (4%)		3 (6%)
Medulla, pheochromocytoma complex				1 (2%)
Medulla, pheochromocytoma benign	3 (6%)	5 (11%)	2 (4%)	3 (6%)
Islets, pancreatic	(48)	(47)	(48)	(47)
Adenoma	5 (10%)	2 (4%)	1 (2%)	4 (9%)
Pituitary gland	(45)	(46)	(48)	(45)
Craniopharyngioma				1 (2%)
Pars distalis, adenoma	30 (67%)	19 (41%)	21 (44%)	13 (29%)

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Endocrine System (continued)				
Thyroid gland	(47)	(47)	(48)	(46)
Fibrous histiocytoma				1 (2%)
Bilateral, follicular cell, carcinoma				1 (2%)
C-cell, adenoma	6 (13%)	7 (15%)	7 (15%)	4 (9%)
C-cell, carcinoma	1 (2%)	2 (4%)		
Follicular cell, adenoma		2 (4%)		1 (2%)
Follicular cell, carcinoma			1 (2%)	1 (2%)
General Body System				
Tissue NOS	(1)			(1)
Genital System				
Epididymis	(48)	(47)	(48)	(47)
Sarcoma, metastatic, skin		2 (4%)		
Preputial gland	(48)	(47)	(48)	(47)
Adenoma	5 (10%)	2 (4%)	7 (15%)	3 (6%)
Carcinoma	6 (13%)		4 (8%)	9 (19%)
Sarcoma, metastatic, skin		2 (4%)		
Squamous cell papilloma				1 (2%)
Prostate	(48)	(47)	(48)	(47)
Testes	(48)	(47)	(48)	(47)
Bilateral, interstitial cell, adenoma	22 (46%)	30 (64%)	38 (79%)	39 (83%)
Interstitial cell, adenoma	15 (31%)	12 (26%)	5 (10%)	6 (13%)
Hematopoietic System				
Bone marrow	(48)	(47)	(48)	(47)
Lymph node	(48)	(47)	(48)	(47)
Spleen	(48)	(47)	(48)	(47)
Adenocarcinoma, metastatic, pancreas				1 (2%)
Thymus	(43)	(42)	(43)	(42)
Fibrous histiocytoma		1 (2%)		
Histiocytic sarcoma		1 (2%)		
Integumentary System				
Mammary gland	(43)	(41)	(46)	(41)
Carcinoma			1 (2%)	
Fibroadenoma	1 (2%)	2 (5%)	1 (2%)	4 (10%)
Skin	(48)	(47)	(47)	(47)
Basal cell adenoma				1 (2%)
Basal cell carcinoma			1 (2%)	
Fibroma	4 (8%)	1 (2%)	1 (2%)	5 (11%)
Fibrous histiocytoma		1 (2%)	1 (2%)	1 (2%)
Keratoacanthoma	2 (4%)	3 (6%)		2 (4%)
Lipoma		3 (6%)	1 (2%)	
Sarcoma		3 (6%)		4 (9%)
Squamous cell carcinoma				1 (2%)
Squamous cell papilloma		1 (2%)		
Face, squamous cell papilloma		1 (2%)		
Sebaceous gland, adenoma		1 (2%)		

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Musculoskeletal System				
Bone	(48)	(47)	(48)	(47)
Femur, sarcoma, metastatic, skin		1 (2%)		
Skeletal muscle	(48)	(47)	(48)	(47)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Nervous System				
Brain	(48)	(47)	(48)	(47)
Cerebrum, astrocytoma malignant		1 (2%)		
Spinal cord	(48)	(47)	(48)	(47)
Respiratory System				
Lung	(48)	(47)	(48)	(47)
Alveolar/bronchiolar adenoma	1 (2%)		3 (6%)	4 (9%)
Carcinoma, metastatic, thyroid gland			1 (2%)	
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Histiocytic sarcoma		1 (2%)		
Sarcoma, metastatic, skin		1 (2%)		1 (2%)
Squamous cell carcinoma, metastatic, preputial gland				1 (2%)
Alveolar/bronchiolar, adenoma		1 (2%)		
Nose	(48)	(47)	(48)	(47)
Trachea	(48)	(47)	(48)	(47)
Fibrous histiocytoma				1 (2%)
Special Senses System				
Ear	(1)		(2)	
Pinna, neural crest tumor	1 (100%)		1 (50%)	
Eye	(48)	(47)	(48)	(47)
Lids, sarcoma	1 (2%)			
Harderian gland	(48)	(47)	(48)	(47)
Zymbal's gland	(46)	(43)	(47)	(47)
Carcinoma	1 (2%)			1 (2%)
Squamous cell carcinoma			1 (2%)	
Urinary System				
Kidney	(48)	(47)	(48)	(47)
Histiocytic sarcoma		1 (2%)		
Urinary bladder	(48)	(47)	(48)	(47)
Transitional epithelium, papilloma		1 (2%)		
Systemic Lesions				
Multiple organs[b]	(48)	(47)	(48)	(47)
Leukemia mononuclear	29 (60%)	16 (34%)	19 (40%)	7 (15%)
Mesothelioma malignant	2 (4%)		1 (2%)	1 (2%)

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	47	47	47
Total primary neoplasms	140	129	122	128
Total animals with benign neoplasms	45	47	47	47
Total benign neoplasms	98	98	92	94
Total animals with malignant neoplasms	34	23	25	26
Total malignant neoplasms	41	31	29	34
Total animals with metastatic neoplasms		3	1	5
Total metastatic neoplasms		10	1	6
Total animals with uncertain neoplasms-benign or malignant	1		1	
Total uncertain neoplasms	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 A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	541	564	564	577	599	613	613	623	632	639	653	659	662	662	666	690	698	701	706	717	718	725	731	732	733
Carcass ID Number	017	230	232	382	026	025	237	238	030	245	038	035	040	042	041	048	050	052	258	056	057	058	273	309	274
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ileum, leiomyoma																									
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma										X							X								
Histiocytic sarcoma																									
Mesentery														+	+	+									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortex, adenoma																									
Medulla, pheochromocytoma benign												X													
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																	X				X				
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Pituitary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Pars distalis, adenoma	X			X		X	X	X		X	X	X	X	X	X				X		X	X	X		X
Thyroid gland	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma						X					X											X			X
C-cell, carcinoma																							X		

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	733	733	733	733	733	733	733	734	734	734	734	735	735	735	735	735	735	735	735	735	735	735	735	
Carcass ID Number	276	277	278	279	280	281	310	365	366	367	368	060	061	062	063	064	065	088	089	311	312	313	314	Total Tissues/ Tumors
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Ileum, leiomyoma																					X			1
Intestine small, duodenum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocellular adenoma																								2
Histiocytic sarcoma	X																							1
Mesentery		+	+																					5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cortex, adenoma																X								1
Medulla, pheochromocytoma benign									X											X				3
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	47
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma								X	X		X													5
Parathyroid gland	+	+	M	+	+	+	M	+	+	M	M	M	+	+	+	+	M	+	M	+	+	M	+	38
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	45
Pars distalis, adenoma		X	X	X		X	X	X		X	X	X	X			X		X	X	X				30
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
C-cell, adenoma						X													X					6
C-cell, carcinoma																								1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	541	564	564	577	599	613	613	623	632	639	653	659	662	662	666	690	698	701	706	717	718	725	731	732	733
Carcass ID Number	017	230	232	382	026	025	237	238	030	245	038	035	040	042	041	048	050	052	058	056	057	058	073	090	274
General Body System																									
Tissue NOS																									
Genital System																									
Coagulating gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																					X				
Carcinoma			X										X												
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma			X		X				X									X			X		X		X
Interstitial cell, adenoma							X			X	X	X	X			X	X					X			
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma								X																	
Keratoacanthoma													X												
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	733	733	733	733	733	733	733	734	734	734	734	735	735	735	735	735	735	735	735	735	735	735	735	
Carcass ID Number	276	277	278	279	280	281	310	365	366	367	368	060	061	062	063	064	065	088	089	311	312	313	314	Total Tissues/ Tumors
General Body System																								
Tissue NOS			+																					1
Genital System																								
Coagulating gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	46
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma							X		X										X				X	5
Carcinoma								X		X					X						X			6
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Bilateral, interstitial cell, adenoma	X	X			X			X		X	X	X	X	X	X		X	X		X		X	X	22
Interstitial cell, adenoma			X	X		X	X		X										X		X			15
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	43
Integumentary System																								
Mammary gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Fibroadenoma																						X		1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibroma										X									X	X				4
Keratoacanthoma		X																						2
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	541	564	564	577	599	613	613	623	632	639	653	659	662	662	666	690	698	701	706	717	718	725	731	732	733
Carcass ID Number	017	230	232	382	026	025	237	238	030	245	038	035	040	042	041	048	050	052	058	056	057	058	273	309	274
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Pinna, neural crest tumor																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lids, sarcoma																									
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma									X																
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X	X		X				X	X	X	X	X	X	X	X	X	X	X	X	X			X	
Mesothelioma malignant							X																		

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	733	733	733	733	733	733	733	734	734	734	734	735	735	735	735	735	735	735	735	735	735	735	735	735	
Carcass ID Number	276	277	278	279	280	281	310	365	366	367	368	060	061	062	063	064	065	068	089	311	312	313	314		Total Tissues/ Tumors
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Alveolar/bronchiolar adenoma															X										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Special Senses System																									
Ear																		+							1
Pinna, neural crest tumor																		X							1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Lids, sarcoma													X												1
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Zymbal's gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		46
Carcinoma																									1
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Leukemia mononuclear	X	X	X	X						X			X			X	X	X		X		X	X		29
Mesothelioma malignant			X																						2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	487	567	579	604	606	615	620	629	636	667	671	697	709	724	729	729	732	733	733	733	733	733	733	733
Carcass ID Number	015	233	024	235	028	029	027	240	244	251	254	256	053	272	307	308	059	282	283	284	285	286	287	288
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rectum, sarcoma, metastatic, skin				X																				
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ileum, leiomyoma								X																
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																					X			
Sarcoma, metastatic, skin				X																				
Mesentery																				+				
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin				X																				
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																		
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																		
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, medulla, pheochromocytoma benign																								
Cortex, adenoma																								
Medulla, pheochromocytoma malignant												X							X					
Medulla, pheochromocytoma benign						X												X						X
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma												X												
Parathyroid gland	+	+	+	M	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X	X			X		X	X	X		X			X		X			X	X				
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma					X																X			
C-cell, carcinoma															X									
Follicular cell, adenoma																								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	734	734	734	734	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	
Carcass ID Number	318	369	370	371	066	067	068	069	070	071	072	090	091	092	093	094	095	096	315	316	317	319	320		Total Tissues/ Tumors
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Rectum, sarcoma, metastatic, skin																									1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Ileum, leiomyoma																									1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Hepatocellular adenoma																X									2
Sarcoma, metastatic, skin																									1
Mesentery																		+		+					3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Sarcoma, metastatic, skin																									1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Histiocytic sarcoma																									1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Histiocytic sarcoma																									1
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Bilateral, medulla, pheochromocytoma benign	X																								1
Cortex, adenoma						X																			1
Medulla, pheochromocytoma malignant																									2
Medulla, pheochromocytoma benign																X					X				5
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+		46
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Adenoma													X												2
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+		41
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		46
Pars distalis, adenoma	X			X	X	X							X	X					X			X			19
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
C-cell, adenoma			X										X		X						X	X			7
C-cell, carcinoma																	X								2
Follicular cell, adenoma			X		X																				2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	487	567	579	604	606	615	620	629	636	667	671	697	709	724	729	729	732	733	733	733	733	733	733	733	733
Carcass ID Number	015	233	024	235	028	029	027	240	244	251	254	256	053	272	307	308	059	282	283	284	285	286	287	288
General Body System																									
None																									
Genital System																									
Coagulating gland	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin				X																					
Penis																									
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Sarcoma, metastatic, skin				X																					
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma			X	X		X				X	X	X	X	X	X						X	X	X		
Interstitial cell, adenoma					X			X								X	X	X	X						X
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Fibrous histiocytoma															X										
Histiocytic sarcoma						X																			
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	M	+	+	+	M	M	+	M	+	+	+	+	M	+	+	+	+	+
Fibroadenoma										X															
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									
Fibrous histiocytoma															X										
Keratoacanthoma			X																						
Lipoma																		X							
Sarcoma				X																					
Squamous cell papilloma																									
Face, squamous cell papilloma																									
Sebaceous gland, adenoma																			X						
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Femur, sarcoma, metastatic, skin				X																					
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	734	734	734	734	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	
Carcass ID Number	318	369	370	371	066	067	068	069	070	071	072	090	091	092	093	094	095	096	315	316	317	319	320	Total Tissues/ Tumors
General Body System																								
None																								
Genital System																								
Coagulating gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Sarcoma, metastatic, skin	X																							2
Penis	+																							1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma						X												X						2
Sarcoma, metastatic, skin	X																							2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Bilateral, interstitial cell, adenoma		X	X	X	X	X		X	X	X	X	X		X	X		X	X	X	X		X	X	30
Interstitial cell, adenoma	X						X						X			X					X			12
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	42
Fibrous histiocytoma																								1
Histiocytic sarcoma																								1
Integumentary System																								
Mammary gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	41
Fibroadenoma							X																	2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Fibroma																	X							1
Fibrous histiocytoma																								1
Keratoacanthoma							X							X										3
Lipoma					X														X					3
Sarcoma	X																					X		3
Squamous cell papilloma	X																							1
Face, squamous cell papilloma								X																1
Sebaceous gland, adenoma																								1
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Femur, sarcoma, metastatic, skin																								1
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	487	567	579	604	606	615	620	629	636	667	671	697	709	724	729	729	732	733	733	733	733	733	733	733	733
Carcass ID Number	015	233	024	235	028	029	027	240	244	251	254	256	053	272	307	308	059	282	283	284	285	286	287	288	288
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cerebrum, astrocytoma malignant															X										
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin																X									
Histiocytic sarcoma						X																			
Sarcoma, metastatic, skin				X																					
Alveolar/bronchiolar, adenoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	M	M	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																			
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma																			X						
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X		X					X	X	X	X				X				X		X	X

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	734	734	734	734	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	
Carcass ID Number	318	369	370	371	066	067	068	069	070	071	072	090	091	092	093	094	095	096	315	316	317	319	320	Total Tissues/ Tumors
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Cerebrum, astrocytoma malignant																								1
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Fibrous histiocytoma, metastatic, skin																								1
Histiocytic sarcoma																								1
Sarcoma, metastatic, skin																								1
Alveolar/bronchiolar, adenoma						X																		1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Special Senses System																								
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Histiocytic sarcoma																								1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Transitional epithelium, papilloma																								1
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Leukemia mononuclear		X					X				X				X			X	X					16

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 272 ppm

Number of Days on Study	435	439	477	543	599	629	652	662	668	698	701	703	722	724	733	733	733	733	733	733	734	734	734	734	734
Carcass ID Number	011	012	013	019	018	241	246	034	043	049	051	260	269	271	289	290	291	292	293	294	372	373	374	375	376
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Colon, adenoma																									
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																								X	X
Mesentery	+							+				+		+						+					
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth							+																		
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortex, adenoma																									
Medulla, pheochromocytoma benign																		X							X
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma					X			X	X	X		X			X				X				X	X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma						X							X			X									
Follicular cell, carcinoma								X																	
General Body System																									
None																									

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 272 ppm

Number of Days on Study	734	734	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	736	
Carcass ID Number	377	378	073	074	075	076	077	097	098	099	100	301	302	321	322	323	324	325	326	327	328	329	086	Total Tissues/ Tumors
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Colon, adenoma																				X				1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocellular adenoma													X											3
Mesentery	+																							6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tooth																								1
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cortex, adenoma				X																				1
Medulla, pheochromocytoma benign																								2
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, medulla	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma														X										1
Parathyroid gland	M	+	+	+	+	+	M	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	M	40
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma	X	X	X					X	X	X		X		X		X		X	X				X	21
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
C-cell, adenoma			X					X						X								X		7
Follicular cell, carcinoma																								1
General Body System																								
None																								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 272 ppm

Number of Days on Study	435	439	447	543	559	629	652	662	668	698	701	703	722	724	733	733	733	733	733	733	734	734	734	734	734
Carcass ID Number	011	012	013	019	018	241	246	034	043	049	051	260	269	271	289	290	291	292	293	294	372	373	374	375	376
Genital System																									
Coagulating gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																		X							
Carcinoma				X					X					X											
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma			X	X		X	X	X	X		X	X	X	X	X	X	X	X		X	X	X	X	X	X
Interstitial cell, adenoma	X																		X						
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Fibroadenoma																									
Skin	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma			X																						
Fibroma																		X							
Fibrous histiocytoma																									
Lipoma										X															
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 272 ppm

Number of Days on Study	734	734	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	736	
Carcass ID Number	377	378	073	074	075	076	077	097	098	099	100	301	302	321	322	323	324	325	326	327	328	329	086	Total Tissues/ Tumors
Genital System																								
Coagulating gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	47
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma		X		X		X					X	X						X						7
Carcinoma																		X						4
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Bilateral, interstitial cell, adenoma	X	X		X	X	X	X	X		X	X		X		X	X	X	X	X	X	X	X		38
Interstitial cell, adenoma			X									X											X	5
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	43
Integumentary System																								
Mammary gland	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	46
Carcinoma										X														1
Fibroadenoma											X													1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Basal cell carcinoma																								1
Fibroma																								1
Fibrous histiocytoma									X															1
Lipoma																								1
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	47
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 272 ppm

Number of Days on Study	435	439	477	543	559	629	652	662	668	698	701	703	722	724	733	733	733	733	733	733	734	734	734	734	734
Carcass ID Number	011	012	013	019	018	241	246	034	043	049	051	060	069	071	089	090	091	092	093	094	072	073	074	075	076
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Carcinoma, metastatic, thyroid gland								X																	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear										+				+											
Pinna, neural crest tumor										X															
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Squamous cell carcinoma							X																		
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urethra																									+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X				X	X	X		X	X	X	X	X	X		X	X	X	X		X	X			
Mesothelioma malignant																			X						

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 272 ppm

Number of Days on Study	734	734	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	736
Carcass ID Number	377	378	073	074	075	076	077	097	098	099	100	301	302	221	222	223	224	225	226	227	228	229	086	Total Tissues/ Tumors
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma							X					X					X							3
Carcinoma, metastatic, thyroid gland																								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Special Senses System																								
Ear																								2
Pinna, neural crest tumor																								1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Squamous cell carcinoma																								1
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Urethra																								1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leukemia mononuclear	X		X																		X			19
Mesothelioma malignant																								1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 543 ppm

Number of Days on Study	475	505	601	645	645	658	660	661	661	676	679	687	698	707	713	717	724	733	733	733	733	733	733	733
Carcass ID Number	014	016	036	031	033	032	037	039	052	044	046	047	057	065	064	070	087	095	096	097	098	099	030	036
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Jejunum, leiomyoma																								
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin																								
Hepatocellular adenoma																								
Mesentery																+							+	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell, adenocarcinoma						X																		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma														X										
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin																								
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Medulla, pheochromocytoma malignant										X						X								
Medulla, pheochromocytoma complex					X																			
Medulla, pheochromocytoma benign																								
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma									X															X
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Craniopharyngioma																								
Pars distalis, adenoma					X						X												X	X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 543 ppm

Number of Days on Study	733	733	734	734	734	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	
Carcass ID Number	337	338	379	380	383	078	079	080	081	082	083	084	085	303	304	305	306	330	331	332	333	334	335	Total Tissues/ Tumors
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Jejunum, leiomyoma										X														1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Fibrous histiocytoma, metastatic, skin										X														1
Hepatocellular adenoma			X											X										2
Mesentery								+																3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Acinar cell, adenocarcinoma																								1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Fibrous histiocytoma																								1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Sarcoma, metastatic, skin																					X			1
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Medulla, pheochromocytoma malignant										X														3
Medulla, pheochromocytoma complex																								1
Medulla, pheochromocytoma benign	X			X														X						3
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma	X														X									4
Parathyroid gland	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Craniopharyngioma			X																					1
Pars distalis, adenoma					X		X		X		X	X	X			X			X				X	13

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 543 ppm

Number of Days on Study	475	505	600	644	645	658	660	661	661	676	679	687	689	707	713	717	724	733	733	733	733	733	733	733	733
Carcass ID Number	014	016	236	031	033	032	037	039	025	044	046	047	057	265	264	270	087	295	296	297	298	299	300	336
Endocrine System (continued)																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma														X											
Bilateral, follicular cell, carcinoma																									
C-cell, adenoma																				X					
Follicular cell, adenoma																									
Follicular cell, carcinoma																									
General Body System																									
Tissue NOS															+										
Genital System																									
Coagulating gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma						X											X								
Carcinoma		X														X									
Squamous cell papilloma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma		X	X	X		X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X
Interstitial cell, adenoma	X				X											X	X								
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, pancreas						X																			
Thymus	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	M	+	+	+	M	+	+	M	+	+	M	+	+	+	M	M	+	+	+	+	+	+
Fibroadenoma																						X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Fibroma												X		X	X										
Fibrous histiocytoma																									
Keratoacanthoma																									
Sarcoma																			X						
Squamous cell carcinoma																									

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Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 543 ppm

Number of Days on Study	733	733	734	734	734	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	
Carcass ID Number	337	338	379	380	383	078	079	080	081	082	083	084	085	303	304	305	306	330	331	332	333	334	335		Total Tissues/ Tumors
Endocrine System (continued)																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		46
Fibrous histiocytoma																									1
Bilateral, follicular cell, carcinoma																		X							1
C-cell, adenoma						X		X												X					4
Follicular cell, adenoma													X												1
Follicular cell, carcinoma																							X		1
General Body System																									
Tissue NOS																									1
Genital System																									
Coagulating gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		46
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Adenoma																						X			3
Carcinoma				X	X			X				X	X					X			X				9
Squamous cell papilloma							X																		1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X		X		X	X	X	X	X	X		X	X		X	X	X	X		39
Interstitial cell, adenoma							X		X																6
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Adenocarcinoma, metastatic, pancreas																									1
Thymus	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M		42
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		41
Fibroadenoma		X							X			X													4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Basal cell adenoma															X										1
Fibroma	X																						X		5
Fibrous histiocytoma										X															1
Keratoacanthoma							X												X						2
Sarcoma				X	X																X				4
Squamous cell carcinoma						X																			1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 543 ppm

Number of Days on Study	475	505	601	645	645	658	660	661	661	676	679	687	698	707	713	717	724	733	733	733	733	733	733	733	733
Carcass ID Number	014	016	236	031	033	032	037	039	025	044	046	047	257	265	264	270	087	295	296	297	298	299	300	336
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma							X				X							X							
Sarcoma, metastatic, skin																			X						
Squamous cell carcinoma, metastatic, preputial gland																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma														X											
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma				X																					
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X				X	X	X								X								
Mesothelioma malignant	X																								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Leucomalachite Green: 543 ppm

Number of Days on Study	733	733	734	734	734	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	
Carcass ID Number	337	338	379	380	383	078	079	080	081	082	083	084	085	303	304	305	306	330	331	332	333	334	335	Total Tissues/ Tumors
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Fibrous histiocytoma, metastatic, skin										X														1
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Alveolar/bronchiolar adenoma														X										4
Sarcoma, metastatic, skin																								1
Squamous cell carcinoma, metastatic, preputial gland				X																				1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Fibrous histiocytoma																								1
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Carcinoma																								1
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Leukemia mononuclear		X																		X				7
Mesothelioma malignant																								1

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	3/47 (6%)	6/46 (13%)	2/47 (4%)	3/47 (6%)
Adjusted rate[b]	7.4%	14.5%	4.8%	7.1%
Terminal rate[c]	2/23 (9%)	5/29 (17%)	2/33 (6%)	3/30 (10%)
First incidence (days)	659	615	733 (T)	733 (T)
Poly-3 test[d]	P=0.311N	P=0.250	P=0.485N	P=0.643N
Adrenal Medulla: Malignant Pheochromocytoma				
Overall rate	0/47 (0%)	2/46 (4%)	0/47 (0%)	3/47 (6%)
Adjusted rate	0.0%	4.9%	0.0%	7.0%
Terminal rate	0/23 (0%)	1/29 (3%)	0/33 (0%)	1/30 (3%)
First incidence (days)	—[e]	697	—	676
Poly-3 test	P=0.127	P=0.241	—[f]	P=0.128
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	3/47 (6%)	8/46 (17%)	2/47 (4%)	7/47 (15%)
Adjusted rate	7.4%	19.2%	4.8%	16.3%
Terminal rate	2/23 (9%)	6/29 (21%)	2/33 (6%)	4/30 (13%)
First incidence (days)	659	615	733 (T)	645
Poly-3 test	P=0.357	P=0.103	P=0.485N	P=0.179
Liver: Hepatocellular Adenoma				
Overall rate	2/48 (4%)	2/47 (4%)	3/48 (6%)	2/47 (4%)
Adjusted rate	4.8%	4.8%	7.0%	4.7%
Terminal rate	0/24 (0%)	2/30 (7%)	3/34 (9%)	2/30 (7%)
First incidence (days)	639	733 (T)	733 (T)	733 (T)
Poly-3 test	P=0.568	P=0.692N	P=0.512	P=0.690N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	1/48 (2%)	1/47 (2%)	3/48 (6%)	4/47 (9%)
Adjusted rate	2.4%	2.4%	7.0%	9.3%
Terminal rate	1/24 (4%)	1/30 (3%)	3/34 (9%)	2/30 (7%)
First incidence (days)	733 (T)	733 (T)	733 (T)	660
Poly-3 test	P=0.073	P=0.757N	P=0.319	P=0.189
Mammary Gland: Fibroadenoma				
Overall rate	1/48 (2%)	2/47 (4%)	1/48 (2%)	4/47 (9%)
Adjusted rate	2.4%	4.7%	2.3%	9.4%
Terminal rate	1/24 (4%)	1/30 (3%)	1/34 (3%)	4/30 (13%)
First incidence (days)	733 (T)	667	733 (T)	733 (T)
Poly-3 test	P=0.122	P=0.508	P=0.752N	P=0.185
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	1/48 (2%)	2/47 (4%)	2/48 (4%)	4/47 (9%)
Adjusted rate	2.4%	4.7%	4.7%	9.4%
Terminal rate	1/24 (4%)	1/30 (3%)	2/34 (6%)	4/30 (13%)
First incidence (days)	733 (T)	667	733 (T)	733 (T)
Poly-3 test	P=0.122	P=0.508	P=0.514	P=0.185
Pancreatic Islets: Adenoma				
Overall rate	5/48 (10%)	2/47 (4%)	1/48 (2%)	4/47 (9%)
Adjusted rate	12.0%	4.7%	2.3%	9.4%
Terminal rate	3/24 (13%)	1/30 (3%)	1/34 (3%)	3/30 (10%)
First incidence (days)	698	697	733 (T)	661
Poly-3 test	P=0.525N	P=0.209N	P=0.093N	P=0.484N

Table A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	30/45 (67%)	19/46 (41%)	21/48 (44%)	13/45 (29%)
Adjusted rate	69.6%	43.0%	47.5%	31.3%
Terminal rate	15/23 (65%)	10/30 (33%)	16/34 (47%)	11/30 (37%)
First incidence (days)	541	487	599	645
Poly-3 test	P<0.001N	P=0.008N	P=0.026N	P<0.001N
Preputial Gland: Adenoma				
Overall rate	5/48 (10%)	2/47 (4%)	7/48 (15%)	3/47 (6%)
Adjusted rate	12.1%	4.8%	16.3%	7.0%
Terminal rate	4/24 (17%)	2/30 (7%)	7/34 (21%)	1/30 (3%)
First incidence (days)	718	733 (T)	733 (T)	658
Poly-3 test	P=0.482N	P=0.209N	P=0.405	P=0.339N
Preputial Gland: Carcinoma				
Overall rate	6/48 (13%)	0/47 (0%)	4/48 (8%)	9/47 (19%)
Adjusted rate	14.2%	0.0%	9.1%	20.9%
Terminal rate	4/24 (17%)	0/30 (0%)	1/34 (3%)	7/30 (23%)
First incidence (days)	564	—	543	505
Poly-3 test	P=0.035	P=0.015N	P=0.344N	P=0.301
Preputial Gland: Adenoma or Carcinoma				
Overall rate	11/48 (23%)	2/47 (4%)	10/48 (21%)	12/47 (26%)
Adjusted rate	26.0%	4.8%	22.8%	27.6%
Terminal rate	8/24 (33%)	2/30 (7%)	7/34 (21%)	8/30 (27%)
First incidence (days)	564	733 (T)	543	505
Poly-3 test	P=0.116	P=0.006N	P=0.460N	P=0.532
Skin: Lipoma				
Overall rate	0/48 (0%)	3/47 (6%)	1/48 (2%)	0/47 (0%)
Adjusted rate	0.0%	7.1%	2.3%	0.0%
Terminal rate	0/24 (0%)	3/30 (10%)	0/34 (0%)	0/30 (0%)
First incidence (days)	—	733 (T)	698	—
Poly-3 test	P=0.274N	P=0.121	P=0.508	—
Skin: Fibroma				
Overall rate	4/48 (8%)	1/47 (2%)	1/48 (2%)	5/47 (11%)
Adjusted rate	9.6%	2.4%	2.3%	11.7%
Terminal rate	3/24 (13%)	1/30 (3%)	1/34 (3%)	2/30 (7%)
First incidence (days)	623	733 (T)	733 (T)	687
Poly-3 test	P=0.250	P=0.176N	P=0.169N	P=0.515
Skin: Sarcoma				
Overall rate	0/48 (0%)	3/47 (6%)	0/48 (0%)	4/47 (9%)
Adjusted rate	0.0%	7.1%	0.0%	9.4%
Terminal rate	0/24 (0%)	2/30 (7%)	0/34 (0%)	4/30 (13%)
First incidence (days)	—	604	—	733 (T)
Poly-3 test	P=0.092	P=0.123	—	P=0.063
Skin: Fibrous Histiocytoma or Sarcoma				
Overall rate	0/48 (0%)	4/47 (9%)	1/48 (2%)	5/47 (11%)
Adjusted rate	0.0%	9.4%	2.3%	11.8%
Terminal rate	0/24 (0%)	2/30 (7%)	1/34 (3%)	5/30 (17%)
First incidence (days)	—	604	733 (T)	733 (T)
Poly-3 test	P=0.071	P=0.063	P=0.508	P=0.032

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Skin: Fibroma, Fibrous Histocytoma, or Sarcoma				
Overall rate	4/48 (8%)	5/47 (11%)	2/48 (4%)	10/47 (21%)
Adjusted rate	9.6%	11.8%	4.7%	23.4%
Terminal rate	3/24 (13%)	3/30 (10%)	2/34 (6%)	7/30 (23%)
First incidence (days)	623	604	733 (T)	687
Poly-3 test	P=0.042	P=0.511	P=0.323N	P=0.076
Skin: Keratoacanthoma				
Overall rate	2/48 (4%)	3/47 (6%)	0/48 (0%)	2/47 (4%)
Adjusted rate	4.8%	7.1%	0.0%	4.7%
Terminal rate	1/24 (4%)	2/30 (7%)	0/34 (0%)	2/30 (7%)
First incidence (days)	662	579	—	733 (T)
Poly-3 test	P=0.428N	P=0.510	P=0.230N	P=0.688N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	2/48 (4%)	5/47 (11%)	0/48 (0%)	2/47 (4%)
Adjusted rate	4.8%	11.7%	0.0%	4.7%
Terminal rate	1/24 (4%)	4/30 (13%)	0/34 (0%)	2/30 (7%)
First incidence (days)	662	579	—	733 (T)
Poly-3 test	P=0.264N	P=0.224	P=0.230N	P=0.688N
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	2/48 (4%)	5/47 (11%)	0/48 (0%)	3/47 (6%)
Adjusted rate	4.8%	11.7%	0.0%	7.1%
Terminal rate	1/24 (4%)	4/30 (13%)	0/34 (0%)	3/30 (10%)
First incidence (days)	662	579	—	733 (T)
Poly-3 test	P=0.461N	P=0.224	P=0.230N	P=0.508
Skin: Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Adenoma, Basal Cell Carcinoma, or Squamous Cell Carcinoma				
Overall rate	2/48 (4%)	5/47 (11%)	1/48 (2%)	4/47 (9%)
Adjusted rate	4.8%	11.7%	2.3%	9.4%
Terminal rate	1/24 (4%)	4/30 (13%)	0/34 (0%)	4/30 (13%)
First incidence (days)	662	579	477	733 (T)
Poly-3 test	P=0.488	P=0.224	P=0.483N	P=0.345
Testes: Adenoma				
Overall rate	37/48 (77%)	42/47 (89%)	43/48 (90%)	45/47 (96%)
Adjusted rate	82.5%	93.3%	92.2%	95.7%
Terminal rate	23/24 (96%)	29/30 (97%)	32/34 (94%)	28/30 (93%)
First incidence (days)	564	579	435	475
Poly-3 test	P=0.036	P=0.078	P=0.115	P=0.029
Thyroid Gland (C-Cell): Adenoma				
Overall rate	6/47 (13%)	7/47 (15%)	7/48 (15%)	4/46 (9%)
Adjusted rate	14.4%	16.5%	16.1%	9.6%
Terminal rate	3/24 (13%)	6/30 (20%)	5/34 (15%)	4/30 (13%)
First incidence (days)	613	606	629	733 (T)
Poly-3 test	P=0.266N	P=0.518	P=0.535	P=0.368N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	7/47 (15%)	9/47 (19%)	7/48 (15%)	4/46 (9%)
Adjusted rate	16.9%	21.2%	16.1%	9.6%
Terminal rate	3/24 (13%)	7/30 (23%)	5/34 (15%)	4/30 (13%)
First incidence (days)	613	606	629	733 (T)
Poly-3 test	P=0.135N	P=0.410	P=0.580N	P=0.259N

Table A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	0/47 (0%)	2/47 (4%)	1/48 (2%)	3/46 (7%)
Adjusted rate	0.0%	4.8%	2.3%	7.2%
Terminal rate	0/24 (0%)	2/30 (7%)	0/34 (0%)	3/30 (10%)
First incidence (days)	—	733 (T)	662	733 (T)
Poly-3 test	P=0.122	P=0.244	P=0.512	P=0.121
All Organs: Mononuclear Cell Leukemia				
Overall rate	29/48 (60%)	16/47 (34%)	19/48 (40%)	7/47 (15%)
Adjusted rate	63.6%	36.6%	41.6%	16.0%
Terminal rate	12/24 (50%)	9/30 (30%)	9/34 (27%)	2/30 (7%)
First incidence (days)	564	579	435	601
Poly-3 test	P<0.001N	P=0.007N	P=0.026N	P<0.001N
All Organs: Benign Neoplasms				
Overall rate	45/48 (94%)	47/47 (100%)	47/48 (98%)	47/47 (100%)
Adjusted rate	95.0%	100.0%	99.6%	100.0%
Terminal rate	24/24 (100%)	30/30 (100%)	34/34 (100%)	30/30 (100%)
First incidence (days)	541	487	435	475
Poly-3 test	P=0.094	P=0.166	P=0.208	P=0.166
All Organs: Malignant Neoplasms				
Overall rate	34/48 (71%)	23/47 (49%)	25/48 (52%)	26/47 (55%)
Adjusted rate	73.8%	51.6%	53.0%	56.0%
Terminal rate	15/24 (63%)	13/30 (43%)	12/34 (35%)	13/30 (43%)
First incidence (days)	564	579	435	475
Poly-3 test	P=0.132N	P=0.020N	P=0.027N	P=0.054N
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/48 (100%)	47/47 (100%)	47/48 (98%)	47/47 (100%)
Adjusted rate	100.0%	100.0%	99.6%	100.0%
Terminal rate	24/24 (100%)	30/30 (100%)	34/34 (100%)	30/30 (100%)
First incidence (days)	541	487	435	475
Poly-3 test	P=0.998N	—	P=1.000N	—

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

TABLE A4a
Historical Incidence of Thyroid Gland (Follicular Cell) Neoplasms in Control Male F344 Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Doxylamine	1/48	0/48	1/48
Fumonisin B_1	0/48	0/48	0/48
Gentian violet	1/163	0/163	1/163
Pyrilamine	0/42	0/42	0/42
Sulfamethazine	0/170	0/170	0/170
Triprolidine	0/40	0/40	0/40
Total (%)	2/511 (0.4%)	0/511 (0%)	2/511 (0.4%)
Range	0%-2%		0%-2%

[a] Data as of April 30, 2003. Studies were performed at the National Center for Toxicological Research in animals given NIH-31 feed.

TABLE A4b
Historical Incidence of Testes Adenoma in Control Male F344 Rats[a]

Study	Incidence in Controls
Doxylamine	36/48
Fumonisin B_1	33/48
Gentian violet	160/177
Pyrilamine	39/48
Sulfamethazine	161/179
Triprolidine	40/47
Total (%)	469/547 (85.7%)
Range	69%-90%

[a] Data as of April 30, 2003. Studies were performed at the National Center for Toxicological Research in animals given NIH-31 feed.

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Leucomalachite Green[a]

	0 ppm	91 ppm	272 ppm	543 ppm
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund	20	12	11	10
Natural deaths	4	5	3	7
Survivors				
Died last week of study	1	1		
Terminal sacrifice	23	29	34	30
Missexed		1		1
Animals examined microscopically	48	47	48	47
Alimentary System				
Intestine large	(48)	(47)	(48)	(47)
Artery, colon, serosa, dilatation			1 (2%)	
Cecum, lymphoid tissue, hyperplasia	3 (6%)	1 (2%)		
Colon, lymphoid tissue, hyperplasia			2 (4%)	1 (2%)
Intestine small	(47)	(47)	(48)	(47)
Duodenum, autolysis		1 (2%)		
Ileum, hyperplasia			1 (2%)	
Ileum, inflammation			1 (2%)	
Ileum, lymphoid tissue, hyperplasia	4 (9%)	1 (2%)	1 (2%)	3 (6%)
Jejunum, autolysis		1 (2%)		
Jejunum, diverticulum				1 (2%)
Jejunum, lymphoid tissue, hyperplasia	1 (2%)			2 (4%)
Liver	(48)	(47)	(48)	(47)
Basophilic focus		2 (4%)		2 (4%)
Degeneration, cystic	4 (8%)	18 (38%)	13 (27%)	19 (40%)
Developmental malformation	1 (2%)	1 (2%)		1 (2%)
Eosinophilic focus	2 (4%)	8 (17%)	9 (19%)	8 (17%)
Eosinophilic focus, multiple	1 (2%)	6 (13%)	10 (21%)	25 (53%)
Hematopoietic cell proliferation	1 (2%)			
Hepatodiaphragmatic nodule	2 (4%)	2 (4%)	3 (6%)	
Infiltration cellular, lymphocyte				1 (2%)
Inflammation, focal, granulomatous	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Mixed cell focus	1 (2%)			2 (4%)
Necrosis	1 (2%)		1 (2%)	
Pigmentation			1 (2%)	
Vacuolization cytoplasmic	9 (19%)	21 (45%)	10 (21%)	13 (28%)
Bile duct, cyst	1 (2%)		1 (2%)	
Bile duct, hyperplasia	41 (85%)	42 (89%)	42 (88%)	38 (81%)
Centrilobular, necrosis		1 (2%)	1 (2%)	3 (6%)
Mesentery	(5)	(3)	(6)	(3)
Accessory spleen			2 (33%)	
Polyarteritis			2 (33%)	1 (33%)
Fat, necrosis	3 (60%)	3 (100%)	2 (33%)	2 (67%)
Pancreas	(48)	(47)	(48)	(47)
Accessory spleen			1 (2%)	1 (2%)
Hyperplasia			1 (2%)	
Infiltration cellular, lymphocyte	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Polyarteritis				1 (2%)
Acinus, atrophy	28 (58%)	16 (34%)	24 (50%)	22 (47%)
Artery, inflammation, chronic		1 (2%)	2 (4%)	4 (9%)
Artery, media, hypertrophy		1 (2%)		1 (2%)

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

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Alimentary System (continued)				
Salivary glands	(48)	(47)	(48)	(47)
Atrophy			1 (2%)	
Stomach	(48)	(47)	(47)	(47)
Forestomach, edema	2 (4%)	1 (2%)	2 (4%)	
Forestomach, inflammation	2 (4%)	1 (2%)	1 (2%)	2 (4%)
Forestomach, necrosis		1 (2%)		
Forestomach, epithelium, hyperplasia	1 (2%)		1 (2%)	2 (4%)
Glandular, edema	1 (2%)	1 (2%)		
Glandular, erosion	1 (2%)	1 (2%)		
Glandular, mineralization				1 (2%)
Tooth			(1)	
Gingiva, hyperplasia			1 (100%)	
Gingiva, inflammation			1 (100%)	
Cardiovascular System				
Heart	(48)	(47)	(48)	(47)
Cardiomyopathy	37 (77%)	36 (77%)	40 (83%)	40 (85%)
Atrium, thrombosis	1 (2%)	2 (4%)		3 (6%)
Myocardium, infarct				1 (2%)
Ventricle right, dilatation	1 (2%)			
Endocrine System				
Adrenal gland	(48)	(47)	(48)	(47)
Degeneration				1 (2%)
Cortex, accessory adrenal cortical nodule			2 (4%)	1 (2%)
Cortex, cytoplasmic alteration				1 (2%)
Cortex, hyperplasia, focal		5 (11%)	3 (6%)	5 (11%)
Cortex, hypertrophy, focal		1 (2%)		1 (2%)
Cortex, vacuolization cytoplasmic	1 (2%)	2 (4%)	4 (8%)	2 (4%)
Medulla, hyperplasia	1 (2%)		3 (6%)	2 (4%)
Islets, pancreatic	(48)	(47)	(48)	(47)
Hyperplasia		1 (2%)	1 (2%)	1 (2%)
Parathyroid gland	(38)	(41)	(40)	(42)
Hyperplasia, focal	2 (5%)	2 (5%)		1 (2%)
Pituitary gland	(45)	(46)	(48)	(45)
Angiectasis		2 (4%)	1 (2%)	
Infiltration cellular, histiocytic		1 (2%)		
Pigmentation		1 (2%)		
Pars distalis, cyst	2 (4%)	3 (7%)	5 (10%)	3 (7%)
Pars distalis, hyperplasia	1 (2%)	7 (15%)	2 (4%)	7 (16%)
Pars intermedia, cyst				1 (2%)
Thyroid gland	(47)	(47)	(48)	(46)
Ultimobranchial cyst			1 (2%)	
C-cell, hyperplasia	8 (17%)	6 (13%)	3 (6%)	3 (7%)
Follicle, cyst				3 (7%)
Follicular cell, hyperplasia	2 (4%)	1 (2%)	3 (6%)	3 (7%)
General Body System				
Tissue NOS	(1)			(1)
Abdominal, pigmentation	1 (100%)			
Abdominal, fat, necrosis				1 (100%)

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Genital System				
Coagulating gland	(46)	(45)	(47)	(46)
Decreased secretory fluid	2 (4%)			3 (7%)
Inflammation, suppurative		1 (2%)		
Epididymis	(48)	(47)	(48)	(47)
Atrophy				1 (2%)
Granuloma sperm		1 (2%)		
Inflammation, chronic				1 (2%)
Serosa, cyst	1 (2%)			
Penis		(1)		
Thrombosis		1 (100%)		
Preputial gland	(48)	(47)	(48)	(47)
Atrophy	1 (2%)			3 (6%)
Hyperplasia	1 (2%)	1 (2%)		3 (6%)
Infiltration cellular, chronic	1 (2%)			
Inflammation	35 (73%)	36 (77%)	38 (79%)	29 (62%)
Duct, ectasia	7 (15%)	8 (17%)	11 (23%)	9 (19%)
Prostate	(48)	(47)	(48)	(47)
Decreased secretory fluid	1 (2%)	1 (2%)		1 (2%)
Inflammation	29 (60%)	21 (45%)	22 (46%)	18 (38%)
Ventral, hyperplasia	1 (2%)	3 (6%)	1 (2%)	6 (13%)
Seminal vesicle	(48)	(47)	(48)	(47)
Atrophy			1 (2%)	1 (2%)
Decreased secretory fluid	28 (58%)	31 (66%)	33 (69%)	36 (77%)
Lumen, distended	1 (2%)			
Serosa, inflammation, chronic	1 (2%)			
Testes	(48)	(47)	(48)	(47)
Mineralization	1 (2%)		1 (2%)	
Interstitial cell, hyperplasia	17 (35%)	11 (23%)	5 (10%)	3 (6%)
Seminiferous tubule, atrophy	11 (23%)	7 (15%)	3 (6%)	8 (17%)
Hematopoietic System				
Bone marrow	(48)	(47)	(48)	(47)
Atrophy	1 (2%)	1 (2%)		1 (2%)
Hyperplasia	2 (4%)			2 (4%)
Myeloid cell, hyperplasia		1 (2%)	2 (4%)	1 (2%)
Lymph node	(48)	(47)	(48)	(47)
Infiltration cellular, plasma cell	1 (2%)			
Axillary, hyperplasia, lymphoid			1 (2%)	
Deep cervical, infiltration cellular, plasma cell			1 (2%)	
Lumbar, hematopoietic cell proliferation			1 (2%)	
Lumbar, hyperplasia, lymphoid	2 (4%)	1 (2%)	2 (4%)	
Lumbar, pigmentation		1 (2%)		
Mandibular, ectasia	3 (6%)	5 (11%)	6 (13%)	4 (9%)
Mandibular, hemorrhage	1 (2%)			1 (2%)
Mandibular, hyperplasia, lymphoid		2 (4%)	3 (6%)	1 (2%)
Mandibular, infiltration cellular, plasma cell	5 (10%)	6 (13%)	11 (23%)	7 (15%)
Mediastinal, ectasia	1 (2%)			
Mediastinal, hemorrhage		1 (2%)	1 (2%)	1 (2%)
Mesenteric, congestion		1 (2%)		
Mesenteric, ectasia	4 (8%)	6 (13%)	6 (13%)	1 (2%)
Mesenteric, hemorrhage	1 (2%)	3 (6%)	2 (4%)	1 (2%)

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Hematopoietic System (continued)				
Lymph node (continued)	(48)	(47)	(48)	(47)
Mesenteric, hyperplasia, lymphoid	1 (2%)		1 (2%)	1 (2%)
Mesenteric, infiltration cellular, plasma cell	2 (4%)			1 (2%)
Pancreatic, hyperplasia, lymphoid	1 (2%)	1 (2%)		
Pancreatic, infiltration cellular, plasma cell	1 (2%)			
Pancreatic, pigmentation		1 (2%)		
Renal, hemorrhage	1 (2%)	2 (4%)		
Renal, hyperplasia, lymphoid		2 (4%)		
Renal, infiltration cellular, diffuse, histiocytic		1 (2%)		
Spleen	(48)	(47)	(48)	(47)
Angiectasis		1 (2%)		
Fibrosis	5 (10%)	6 (13%)	1 (2%)	5 (11%)
Hematopoietic cell proliferation	2 (4%)	3 (6%)	1 (2%)	8 (17%)
Hyperplasia, focal			1 (2%)	
Hyperplasia, lymphoid		1 (2%)	2 (4%)	4 (9%)
Infarct		1 (2%)		2 (4%)
Pigmentation	3 (6%)	2 (4%)		2 (4%)
Stromal hyperplasia	1 (2%)			
Thymus	(43)	(42)	(43)	(42)
Ectopic parathyroid gland	1 (2%)			
Epithelial cell, hyperplasia				1 (2%)
Integumentary System				
Mammary gland	(43)	(41)	(46)	(41)
Galactocele	6 (14%)	1 (2%)	6 (13%)	2 (5%)
Hyperplasia	5 (12%)	4 (10%)	4 (9%)	5 (12%)
Duct, dilatation	12 (28%)	6 (15%)	3 (7%)	4 (10%)
Skin	(48)	(47)	(47)	(47)
Abscess	1 (2%)			
Cyst				1 (2%)
Cyst epithelial inclusion	2 (4%)	2 (4%)		1 (2%)
Hyperkeratosis	1 (2%)			
Inflammation			1 (2%)	
Inflammation, chronic			1 (2%)	
Epidermis, hyperplasia	1 (2%)			1 (2%)
Epidermis, necrosis				1 (2%)
Epithelium, hyperplasia				2 (4%)
Foot, inflammation, chronic		1 (2%)	1 (2%)	1 (2%)
Musculoskeletal System				
Bone	(48)	(47)	(48)	(47)
Femur, fibrous osteodystrophy	1 (2%)			2 (4%)
Sternum, fibrous osteodystrophy		1 (2%)		
Skeletal muscle	(48)	(47)	(48)	(47)
Arteriole, mandibular, proliferation			1 (2%)	
Fiber, mandibular, hypertrophy			1 (2%)	

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Nervous System				
Brain	(48)	(47)	(48)	(47)
Brain stem, hemorrhage	1 (2%)	2 (4%)		
Brain stem, hypothalamus, compression	9 (19%)	4 (9%)	6 (13%)	1 (2%)
Cerebrum, compression				1 (2%)
Cerebrum, hemorrhage				1 (2%)
Cerebrum, hydrocephalus		1 (2%)		
Cerebrum, hypothalamus, compression		1 (2%)		
Spinal cord	(48)	(47)	(48)	(47)
Thoracic, hemorrhage		1 (2%)		
Respiratory System				
Lung	(48)	(47)	(48)	(47)
Granuloma		1 (2%)		
Hemorrhage	1 (2%)		2 (4%)	
Alveolar epithelium, hyperplasia	2 (4%)	2 (4%)	4 (8%)	6 (13%)
Alveolus, infiltration cellular, histiocytic				1 (2%)
Alveolus, infiltration cellular, histiocyte	3 (6%)	4 (9%)	2 (4%)	4 (9%)
Alveolus, inflammation, multifocal			1 (2%)	
Mediastinum, hemorrhage		1 (2%)		
Nose	(48)	(47)	(48)	(47)
Inflammation, suppurative		1 (2%)		
Nasolacrimal duct, inflammation	10 (21%)	15 (32%)	16 (33%)	12 (26%)
Nasolacrimal duct, mineralization				1 (2%)
Respiratory epithelium, hyperplasia	1 (2%)	1 (2%)	1 (2%)	
Respiratory epithelium, inflammation, chronic	1 (2%)			
Septum, inflammation, chronic	1 (2%)		1 (2%)	
Special Senses System				
Ear	(1)		(2)	
Canal, external ear, exudate			1 (50%)	
Eye	(48)	(47)	(48)	(47)
Cataract	3 (6%)	3 (6%)	2 (4%)	1 (2%)
Hemorrhage	2 (4%)			
Inflammation	1 (2%)			
Bilateral, cataract	2 (4%)		1 (2%)	3 (6%)
Bilateral, retina, degeneration	1 (2%)			5 (11%)
Cornea, edema	1 (2%)			
Retina, autolysis	4 (8%)	5 (11%)	3 (6%)	3 (6%)
Retina, degeneration	8 (17%)	3 (6%)	3 (6%)	2 (4%)
Sclera, metaplasia, osseous	2 (4%)		1 (2%)	1 (2%)
Harderian gland	(48)	(47)	(48)	(47)
Hyperplasia		1 (2%)		
Infiltration cellular, lymphocyte	16 (33%)	16 (34%)	16 (33%)	18 (38%)
Inflammation	1 (2%)	1 (2%)		1 (2%)
Zymbal's gland	(46)	(43)	(47)	(47)
Hyperplasia			1 (2%)	
Inflammation, chronic	1 (2%)			
Duct, ectasia				1 (2%)

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Urinary System				
Kidney	(48)	(47)	(48)	(47)
Accumulation, hyaline droplet				1 (2%)
Infiltration cellular, lymphocyte	1 (2%)			
Nephropathy	43 (90%)	45 (96%)	46 (96%)	47 (100%)
Pigmentation	3 (6%)	1 (2%)	1 (2%)	
Thrombosis				1 (2%)
Cortex, cyst			1 (2%)	1 (2%)
Renal tubule, hyperplasia			1 (2%)	
Urinary bladder	(48)	(47)	(48)	(47)
Calculus microscopic observation only				1 (2%)
Hemorrhage		1 (2%)		
Inflammation		1 (2%)		
Artery, inflammation				1 (2%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR FEED STUDIES OF MALACHITE GREEN CHLORIDE AND LEUCOMALACHITE GREEN

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TABLE B1a
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Malachite Green Chloride[a]

	0 ppm	100 ppm	300 ppm	600 ppm
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund	18	22	15	17
Natural deaths	1	3	1	6
Survivors				
Terminal sacrifice	29	23	32	25
Animals examined microscopically	48	48	48	48
Alimentary System				
Intestine large, colon	(47)	(47)	(48)	(47)
Polyp adenomatous			1 (2%)	
Intestine large, rectum	(48)	(46)	(48)	(48)
Polyp adenomatous				1 (2%)
Intestine small, ileum	(47)	(46)	(48)	(47)
Liver	(48)	(48)	(48)	(48)
Carcinoma, metastatic, uterus		1 (2%)		
Hepatocellular carcinoma			1 (2%)	
Hepatocellular adenoma		1 (2%)	3 (6%)	3 (6%)
Hepatocellular adenoma, multiple	1 (2%)			1 (2%)
Sarcoma, metastatic, ovary				1 (2%)
Mesentery	(9)	(5)	(2)	(4)
Carcinoma, metastatic, uterus		1 (20%)		
Sarcoma	1 (11%)			
Sarcoma, metastatic, ovary				1 (25%)
Pancreas	(48)	(48)	(48)	(48)
Carcinoma, metastatic, uterus		1 (2%)		
Salivary glands	(48)	(47)	(48)	(48)
Tongue	(48)	(48)	(48)	(48)
Sarcoma, metastatic, ovary				1 (2%)
Squamous cell carcinoma				1 (2%)
Squamous cell papilloma		1 (2%)		
Cardiovascular System				
Heart	(48)	(48)	(48)	(48)
Sarcoma, metastatic, ovary				1 (2%)
Endocrine System				
Adrenal cortex	(48)	(48)	(48)	(48)
Adenoma	2 (4%)			
Pheochromocytoma malignant, metastatic, adrenal medulla	1 (2%)			
Sarcoma, metastatic, ovary				1 (2%)
Adrenal medulla	(47)	(47)	(48)	(48)
Pheochromocytoma malignant	1 (2%)			
Pheochromocytoma benign		1 (2%)		1 (2%)
Sarcoma, metastatic, ovary				1 (2%)
Islets, pancreatic	(48)	(48)	(48)	(48)
Adenoma		2 (4%)	1 (2%)	1 (2%)
Carcinoma			1 (2%)	

Table B1a
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
Endocrine System (continued)				
Pituitary gland	(48)	(47)	(46)	(45)
Carcinoma, metastatic, Zymbal's gland				1 (2%)
Pars distalis, adenoma	26 (54%)	36 (77%)	32 (70%)	29 (64%)
Pars distalis, carcinoma				1 (2%)
Thyroid gland	(46)	(48)	(47)	(46)
Sarcoma, metastatic, ovary				1 (2%)
C-cell, adenoma	4 (9%)	5 (10%)	3 (6%)	3 (7%)
C-cell, carcinoma	3 (7%)	1 (2%)	2 (4%)	1 (2%)
Follicular cell, adenoma			1 (2%)	1 (2%)
Follicular cell, carcinoma			2 (4%)	1 (2%)
General Body System				
Tissue NOS		(1)		
Genital System				
Clitoral gland	(48)	(48)	(47)	(47)
Adenoma	7 (15%)	5 (10%)	8 (17%)	8 (17%)
Carcinoma	5 (10%)	1 (2%)	5 (11%)	4 (9%)
Bilateral, carcinoma		1 (2%)	1 (2%)	1 (2%)
Ovary	(48)	(48)	(47)	(48)
Carcinoma, metastatic, clitoral gland			1 (2%)	
Granulosa cell tumor malignant		1 (2%)		
Sarcoma				1 (2%)
Uterus	(48)	(48)	(48)	(48)
Polyp stromal	14 (29%)	7 (15%)	5 (10%)	16 (33%)
Sarcoma, metastatic, ovary				1 (2%)
Sarcoma stromal	2 (4%)	1 (2%)	1 (2%)	
Schwannoma malignant				1 (2%)
Endometrium, adenoma				1 (2%)
Endometrium, carcinoma		1 (2%)		
Vagina	(48)	(48)	(48)	(48)
Sarcoma				1 (2%)
Hematopoietic System				
Bone marrow	(48)	(48)	(48)	(48)
Lymph node	(10)	(7)	(7)	(5)
Lymph node, mandibular	(47)	(46)	(47)	(48)
Lymph node, mesenteric	(47)	(47)	(48)	(48)
Spleen	(48)	(48)	(48)	(48)
Carcinoma, metastatic, uterus		1 (2%)		
Hemangiosarcoma				1 (2%)
Sarcoma, metastatic, ovary				1 (2%)
Thymus	(40)	(42)	(43)	(40)
Sarcoma, metastatic, ovary				1 (3%)
Sarcoma, metastatic, uncertain primary site				1 (3%)

TABLE B1a
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
Integumentary System				
Mammary gland	(46)	(48)	(48)	(48)
Carcinoma	2 (4%)	2 (4%)	1 (2%)	5 (10%)
Fibroadenoma	15 (33%)	13 (27%)	12 (25%)	9 (19%)
Skin	(48)	(48)	(48)	(48)
Basal cell adenoma	1 (2%)			
Keratoacanthoma			1 (2%)	
Squamous cell papilloma			1 (2%)	
Subcutaneous tissue, fibroma		1 (2%)		
Subcutaneous tissue, sarcoma				2 (4%)
Musculoskeletal System				
Bone	(48)	(48)	(48)	(48)
Sarcoma				1 (2%)
Skeletal muscle	(48)	(48)	(48)	(48)
Nervous System				
Brain	(48)	(48)	(48)	(48)
Oligodendroglioma malignant				1 (2%)
Ventricle, carcinoma, metastatic, pituitary gland				1 (2%)
Respiratory System				
Lung	(48)	(48)	(48)	(48)
Alveolar/bronchiolar adenoma		1 (2%)	1 (2%)	
Carcinoma, metastatic, thyroid gland		1 (2%)		
Carcinoma, metastatic, Zymbal's gland				1 (2%)
Sarcoma, metastatic, mesentery	1 (2%)			
Sarcoma, metastatic, uncertain primary site				1 (2%)
Sarcoma stromal, metastatic, uterus		1 (2%)		
Mediastinum, sarcoma, metastatic, ovary				1 (2%)
Special Senses System				
Ear				(1)
Pinna, neural crest tumor				1 (100%)
Eye	(47)	(48)	(48)	(48)
Harderian gland	(47)	(48)	(48)	(48)
Adenoma				1 (2%)
Zymbal's gland	(47)	(48)	(48)	(46)
Carcinoma	1 (2%)		1 (2%)	2 (4%)
Urinary System				
Kidney	(48)	(48)	(48)	(48)
Urinary bladder	(47)	(48)	(47)	(47)
Sarcoma, metastatic, skin				1 (2%)
Transitional epithelium, carcinoma				1 (2%)
Transitional epithelium, papilloma		1 (2%)		
Systemic Lesions				
Multiple organs[b]	(48)	(48)	(48)	(48)
Leukemia mononuclear	19 (40%)	17 (35%)	10 (21%)	1 (2%)

Table B1a
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
Neoplasm Summary				
Total animals with primary neoplasms[c]	47	48	46	42
Total primary neoplasms	104	99	94	102
Total animals with benign neoplasms	41	42	40	35
Total benign neoplasms	70	74	69	75
Total animals with malignant neoplasms	29	24	21	23
Total malignant neoplasms	34	25	25	26
Total animals with metastatic neoplasms	2	3	1	5
Total metastatic neoplasms	2	6	1	17
Total animals with malignant neoplasms of uncertain primary site				1
Total animals with uncertain neoplasms-benign or malignant				1
Total uncertain neoplasms				1

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

TABLE B1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Leucomalachite Green[a]

	0 ppm	91 ppm	272 ppm	543 ppm
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund	12	11	12	10
Natural deaths	3	1	1	4
Survivors				
Died last day of study				1
Terminal sacrifice	33	36	35	33
Animals examined microscopically	48	48	48	48
Alimentary System				
Intestine large	(48)	(48)	(48)	(48)
Colon, adenocarcinoma				1 (2%)
Intestine small	(48)	(48)	(48)	(48)
Liver	(48)	(48)	(48)	(48)
Hepatocellular adenoma	1 (2%)	3 (6%)		3 (6%)
Mesentery	(8)	(7)	(1)	(4)
Sarcoma	1 (13%)			
Schwannoma malignant, metastatic, uterus	1 (13%)			
Pancreas	(48)	(48)	(47)	(48)
Schwannoma malignant, metastatic, uterus	1 (2%)			
Acinar cell, adenoma	1 (2%)			
Salivary glands	(48)	(48)	(48)	(48)
Sarcoma			1 (2%)	
Parotid gland, adenoma				1 (2%)
Stomach	(48)	(48)	(48)	(48)
Tongue	(48)	(48)	(48)	(48)
Squamous cell carcinoma			1 (2%)	
Squamous cell papilloma				1 (2%)
Cardiovascular System				
Blood vessel	(48)	(48)	(48)	(48)
Heart	(48)	(48)	(48)	(48)
Endocardium, schwannoma malignant			1 (2%)	
Myocardium, schwannoma malignant			1 (2%)	
Endocrine System				
Adrenal gland	(47)	(48)	(47)	(48)
Medulla, pheochromocytoma malignant			1 (2%)	
Medulla, pheochromocytoma benign			1 (2%)	
Islets, pancreatic	(48)	(48)	(47)	(48)
Pituitary gland	(47)	(47)	(45)	(46)
Pars distalis, adenoma	26 (55%)	23 (49%)	17 (38%)	20 (43%)
Thyroid gland	(46)	(46)	(47)	(48)
Bilateral, C-cell, adenoma			1 (2%)	1 (2%)
Bilateral, C-cell, carcinoma			1 (2%)	
C-cell, adenoma	4 (9%)	7 (15%)	4 (9%)	4 (8%)
C-cell, carcinoma		1 (2%)		
Follicular cell, adenoma				1 (2%)
Follicular cell, carcinoma		1 (2%)	2 (4%)	

Table B1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
General Body System				
Tissue NOS	(1)		(1)	
Schwannoma malignant, metastatic, uterus	1 (100%)			
Abdominal, lipoma			1 (100%)	
Genital System				
Clitoral gland	(47)	(47)	(46)	(47)
Adenoma	7 (15%)	8 (17%)	3 (7%)	6 (13%)
Carcinoma	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Ovary	(48)	(48)	(48)	(48)
Oviduct	(1)			
Leiomyoma	1 (100%)			
Uterus	(48)	(48)	(48)	(48)
Leiomyoma	1 (2%)			1 (2%)
Polyp stromal	9 (19%)	10 (21%)	16 (33%)	16 (33%)
Sarcoma stromal	1 (2%)			1 (2%)
Schwannoma malignant	1 (2%)		1 (2%)	
Bilateral, polyp stromal	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Cervix, squamous cell papilloma		1 (2%)		
Endometrium, adenoma		1 (2%)		1 (2%)
Endometrium, carcinoma		1 (2%)		
Vagina	(48)	(48)	(48)	(48)
Sarcoma			1 (2%)	
Schwannoma malignant		1 (2%)		
Hematopoietic System				
Lymph node	(48)	(48)	(48)	(48)
Mesenteric, schwannoma malignant, metastatic, uterus	1 (2%)			
Spleen	(48)	(48)	(48)	(48)
Thymus	(41)	(47)	(44)	(42)
Integumentary System				
Mammary gland	(48)	(46)	(48)	(48)
Adenoma		1 (2%)	1 (2%)	2 (4%)
Carcinoma		1 (2%)	2 (4%)	2 (4%)
Fibroadenoma	20 (42%)	12 (26%)	9 (19%)	13 (27%)
Fibroadenoma, multiple			3 (6%)	
Skin	(48)	(48)	(48)	(48)
Basal cell adenoma	1 (2%)	1 (2%)		
Basal cell carcinoma				1 (2%)
Fibroma		1 (2%)		1 (2%)
Fibrous histiocytoma			1 (2%)	
Keratoacanthoma		1 (2%)		1 (2%)
Sarcoma		2 (4%)		
Squamous cell papilloma	1 (2%)			
Musculoskeletal System				
None				

Table B1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Nervous System				
Brain	(48)	(48)	(48)	(48)
Cranial nerve, schwannoma malignant			1 (2%)	
Respiratory System				
Lung	(48)	(48)	(48)	(48)
Alveolar/bronchiolar adenoma	1 (2%)	2 (4%)	3 (6%)	
Carcinoma, metastatic, thyroid gland			1 (2%)	
Nose	(48)	(48)	(48)	(48)
Trachea	(48)	(48)	(48)	(48)
Special Senses System				
Ear		(1)		
Pinna, neural crest tumor		1 (100%)		
Eye	(48)	(48)	(48)	(48)
Lids, sarcoma	1 (2%)			
Harderian gland	(48)	(48)	(48)	(48)
Zymbal's gland	(46)	(47)	(47)	(46)
Adenoma				2 (4%)
Urinary System				
Kidney	(48)	(48)	(48)	(48)
Hemangiosarcoma		1 (2%)		
Mesenchymal tumor benign			1 (2%)	
Sarcoma	1 (2%)			
Renal tubule, carcinoma	1 (2%)			
Urinary bladder	(47)	(47)	(48)	(48)
Transitional epithelium, carcinoma		1 (2%)		
Systemic Lesions				
Multiple organs[b]	(48)	(48)	(48)	(48)
Hemangiosarcoma		1 (2%)		
Leukemia mononuclear	17 (35%)	8 (17%)	5 (10%)	8 (17%)

TABLE B1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Neoplasm Summary				
Total animals with primary neoplasms[c]	46	46	42	43
Total primary neoplasms	99	92	84	90
Total animals with benign neoplasms	41	43	37	38
Total benign neoplasms	74	73	63	75
Total animals with malignant neoplasms	21	16	19	15
Total malignant neoplasms	25	18	21	15
Total animals with metastatic neoplasms	1		1	
Total metastatic neoplasms	4		1	
Total animals with uncertain neoplasms-benign or malignant		1		
Total uncertain neoplasms		1		

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

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 0 ppm

Number of Days on Study	401	443	526	529	533	535	552	589	606	626	626	631	641	641	647	667	704	711	712	728	728	728	728	728	728
Carcass ID Number	012	014	027	028	025	026	029	040	046	051	054	066	064	070	072	075	082	033	032	091	092	093	094	095	096
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma, multiple																									
Mesentery					+					+							+			+	+				
Sarcoma					X																				
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																					X				
Pheochromocytoma malignant, metastatic, adrenal medulla										X															
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant										X															
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	M	+	+	+	+	+	+	+	M
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma							X		X	X	X			X	X	X	X	X	X	X	X	X		X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+
C-cell, adenoma																		X							
C-cell, carcinoma															X										
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 0 ppm

Number of Days on Study	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	
Carcass ID Number	097	111	112	113	114	115	116	117	134	135	136	137	138	162	163	164	165	166	188	189	190	191	192	Total Tissues/ Tumors
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocellular adenoma, multiple																				X				1
Mesentery	+			+				+		+														9
Sarcoma																								1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma	X																							2
Pheochromocytoma malignant, metastatic, adrenal medulla																								1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	47
Pheochromocytoma malignant																								1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma	X	X		X	X		X	X			X				X	X				X		X	X	26
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
C-cell, adenoma						X					X											X		4
C-cell, carcinoma										X							X							3
General Body System																								
None																								

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 0 ppm

Number of Days on Study	401	443	526	529	533	535	552	589	606	626	626	631	641	641	647	667	704	711	722	728	728	728	728	728	728
Carcass ID Number	012	014	027	028	025	026	029	040	046	051	054	066	064	070	072	075	082	013	032	091	092	093	094	095	096
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X												X				X				
Carcinoma	X	X																				X		X	X
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal				X			X						X					X			X	X			X
Sarcoma stromal								X																	
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node						+			+	+					+			+	+	+				+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	M	M	M	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																	X								
Fibroadenoma	X			X		X											X			X			X	X	X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																				X					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, mesentery					X																				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 0 ppm

Number of Days on Study	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	
Carcass ID Number	097	111	112	113	114	115	116	117	134	135	136	137	138	162	163	164	165	166	188	189	190	191	192		Total Tissues/ Tumors
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Adenoma		X		X						X						X									7
Carcinoma																									5
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Polyp stromal			X		X						X	X		X	X						X				14
Sarcoma stromal																						X			2
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Lymph node									+											+					10
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Lymph node, mesenteric	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M		40
Integumentary System																									
Mammary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+		46
Carcinoma										X															2
Fibroadenoma		X					X		X			X							X		X		X		15
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Basal cell adenoma																									1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Sarcoma, metastatic, mesentery																									1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 0 ppm

Number of Days on Study	401	443	526	529	533	535	552	589	606	626	626	631	641	641	647	667	704	711	722	728	728	728	728	728	728
Carcass ID Number	012	014	027	028	025	026	029	040	046	051	054	066	064	070	072	075	082	033	132	091	092	093	094	095	096
Special Senses System																									
Eye	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimal gland										+	+	+	+	+					+		+			+	+
Zymbal's gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma												X													
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X			X	X			X			X	X	X	X		X	X	X		X	X		X

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 0 ppm

Number of Days on Study	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	
Carcass ID Number	097	111	112	113	114	115	116	117	134	135	136	137	138	162	163	164	165	166	188	189	190	191	192	Total Tissues/ Tumors
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lacrimal gland	+		+	+	+		+	+			+	+	+											18
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Carcinoma																								1
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leukemia mononuclear					X									X	X	X		X						19

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 100 ppm

Number of Days on Study	443	485	494	512	539	540	556	574	577	591	596	598	600	610	613	624	641	648	686	687	707	708	718	718	722
Carcass ID Number	013	018	021	022	023	024	035	041	038	048	042	045	049	043	047	067	062	065	086	081	085	131	089	061	159
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus															X										
Hepatocellular adenoma																									
Mesentery				+						+	+				+										
Carcinoma, metastatic, uterus															X										
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus															X										
Salivary glands	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	M	M	+	+	+	+	M	+	+	M	+	+	+
Pituitary gland	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma		X	X	X	X		X				X	X				X	X	X	X	X	X			X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma					X																				
C-cell, carcinoma						X																			
General Body System																									
Tissue NOS																						+			
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									X
Carcinoma																									
Bilateral, carcinoma																									

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 100 ppm

Number of Days on Study	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	
Carcass ID Number	098	099	100	101	109	110	118	119	139	140	141	142	143	167	168	169	170	171	184	193	194	195	196	Total Tissues/ Tumors
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma, metastatic, uterus																								1
Hepatocellular adenoma																						X		1
Mesentery																+								5
Carcinoma, metastatic, uterus																								1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma, metastatic, uterus																								1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Squamous cell papilloma																		X						1
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pheochromocytoma benign					X																			1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma				X						X														2
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pars distalis, adenoma	X	X	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	36
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
C-cell, adenoma	X										X	X										X		5
C-cell, carcinoma																								1
General Body System																									
Tissue NOS																								1
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma	X					X								X					X					5
Carcinoma					X																			1
Bilateral, carcinoma																						X		1

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 100 ppm

Number of Days on Study	443	485	494	512	539	540	556	574	577	591	596	598	600	610	613	624	641	648	686	687	707	708	718	718	722
Carcass ID Number	013	018	021	022	023	024	035	041	038	048	042	045	049	043	047	067	062	065	086	081	085	131	089	061	159
Genital System (continued)																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor malignant																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal											X		X		X	X									
Sarcoma stromal																									
Endometrium, carcinoma															X										
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+							+								+						+	+		
Lymph node, mandibular	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus															X										
Thymus	+	+	+	+	M	+	M	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																			X						
Fibroadenoma			X	X				X									X						X		X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Carcinoma, metastatic, thyroid gland						X																			
Sarcoma stromal, metastatic, uterus																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 100 ppm

Number of Days on Study	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	
Carcass ID Number	098	099	100	101	109	110	118	119	139	140	141	142	143	167	168	169	170	171	184	193	194	195	196	Total Tissues/ Tumors
Genital System (continued)																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Granulosa cell tumor malignant				X																				1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Polyp stromal				X									X				X							7
Sarcoma stromal										X														1
Endometrium, carcinoma																								1
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node																+						+		7
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma, metastatic, uterus																								1
Thymus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma												X												2
Fibroadenoma	X			X						X		X		X								X	X	13
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Subcutaneous tissue, fibroma																				X				1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Peripheral nerve	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma														X										1
Carcinoma, metastatic, thyroid gland																								1
Sarcoma stromal, metastatic, uterus										X														1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 100 ppm

Number of Days on Study	443	485	494	512	539	540	556	574	577	591	596	598	600	601	613	624	641	648	686	687	707	708	718	718	722
Carcass ID Number	013	018	021	022	023	024	035	041	038	048	042	045	049	043	047	067	062	065	086	081	085	131	089	061	059
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimal gland								+									+	+	+			+	+		
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma																								X	
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X							X	X	X			X	X		X		X	X	X	X	X	X		

Table B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 100 ppm

Number of Days on Study	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	
Carcass ID Number	098	099	100	101	109	110	118	119	139	140	141	142	143	167	168	169	170	171	184	193	194	195	196	Total Tissues/ Tumors
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lacrimal gland	+	+		+	+		+	+	+					+										14
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Transitional epithelium, papilloma																								1
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leukemia mononuclear		X							X					X		X								17

Table B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 300 ppm

Number of Days on Study	484	508	603	619	626	627	652	669	659	666	673	692	697	697	715	719	728	728	728	721	728	728	728	728	728
Carcass ID Number	017	019	050	060	053	056	068	069	073	071	080	088	078	090	060	087	102	103	120	121	122	123	124	125	126
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																									
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Hepatocellular adenoma							X																		
Mesentery	+								+																
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																									
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X	X		X	X	X	X	X		X		X		X	X		X		X	X		X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																X									X
C-cell, carcinoma																X							X		
Follicular cell, adenoma																									
Follicular cell, carcinoma																							X		
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Adenoma												X			X								X		
Carcinoma	X	X																X							
Bilateral, carcinoma																									

Table B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 300 ppm

Number of Days on Study	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	
Carcass ID Number	127	144	145	146	147	148	149	150	172	173	174	175	176	177	178	179	197	198	199	200	201	202	203	Total Tissues/ Tumors
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Polyp adenomatous										X														1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocellular carcinoma										X														1
Hepatocellular adenoma							X			X														3
Mesentery																								2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																	X							1
Carcinoma																	X							1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pars distalis, adenoma	X	X	X				X	X		X	X	X	X	X	X	X	X		X	X		X	X	32
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	47
C-cell, adenoma	X																							3
C-cell, carcinoma																								2
Follicular cell, adenoma											X													1
Follicular cell, carcinoma																						X		2
General Body System																								
None																								
Genital System																								
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma									X			X			X	X	X							8
Carcinoma																		X			X			5
Bilateral, carcinoma																				X				1

Table B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 300 ppm

Number of Days on Study	484	508	603	619	626	627	652	669	659	666	673	692	697	697	715	719	728	728	728	728	728	728	728	728	728
Carcass ID Number	017	019	050	060	053	056	068	069	073	071	080	088	078	090	160	087	102	103	120	121	122	123	124	125	126
Genital System (continued)																									
Ovary	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, clitoral gland																		X							
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal			X					X																	
Sarcoma stromal																		X							
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+	+											+		+							+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	M	+	+	+	+	M	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Fibroadenoma					X	X		X						X		X									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Squamous cell papilloma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimal gland			+			+	+	+	+		+		+					+		+	+	+	+	+	+
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma							X																		

Table B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 300 ppm

Number of Days on Study	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	
Carcass ID Number	127	144	145	146	147	148	149	150	172	173	174	175	176	177	178	179	197	198	199	200	201	202	203	Total Tissues/ Tumors
Genital System (continued)																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Carcinoma, metastatic, clitoral gland																								1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Polyp stromal			X		X			X																5
Sarcoma stromal																								1
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node										+				+										7
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma													X											1
Fibroadenoma		X				X				X			X							X	X	X		12
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Keratoacanthoma														X										1
Squamous cell papilloma																X								1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma																	X							1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lacrimal gland	+																							15
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma																								1

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 300 ppm

Number of Days on Study	484	508	603	619	626	627	652	659	659	666	673	692	697	697	715	719	728	728	728	728	728	728	728	728	728
Carcass ID Number	017	019	050	060	053	056	068	069	073	071	080	088	078	090	060	087	102	103	120	121	122	123	124	125	126
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X				X			X	X	X			X					X				

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 300 ppm

Number of Days on Study	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	
Carcass ID Number	127	144	145	146	147	148	149	150	172	173	174	175	176	177	178	179	197	198	199	200	201	202	203	Total Tissues/ Tumors
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leukemia mononuclear			X							X													X	10

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 600 ppm

Number of Days on Study	1	3	3	4	4	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	7	7
	1	2	7	4	5	0	4	6	7	8	8	0	1	2	3	3	3	5	7	8	9	9	9	2	2
	1	3	8	3	4	8	6	2	0	1	9	5	9	0	0	3	4	4	0	5	4	7	9	8	8
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	0	0	1	1	1	2	3	3	3	3	4	5	5	5	6	5	6	7	7	8	7	7	8	0	0
	1	6	1	6	5	0	6	7	4	9	4	2	9	5	1	8	3	4	6	3	7	9	4	4	5
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																									
Intestine large, cecum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Hepatocellular adenoma, multiple																									X
Sarcoma, metastatic, ovary											X														
Mesentery											+				+										
Sarcoma, metastatic, ovary											X														
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, ovary											X														
Squamous cell carcinoma																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, ovary											X														
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, ovary											X														
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Sarcoma, metastatic, ovary											X														
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	M	+	+	M	+	M	+	M	+	M	+	+	+	+	+
Pituitary gland	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland								X																	
Pars distalis, adenoma									X		X					X	X	X			X	X	X		X
Pars distalis, carcinoma																				X					
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Sarcoma, metastatic, ovary											X														
C-cell, adenoma																									
C-cell, carcinoma																									
Follicular cell, adenoma																									
Follicular cell, carcinoma																									

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 600 ppm

Number of Days on Study	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	
Carcass ID Number	106	107	108	128	129	130	151	152	153	154	155	156	157	158	180	181	182	183	204	205	206	207	208	Total Tissues/ Tumors
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Polyp adenomatous																							X	1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocellular adenoma								X		X											X			3
Hepatocellular adenoma, multiple																								1
Sarcoma, metastatic, ovary																								1
Mesentery									+							+								4
Sarcoma, metastatic, ovary																								1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma, metastatic, ovary																								1
Squamous cell carcinoma																		X						1
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma, metastatic, ovary																								1
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma, metastatic, ovary																								1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pheochromocytoma benign																X								1
Sarcoma, metastatic, ovary																								1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																					X			1
Parathyroid gland	+	+	+	M	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	39
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Carcinoma, metastatic, Zymbal's gland																								1
Pars distalis, adenoma	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X		X		X	X	X	29
Pars distalis, carcinoma																								1
Thyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Sarcoma, metastatic, ovary																								1
C-cell, adenoma		X							X										X					3
C-cell, carcinoma											X													1
Follicular cell, adenoma														X										1
Follicular cell, carcinoma															X									1

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 600 ppm

Number of Days on Study	1	3	3	4	4	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	7	7
	1	2	7	4	5	0	4	6	7	8	8	0	1	2	3	3	3	5	7	8	9	9	9	2	2
	1	3	8	3	4	8	6	2	0	1	9	5	9	0	0	3	4	4	0	5	4	7	9	8	8
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	0	0	1	1	1	2	3	3	3	3	4	5	5	5	6	5	6	7	7	8	7	7	8	0	0
	0	6	1	6	5	0	6	7	4	9	4	2	9	5	1	8	3	4	6	3	7	9	4	4	5
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Adenoma									X				X				X			X					
Carcinoma		X		X													X					X			
Bilateral, carcinoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma											X														
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal						X		X							X					X	X				
Sarcoma, metastatic, ovary											X														
Schwannoma malignant														X											
Endometrium, adenoma																									
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																									X
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node							+											+			+				
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Sarcoma, metastatic, ovary											X														
Thymus	+	+	M	+	+	+	M	M	M	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+
Sarcoma, metastatic, ovary											X														
Sarcoma, metastatic, uncertain primary site																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma						X				X										X					
Fibroadenoma														X	X							X	X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, sarcoma																X		X							
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																									
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 600 ppm

Number of Days on Study	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	
Carcass ID Number	106	107	108	128	129	130	151	152	153	154	155	156	157	158	180	181	182	183	204	205	206	207	208	Total Tissues/ Tumors
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma			X									X	X			X								8
Carcinoma																								4
Bilateral, carcinoma																						X		1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma																								1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Polyp stromal			X		X			X	X	X			X	X			X		X		X		X	16
Sarcoma, metastatic, ovary																								1
Schwannoma malignant																								1
Endometrium, adenoma														X										1
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma																								1
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node				+																		+		5
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangiosarcoma																		X						1
Sarcoma, metastatic, ovary																								1
Thymus	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	40
Sarcoma, metastatic, ovary																								1
Sarcoma, metastatic, uncertain primary site																		X						1
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma							X												X					5
Fibroadenoma	X							X		X	X										X			9
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Subcutaneous tissue, sarcoma																								2
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma															X									1
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

Table B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 600 ppm

Number of Days on Study	1	3	3	4	4	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	7	7
	1	2	7	4	5	0	4	6	7	8	8	0	1	2	3	3	3	5	7	8	9	9	9	2	2
	1	3	8	3	4	8	6	2	0	1	9	5	9	0	0	3	4	4	0	5	4	7	9	8	8
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	0	0	1	1	1	2	3	3	3	3	4	5	5	5	6	5	6	7	7	8	7	7	8	0	0
	1	6	1	6	5	0	6	7	4	9	4	2	9	5	1	8	3	4	6	3	7	9	4	4	5
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oligodendroglioma malignant																									
Ventricle, carcinoma, metastatic, pituitary gland																				X					
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland								X																	
Sarcoma, metastatic, uncertain primary site																									
Mediastinum, sarcoma, metastatic, ovary											X														
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Pinna, neural crest tumor																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																							X		
Lacrimal gland															+		+								+
Zymbal's gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Carcinoma								X	X																
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin																X									
Transitional epithelium, carcinoma																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							X																		

TABLE B2a
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Malachite Green Chloride: 600 ppm

Number of Days on Study	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	728	
Carcass ID Number	106	107	108	128	129	130	151	152	153	154	155	156	157	158	180	181	182	183	204	205	206	207	208	Total Tissues/ Tumors
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Oligodendroglioma malignant									X															1
Ventricle, carcinoma, metastatic, pituitary gland																								1
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma, metastatic, Zymbal's gland																								1
Sarcoma, metastatic, uncertain primary site																		X						1
Mediastinum, sarcoma, metastatic, ovary																								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Special Senses System																									
Ear													+											1
Pinna, neural crest tumor													X											1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																								1
Lacrimal gland	+		+	+	+	+									+									9
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Carcinoma																								2
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	47
Sarcoma, metastatic, skin																								1
Transitional epithelium, carcinoma																				X				1
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leukemia mononuclear																								1

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	552	578	585	589	627	635	653	662	662	669	673	714	718	719	719	734	734	734	734	734	734	734	734	734	735
Carcass ID Number	220	119	122	234	125	126	248	130	131	134	253	268	140	141	142	357	358	359	360	361	362	363	364	438	395
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery	+	+			+			+	+						+			+							
Sarcoma	X																								
Schwannoma malignant, metastatic, uterus		X																							
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, uterus		X																							
Acinar cell, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	A	+	M	+	+	+	+	M	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma					X			X	X		X	X	X		X			X		X		X	X	X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma				X																				X	
General Body System																									
Tissue NOS		+																							
Schwannoma malignant, metastatic, uterus		X																							

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	735	735	735	735	735	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	
Carcass ID Number	396	397	398	399	437	146	147	148	149	173	174	175	176	177	178	179	180	406	407	408	409	410	411	Total Tissues/ Tumors
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocellular adenoma					X																			1
Mesentery																	+							8
Sarcoma																								1
Schwannoma malignant, metastatic, uterus																								1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Schwannoma malignant, metastatic, uterus																								1
Acinar cell, adenoma															X									1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adrenal gland, cortex	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adrenal gland, medulla	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	42
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pars distalis, adenoma		X	X	X	X				X			X		X			X	X	X		X	X	X	26
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
C-cell, adenoma									X									X						4
General Body System																								
Tissue NOS																								1
Schwannoma malignant, metastatic, uterus																								1

Table B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	552	578	585	589	627	635	653	662	662	669	673	714	718	719	719	734	734	734	734	734	734	734	734	734	734	735
Carcass ID Number	220	119	122	234	125	126	248	130	131	134	253	268	140	141	142	357	358	359	360	361	362	363	364	438	395
Genital System																										
Clitoral gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																				X						X
Carcinoma			X																							
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oviduct																										
Leiomyoma																										
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma												X														
Polyp stromal			X				X												X							
Sarcoma stromal																				X						
Schwannoma malignant		X																								
Bilateral, polyp stromal																										
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesenteric, schwannoma malignant, metastatic, uterus		X																								
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	M	+
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma				X						X				X		X	X	X	X	X				X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																										
Squamous cell papilloma																								X		
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	735	735	735	735	735	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	
Carcass ID Number	396	397	398	399	437	146	147	148	149	173	174	175	176	177	178	179	180	406	407	408	409	410	411	Total Tissues/ Tumors
Genital System																								
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma							X					X			X				X		X			7
Carcinoma																						X		2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Oviduct																							+	1
Leiomyoma																							X	1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leiomyoma																								1
Polyp stromal	X				X			X			X							X		X				9
Sarcoma stromal																								1
Schwannoma malignant																								1
Bilateral, polyp stromal			X																					1
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Mesenteric, schwannoma malignant, metastatic, uterus																								1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	41
Integumentary System																								
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibroadenoma	X	X					X		X	X	X			X	X				X	X		X		20
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Basal cell adenoma																			X					1
Squamous cell papilloma																								1
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	552	578	585	589	627	635	653	662	662	669	673	714	718	719	719	734	734	734	734	734	734	734	734	734	735
Carcass ID Number	220	119	122	234	125	126	248	130	131	134	253	268	140	141	142	357	358	359	360	361	362	363	364	438	395
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									X
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lids, sarcoma																									
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimal gland																+	+						+		+
Zymbal's gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma															X										
Renal tubule, carcinoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X			X	X	X	X					X	X	X	X		X		X		X	X			X

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	735	735	735	735	735	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	
Carcass ID Number	396	397	398	399	437	146	147	148	149	173	174	175	176	177	178	179	180	406	407	408	409	410	411	Total Tissues/ Tumors
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma																								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lids, sarcoma													X											1
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lacrimal gland	+		+		+				+					+	+									10
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma																								1
Renal tubule, carcinoma																		X						1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leukemia mononuclear																		X		X		X		17

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	060	561	605	625	657	683	690	709	714	716	725	732	734	734	734	734	734	735	735	735	735	735	735	735	735
Carcass ID Number	101	118	123	239	129	138	137	261	266	267	172	405	339	340	341	342	343	344	400	439	440	441	442	443	444
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery																	+	+	+				+		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	M	+	M	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma		X	X				X	X		X					X		X		X		X			X	X
Thyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma											X		X	X					X	X		X			
C-cell, carcinoma			X																						
Follicular cell, carcinoma												X													
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																			X					X	
Carcinoma											X														
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	735	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	
Carcass ID Number	445	150	151	152	153	154	155	156	181	182	183	184	185	186	187	412	413	414	415	416	417	418	419	Total Tissues/ Tumors
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocellular adenoma					X											X		X						3
Mesentery							+			+	+													7
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	M	+	+	+	+	+	M	+	M	+	+	M	+	+	+	+	+	+	+	+	M	+	M	37
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	47
Pars distalis, adenoma	X			X	X			X			X	X	X			X	X		X			X	X	23
Thyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
C-cell, adenoma												X												7
C-cell, carcinoma																								1
Follicular cell, carcinoma																								1
General Body System																								
None																								
Genital System																								
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma		X	X	X			X			X							X							8
Carcinoma																								1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	060	561	605	625	657	683	690	709	714	716	725	732	734	734	734	734	734	735	735	735	735	735	735	735	735
Carcass ID Number	101	118	123	239	129	138	137	261	266	267	172	405	339	340	341	342	343	344	400	439	440	441	442	443	444
Genital System (continued)																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal												X				X	X				X				X
Bilateral, polyp stromal																									
Cervix, squamous cell papilloma																									
Endometrium, adenoma																									
Endometrium, carcinoma					X																				
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant											X														
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma									X																
Carcinoma							X																		
Fibroadenoma				X	X		X	X														X	X	X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Fibroma																				X					
Keratoacanthoma																									
Sarcoma								X		X															
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	735	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	
Carcass ID Number	445	150	151	152	153	154	155	156	181	182	183	184	185	186	187	412	413	414	415	416	417	418	419	Total Tissues/ Tumor
Genital System (continued)																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Polyp stromal		X				X			X		X					X								10
Bilateral, polyp stromal								X										X						2
Cervix, squamous cell papilloma														X										1
Endometrium, adenoma																X								1
Endometrium, carcinoma																								1
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Schwannoma malignant																								1
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Integumentary System																									
Mammary gland	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Adenoma																								1
Carcinoma																								1
Fibroadenoma				X						X			X		X	X								12
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Basal cell adenoma																	X							1
Fibroma																								1
Keratoacanthoma																							X	1
Sarcoma																								2
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	060	561	605	625	657	683	690	709	714	716	725	732	734	734	734	734	734	735	735	735	735	735	735	735	735
Carcass ID Number	101	118	123	239	129	138	137	261	266	267	172	405	339	340	341	342	343	344	400	439	440	441	442	443	444
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma											X														
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Pinna, neural crest tumor																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimal gland																		+		+		+			
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma							X																		
Urinary bladder	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, carcinoma																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma							X																		
Leukemia mononuclear						X			X					X					X						

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	735	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	
Carcass ID Number	445	150	151	152	153	154	155	156	181	182	183	184	185	186	187	412	413	414	415	416	417	418	419	Total Tissues/ Tumor
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma										X														2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Special Senses System																									
Ear																				+				1
Pinna, neural crest tumor																				X				1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lacrimal gland					+							+									+			6
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangiosarcoma																								1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Transitional epithelium, carcinoma					X																			1
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangiosarcoma																								1
Leukemia mononuclear						X					X		X	X										8

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 272 ppm

Number of Days on Study	102	470	482	599	626	657	676	698	709	709	709	719	724	734	734	734	734	734	735	735	735	735	735	735	735
Carcass ID Number	103	111	218	120	243	247	135	139	259	262	263	145	170	345	346	347	348	349	446	447	448	449	450	451	452
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery																			+						
Pancreas	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																									
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocardium, schwannoma malignant																									
Myocardium, schwannoma malignant																									
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Medulla, pheochromocytoma malignant																									X
Medulla, pheochromocytoma benign																									
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	M	+	+	M	+	+
Pituitary gland	M	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X			X		X	X			X	X								X			
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+
Bilateral, C-cell, adenoma												X													
Bilateral, C-cell, carcinoma																									
C-cell, adenoma															X	X									
Follicular cell, carcinoma																							X		
General Body System																									
Tissue NOS										+															
Abdominal, lipoma										X															

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 272 ppm

Number of Days on Study	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736
Carcass ID Number	157	158	159	160	161	162	163	164	188	189	190	191	192	193	194	195	196	420	421	422	423	424	425	Total Tissues/ Tumors
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Mesentery																								1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma																						X		1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Squamous cell carcinoma	X																							1
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endocardium, schwannoma malignant											X													1
Myocardium, schwannoma malignant																			X					1
Endocrine System																								
Adrenal gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Medulla, pheochromocytoma malignant																								1
Medulla, pheochromocytoma benign																	X							1
Adrenal gland, cortex	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adrenal gland, medulla	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	M	+	+	40
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pars distalis, adenoma	X				X			X	X	X		X				X		X	X		X			17
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Bilateral, C-cell, adenoma																								1
Bilateral, C-cell, carcinoma														X										1
C-cell, adenoma										X			X											4
Follicular cell, carcinoma																			X					2
General Body System																								
Tissue NOS																								1
Abdominal, lipoma																								1

Table B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 272 ppm

Number of Days on Study	102	470	482	559	626	657	676	698	709	709	709	719	724	734	734	734	734	735	735	735	735	735	735	735	735
Carcass ID Number	103	111	218	120	243	247	135	139	259	262	263	145	170	345	346	347	348	349	446	447	448	449	450	451	452
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																X									
Carcinoma							X																		X
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal			X	X				X		X		X				X				X		X	X		
Schwannoma malignant																									
Bilateral, polyp stromal																	X								
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma													X												
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma													X												
Carcinoma						X																			
Fibroadenoma								X			X	X		X											X
Fibroadenoma, multiple				X																					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma		X																							
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cranial nerve, schwannoma malignant	X																								
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 272 ppm

Number of Days on Study	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	
Carcass ID Number	157	158	159	160	161	162	163	164	188	189	190	191	192	193	194	195	196	420	421	422	423	424	425		Total Tissues/ Tumors
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		46
Adenoma							X										X								3
Carcinoma																									2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Polyp stromal					X	X	X		X	X										X			X		16
Schwannoma malignant												X													1
Bilateral, polyp stromal			X										X												3
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Sarcoma																									1
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+		44
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Adenoma																									1
Carcinoma																X									2
Fibroadenoma																X	X	X					X		9
Fibroadenoma, multiple									X													X			3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Fibrous histiocytoma																									1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Cranial nerve, schwannoma malignant																									1
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 272 ppm

Number of Days on Study	102	470	482	599	626	657	676	698	709	709	709	719	724	734	734	734	734	734	735	735	735	735	735	735	735	735
Carcass ID Number	103	111	218	120	243	247	135	139	259	262	263	145	170	345	346	347	348	349	446	447	448	449	450	451	452
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X																				
Carcinoma, metastatic, thyroid gland																										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimal gland																			+	+					
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesenchymal tumor benign																							X		
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X	X	X				X										X						

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 272 ppm

Number of Days on Study	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	
Carcass ID Number	157	158	159	160	161	162	163	164	188	189	190	191	192	193	194	195	196	420	421	422	423	424	425	Total Tissues/ Tumors
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma					X						X													3
Carcinoma, metastatic, thyroid gland														X										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Special Senses System																								
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lacrimal gland							+	+			+	+			+						+			8
Zymbal's gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Mesenchymal tumor benign																								1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leukemia mononuclear																								5

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 543 ppm

Number of Days on Study	405	484	536	570	585	632	633	640	648	654	669	676	719	733	734	734	734	734	734	734	735	735	735	735	735
Carcass ID Number	217	112	113	231	121	242	127	128	132	249	136	133	143	144	350	351	352	353	354	355	356	358	359	390	391
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Colon, adenocarcinoma																									
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																X									
Mesentery												+													
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parotid gland, adenoma																									
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																			X						
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	M	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+
Pars distalis, adenoma						X	X				X					X		X	X				X		X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																									
C-cell, adenoma												X		X									X		
Follicular cell, adenoma																									
General Body System																									
None																									

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 543 ppm

Number of Days on Study	735	735	735	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	
Carcass ID number	392	393	394	165	166	167	168	169	197	198	199	200	401	402	403	404	426	427	428	429	430	431	432	Total Tissues/ Tumors
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Colon, adenocarcinoma											X													1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocellular adenoma												X	X											3
Mesentery																+			+		+			4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parotid gland, adenoma				X																				1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Squamous cell papilloma																								1
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	+	+	M	+	+	+	M	M	M	+	+	+	+	+	+	+	+	M	M	+	M	+	36
Pituitary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	46
Pars distalis, adenoma	X						X	X	X	X		X	X		X	X		X		X			X	20
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Bilateral, C-cell, adenoma				X																				1
C-cell, adenoma																			X					4
Follicular cell, adenoma																							X	1
General Body System																								
None																								

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 543 ppm

Number of Days on Study	405	484	536	570	585	632	633	640	648	654	669	676	719	733	734	734	734	734	734	734	734	735	735	735	735	735
Carcass ID Number	217	112	113	231	121	242	127	128	132	249	136	133	143	144	350	351	352	353	354	355	356	358	389	390	391
Genital System																										
Clitoral gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma		X												X					X							X
Carcinoma																								X	X	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma														X												
Polyp stromal				X							X	X		X		X	X									
Sarcoma stromal			X																							
Bilateral, polyp stromal																									X	
Endometrium, adenoma																										
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	M	M	M	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X													X								
Carcinoma																							X			
Fibroadenoma				X		X	X			X									X							
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																X										
Fibroma					X																					
Keratoacanthoma																		X								
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 543 ppm

Number of Days on Study	735	735	735	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	
Carcass ID number	392	393	394	165	166	167	168	169	197	198	199	200	401	402	403	404	426	427	428	429	430	431	432	Total Tissues/ Tumors
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma											X					X								6
Carcinoma																								2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leiomyoma																								1
Polyp stromal		X	X					X			X		X	X			X	X			X		X	16
Sarcoma stromal																								1
Bilateral, polyp stromal																								1
Endometrium, adenoma							X																	1
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																								2
Carcinoma																X								2
Fibroadenoma								X				X		X	X		X	X	X			X		13
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Basal cell carcinoma																								1
Fibroma																								1
Keratoacanthoma																								1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 543 ppm

Number of Days on Study	405	484	536	570	585	632	633	640	648	654	669	676	719	733	734	734	734	734	734	735	734	735	735	735	735
Carcass ID Number	217	112	113	231	121	242	127	128	132	249	136	133	143	144	350	351	352	353	354	355	356	388	389	390	391
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimal gland															+					+					
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X						X	X				X	X											

TABLE B2b
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Leucomalachite Green: 543 ppm

Number of Days on Study	735	735	735	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	
Carcass ID number	392	393	394	165	166	167	168	169	197	198	199	200	401	402	403	404	426	427	428	429	430	431	432	Total Tissues/ Tumors
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Special Senses System																								
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lacrimal gland							+			+														4
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	46
Adenoma						X					X													2
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leukemia mononuclear		X				X													X					8

TABLE B3a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
Clitoral Gland: Adenoma				
Overall rate[a]	7/48 (15%)	5/48 (10%)	8/47 (17%)	8/47 (17%)
Adjusted rate[b]	17.2%	13.1%	18.8%	21.1%
Terminal rate[c]	5/29 (17%)	4/23 (17%)	6/31 (19%)	4/25 (16%)
First incidence (days)	533	722	692	570
Poly-3 test[d]	P=0.282	P=0.423N	P=0.537	P=0.439
Clitoral Gland: Carcinoma				
Overall rate	5/48 (10%)	2/48 (4%)	6/47 (13%)	5/47 (11%)
Adjusted rate	12.0%	5.2%	13.7%	12.9%
Terminal rate	3/29 (10%)	2/23 (9%)	4/31 (13%)	1/25 (4%)
First incidence (days)	401	728 (T)	484	323
Poly-3 test	P=0.337	P=0.251N	P=0.534	P=0.584
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	12/48 (25%)	7/48 (15%)	14/47 (30%)	12/47 (26%)
Adjusted rate	28.3%	18.3%	31.9%	30.2%
Terminal rate	8/29 (28%)	6/23 (26%)	10/31 (32%)	5/25 (20%)
First incidence (days)	401	722	484	323
Poly-3 test	P=0.287	P=0.211N	P=0.451	P=0.524
Liver: Hepatocellular Adenoma				
Overall rate	1/48 (2%)	1/48 (2%)	3/48 (6%)[e]	4/48 (8%)
Adjusted rate	2.5%	2.6%	6.9%	10.8%
Terminal rate	1/29 (3%)	1/23 (4%)	2/32 (6%)	4/25 (16%)
First incidence (days)	728 (T)	728 (T)	652	728 (T)
Poly-3 test	P=0.059	P=0.751	P=0.336	P=0.155
Mammary Gland: Fibroadenoma				
Overall rate	15/48 (31%)	13/48 (27%)	12/48 (25%)	9/48 (19%)
Adjusted rate	35.5%	32.1%	27.0%	23.6%
Terminal rate	11/29 (38%)	7/23 (30%)	7/32 (22%)	5/25 (20%)
First incidence (days)	401	494	626	620
Poly-3 test	P=0.127N	P=0.461N	P=0.263N	P=0.175N
Mammary Gland: Carcinoma				
Overall rate	2/48 (4%)	2/48 (4%)	1/48 (2%)	5/48 (10%)
Adjusted rate	5.0%	5.2%	2.3%	13.0%
Terminal rate	1/29 (3%)	1/23 (4%)	1/32 (3%)	2/25 (8%)
First incidence (days)	704	686	728 (T)	508
Poly-3 test	P=0.113	P=0.679	P=0.473N	P=0.197
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	16/48 (33%)	14/48 (29%)	12/48 (25%)	14/48 (29%)
Adjusted rate	37.9%	34.5%	27.0%	35.5%
Terminal rate	12/29 (41%)	7/23 (30%)	7/32 (22%)	7/25 (28%)
First incidence (days)	401	494	626	508
Poly-3 test	P=0.415N	P=0.460N	P=0.193N	P=0.500N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	26/48 (54%)	36/47 (77%)	32/46 (70%)	29/45 (64%)
Adjusted rate	60.7%	82.7%	71.6%	76.2%
Terminal rate	16/29 (55%)	21/23 (91%)	22/32 (69%)	21/25 (84%)
First incidence (days)	552	485	603	570
Poly-3 test	P=0.233	P=0.014	P=0.193	P=0.092

TABLE B3a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	26/48 (54%)	36/47 (77%)	32/46 (70%)	30/45 (67%)
Adjusted rate	60.7%	82.7%	71.6%	78.5%
Terminal rate	16/29 (55%)	21/23 (91%)	22/32 (69%)	21/25 (84%)
First incidence (days)	552	485	603	570
Poly-3 test	P=0.162	P=0.014	P=0.193	P=0.056
Thyroid Gland (C-Cell): Adenoma				
Overall rate	4/46 (9%)	5/48 (10%)	3/47 (6%)	3/46 (7%)
Adjusted rate	10.4%	12.9%	7.1%	8.5%
Terminal rate	3/29 (10%)	4/23 (17%)	2/31 (7%)	3/24 (13%)
First incidence (days)	711	539	719	728 (T)
Poly-3 test	P=0.348N	P=0.506	P=0.446N	P=0.546N
Thyroid Gland (C-Cell): Carcinoma				
Overall rate	3/46 (7%)	1/48 (2%)	2/47 (4%)	1/46 (2%)
Adjusted rate	7.8%	2.6%	4.7%	2.8%
Terminal rate	2/29 (7%)	0/23 (0%)	1/31 (3%)	1/24 (4%)
First incidence (days)	647	540	719	728 (T)
Poly-3 test	P=0.323N	P=0.303N	P=0.458N	P=0.337N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	7/46 (15%)	6/48 (13%)	4/47 (9%)	4/46 (9%)
Adjusted rate	18.1%	15.2%	9.4%	11.3%
Terminal rate	5/29 (17%)	4/23 (17%)	3/31 (10%)	4/24 (17%)
First incidence (days)	647	539	719	728 (T)
Poly-3 test	P=0.207N	P=0.486N	P=0.209N	P=0.313N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	0/46 (0%)	0/48 (0%)	3/47 (6%)	2/46 (4%)
Adjusted rate	0.0%	0.0%	7.1%	5.7%
Terminal rate	0/29 (0%)	0/23 (0%)	3/31 (10%)	2/24 (8%)
First incidence (days)	—[f]	—	728 (T)	728 (T)
Poly-3 test	P=0.064	—[g]	P=0.137	P=0.218
Uterus: Stromal Polyp				
Overall rate	14/48 (29%)	7/48 (15%)	5/48 (10%)	16/48 (33%)
Adjusted rate	33.6%	17.5%	11.4%	41.0%
Terminal rate	10/29 (35%)	3/23 (13%)	3/32 (9%)	11/25 (44%)
First incidence (days)	529	596	603	508
Poly-3 test	P=0.172	P=0.076N	P=0.011N	P=0.323
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	16/48 (33%)	8/48 (17%)	6/48 (13%)	16/48 (33%)
Adjusted rate	38.0%	20.1%	13.6%	41.0%
Terminal rate	11/29 (38%)	4/23 (17%)	4/32 (13%)	11/25 (44%)
First incidence (days)	529	596	603	508
Poly-3 test	P=0.319	P=0.057N	P=0.007N	P=0.481
All Organs: Mononuclear Cell Leukemia				
Overall rate	19/48 (40%)	17/48 (35%)	10/48 (21%)	1/48 (2%)
Adjusted rate	43.7%	40.0%	22.5%	2.7%
Terminal rate	9/29 (31%)	4/23 (17%)	4/32 (13%)	0/25 (0%)
First incidence (days)	526	443	619	546
Poly-3 test	P<0.001N	P=0.447N	P=0.026N	P<0.001N

TABLE B3a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
All Organs: Benign Neoplasms				
Overall rate	41/48 (85%)	42/48 (88%)	40/48 (83%)	35/48 (73%)
Adjusted rate	89.6%	93.0%	86.4%	83.4%
Terminal rate	26/29 (90%)	23/23 (100%)	28/32 (88%)	21/25 (84%)
First incidence (days)	401	485	603	508
Poly-3 test	P=0.114N	P=0.407	P=0.439N	P=0.279N
All Organs: Malignant Neoplasms				
Overall rate	29/48 (60%)	24/48 (50%)	21/48 (44%)	23/48 (48%)
Adjusted rate	62.2%	55.1%	45.5%	52.6%
Terminal rate	13/29 (45%)	9/23 (39%)	12/32 (38%)	9/25 (36%)
First incidence (days)	401	443	484	323
Poly-3 test	P=0.186N	P=0.317N	P=0.076N	P=0.235N
All Organs: Benign or Malignant Neoplasms				
Overall rate	47/48 (98%)	48/48 (100%)	46/48 (96%)	42/48 (88%)
Adjusted rate	97.9%	100.0%	95.8%	93.9%
Terminal rate	28/29 (97%)	23/23 (100%)	30/32 (94%)	24/25 (96%)
First incidence (days)	401	443	484	323
Poly-3 test	P=0.084N	P=0.500	P=0.500N	P=0.306N

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

TABLE B3b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Clitoral Gland: Adenoma				
Overall rate[a]	7/47 (15%)	8/47 (17%)	3/46 (7%)	6/47 (13%)
Adjusted rate[b]	16.2%	18.2%	7.2%	14.0%
Terminal rate[c]	7/33 (21%)	8/36 (22%)	3/35 (9%)	4/34 (12%)
First incidence (days)	734 (T)	734 (T)	734 (T)	484
Poly-3 test[d]	P=0.310N	P=0.514	P=0.169N	P=0.506N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	9/47 (19%)	9/47 (19%)	5/46 (11%)	8/47 (17%)
Adjusted rate	20.6%	20.5%	11.9%	18.7%
Terminal rate	8/33 (24%)	8/36 (22%)	4/35 (11%)	6/34 (18%)
First incidence (days)	585	725	676	484
Poly-3 test	P=0.386N	P=0.599N	P=0.212N	P=0.517N
Liver: Hepatocellular Adenoma				
Overall rate	1/48 (2%)	3/48 (6%)	0/48 (0%)	3/48 (6%)
Adjusted rate	2.3%	6.7%	0.0%	7.0%
Terminal rate	1/33 (3%)	3/36 (8%)	0/35 (0%)	3/34 (9%)
First incidence (days)	734 (T)	734 (T)	—[e]	734 (T)
Poly-3 test	P=0.360	P=0.314	P=0.500N	P=0.297
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	1/48 (2%)	2/48 (4%)	3/48 (6%)	0/48 (0%)
Adjusted rate	2.3%	4.5%	6.8%	0.0%
Terminal rate	1/33 (3%)	1/36 (3%)	2/35 (6%)	0/34 (0%)
First incidence (days)	734 (T)	725	657	—
Poly-3 test	P=0.340N	P=0.508	P=0.308	P=0.505N
Mammary Gland: Fibroadenoma				
Overall rate	20/48 (42%)	12/48 (25%)	12/48 (25%)	13/48 (27%)
Adjusted rate	45.0%	26.3%	27.0%	29.4%
Terminal rate	17/33 (52%)	8/36 (22%)	8/35 (23%)	9/34 (27%)
First incidence (days)	589	625	599	570
Poly-3 test	P=0.160N	P=0.048N	P=0.058N	P=0.094N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	20/48 (42%)	13/48 (27%)	13/48 (27%)	15/48 (31%)
Adjusted rate	45.0%	28.5%	29.2%	33.6%
Terminal rate	17/33 (52%)	8/36 (22%)	8/35 (23%)	10/34 (29%)
First incidence (days)	589	625	599	570
Poly-3 test	P=0.278N	P=0.076N	P=0.090N	P=0.185N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	0/48 (0%)	2/48 (4%)	3/48 (6%)	4/48 (8%)
Adjusted rate	0.0%	4.5%	6.8%	9.3%
Terminal rate	0/33 (0%)	0/36 (0%)	1/35 (3%)	3/34 (9%)
First incidence (days)	—	690	657	585
Poly-3 test	P=0.047	P=0.244	P=0.120	P=0.058
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	20/48 (42%)	13/48 (27%)	14/48 (29%)	17/48 (35%)
Adjusted rate	45.0%	28.5%	31.3%	38.1%
Terminal rate	17/33 (52%)	8/36 (22%)	8/35 (23%)	12/34 (35%)
First incidence (days)	589	625	599	570
Poly-3 test	P=0.472N	P=0.076N	P=0.130N	P=0.325N

TABLE B3b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	26/47 (55%)	23/47 (49%)	17/45 (38%)	20/46 (43%)
Adjusted rate	58.6%	51.1%	39.7%	48.1%
Terminal rate	19/33 (58%)	18/35 (51%)	12/35 (34%)	17/32 (53%)
First incidence (days)	627	561	599	632
Poly-3 test	P=0.170N	P=0.306N	P=0.056N	P=0.218N
Skin: Fibroma, Fibrous Histiocytoma, or Sarcoma				
Overall rate	0/48 (0%)	3/48 (6%)	1/48 (2%)	1/48 (2%)
Adjusted rate	0.0%	6.7%	2.3%	2.3%
Terminal rate	0/33 (0%)	1/36 (3%)	0/35 (0%)	0/34 (0%)
First incidence (days)	—	709	470	585
Poly-3 test	P=0.579N	P=0.123	P=0.503	P=0.498
Thyroid Gland (C-Cell): Adenoma				
Overall rate	4/46 (9%)	7/46 (15%)	5/47 (11%)	5/48 (10%)
Adjusted rate	9.4%	16.2%	11.7%	11.7%
Terminal rate	3/33 (9%)	6/35 (17%)	4/34 (12%)	3/34 (9%)
First incidence (days)	589	725	719	676
Poly-3 test	P=0.536N	P=0.271	P=0.505	P=0.506
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	4/46 (9%)	8/46 (17%)	6/47 (13%)	5/48 (10%)
Adjusted rate	9.4%	18.3%	14.0%	11.7%
Terminal rate	3/33 (9%)	6/35 (17%)	5/34 (15%)	3/34 (9%)
First incidence (days)	589	605	719	676
Poly-3 test	P=0.499N	P=0.190	P=0.374	P=0.506
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	0/46 (0%)	1/46 (2%)	2/47 (4%)	1/48 (2%)
Adjusted rate	0.0%	2.3%	4.7%	2.4%
Terminal rate	0/33 (0%)	0/35 (0%)	2/34 (6%)	1/34 (3%)
First incidence (days)	—	732	734 (T)	734 (T)
Poly-3 test	P=0.367	P=0.506	P=0.241	P=0.503
Uterus: Stromal Polyp				
Overall rate	10/48 (21%)	12/48 (25%)	19/48 (40%)	17/48 (35%)
Adjusted rate	22.5%	26.9%	42.1%	39.0%
Terminal rate	8/33 (24%)	11/36 (31%)	14/35 (40%)	13/34 (38%)
First incidence (days)	585	732	482	570
Poly-3 test	P=0.035	P=0.409	P=0.037	P=0.071
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	11/48 (23%)	12/48 (25%)	19/48 (40%)	18/48 (38%)
Adjusted rate	24.8%	26.9%	42.1%	40.7%
Terminal rate	9/33 (27%)	11/36 (31%)	14/35 (40%)	13/34 (38%)
First incidence (days)	585	732	482	536
Poly-3 test	P=0.035	P=0.506	P=0.062	P=0.082
All Organs: Mononuclear Leukemia				
Overall rate	17/48 (35%)	8/48 (17%)	5/48 (10%)	8/48 (17%)
Adjusted rate	37.0%	17.8%	11.0%	18.2%
Terminal rate	8/33 (24%)	6/36 (17%)	1/35 (3%)	3/34 (9%)
First incidence (days)	552	683	482	484
Poly-3 test	P=0.038N	P=0.033N	P=0.003N	P=0.037N

TABLE B3b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
All Organs: Benign Neoplasms				
Overall rate	41/48 (85%)	43/48 (90%)	37/48 (77%)	38/48 (79%)
Adjusted rate	88.1%	91.9%	80.6%	82.9%
Terminal rate	29/33 (88%)	33/36 (92%)	27/35 (77%)	29/34 (85%)
First incidence (days)	585	561	482	484
Poly-3 test	P=0.140N	P=0.390	P=0.237N	P=0.334N
All Organs: Malignant Neoplasms				
Overall rate	21/48 (44%)	16/48 (33%)	19/48 (40%)	15/48 (31%)
Adjusted rate	44.7%	34.7%	39.9%	33.6%
Terminal rate	10/33 (30%)	7/36 (19%)	10/35 (29%)	9/34 (27%)
First incidence (days)	552	605	102	484
Poly-3 test	P=0.242N	P=0.221N	P=0.397N	P=0.190N
All Organs: Benign or Malignant Neoplasms				
Overall rate	46/48 (96%)	46/48 (96%)	42/48 (88%)	43/48 (90%)
Adjusted rate	95.8%	97.9%	87.5%	91.2%
Terminal rate	31/33 (94%)	35/36 (97%)	29/35 (83%)	30/34 (88%)
First incidence (days)	552	561	102	484
Poly-3 test	P=0.111N	P=0.507	P=0.134N	P=0.305N

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

TABLE B4a
Historical Incidence of Thyroid Gland (Follicular Cell) Neoplasms in Control Female F344 Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Doxylamine	0/47	0/47	0/47
Fumonisin B_1	0/48	0/48	0/48
Gentian violet	1/159	0/159	1/159
Pyrilamine	0/48	0/48	0/48
Sulfamethazine	5/170	0/170	5/170
Triprolidine	1/45	0/45	1/45
Total (%)	7/517 (1.4%)	0/517 (0%)	7/517 (1.4%)
Range	0%-3%		0%-3%

[a] Data as of April 30, 2003. Studies were performed at the National Center for Toxicological Research in animals given NIH-31 feed.

TABLE B4b
Historical Incidence of Hepatocellular Adenoma in Control Female F344 Rats[a]

Study	Incidence in Controls
Doxylamine	0/48
Fumonisin B_1	0/48
Gentian violet	0/170
Pyrilamine	0/48
Sulfamethazine	1/179
Triprolidine	0/48
Total (%)	1/541 (0.2%)
Range	0%-1%

[a] Data as of April 30, 2003. Studies were performed at the National Center for Toxicological Research in animals given NIH-31 feed.

TABLE B4c
Historical Incidence of Mammary Gland Neoplasms in Control Female F344 Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Doxylamine	1/48	2/48	3/48
Fumonisin B_1	1/47	2/47	3/47
Gentian violet	1/169	0/169	1/169
Pyrilamine	1/47	0/47	1/47
Sulfamethazine	1/177	0/177	1/177
Triprolidine	0/46	0/46	0/46
Total (%)	5/534 (0.9%)	4/534 (0.7%)	9/534 (1.7%)
Range	0%-2%	0%-4%	0%-6%

[a] Data as of April 30, 2003. Studies were performed at the National Center for Toxicological Research in animals given NIH-31 feed.

TABLE B4d
Historical Incidence of Pituitary Gland (Pars Distalis) Neoplasms in Control Female F344 Rats[a]

Study	Incidence in Controls
	Adenoma or Carcinoma
Doxylamine	25/48
Fumonisin B_1	32/47
Gentian violet	82/161
Pyrilamine	32/47
Sulfamethazine	103/177
Triprolidine	32/48
Total (%)	306/528 (58.0%)
Range	51%-68%

[a] Data as of April 30, 2003. Studies were performed at the National Center for Toxicological Research in animals given NIH-31 feed.

Table B5a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Malachite Green Chloride[a]

	0 ppm	100 ppm	300 ppm	600 ppm
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund	18	22	15	17
Natural deaths	1	3	1	6
Survivors				
Terminal sacrifice	29	23	32	25
Animals examined microscopically	48	48	48	48
Alimentary System				
Esophagus	(48)	(48)	(48)	(48)
Dilatation				1 (2%)
Foreign body				1 (2%)
Intestine small, ileum	(47)	(46)	(48)	(47)
Dilatation		1 (2%)		
Liver	(48)	(48)	(48)	(48)
Angiectasis	1 (2%)			
Basophilic focus	2 (4%)	3 (6%)	2 (4%)	7 (15%)
Basophilic focus, multiple	19 (40%)	14 (29%)	18 (38%)	8 (17%)
Clear cell focus		2 (4%)		
Clear cell focus, multiple			1 (2%)	
Cyst		1 (2%)		
Degeneration, cystic			1 (2%)	
Developmental malformation	4 (8%)	7 (15%)	2 (4%)	5 (10%)
Eosinophilic focus	3 (6%)	9 (19%)	3 (6%)	11 (23%)
Eosinophilic focus, multiple	2 (4%)	1 (2%)	10 (21%)	3 (6%)
Fatty change				1 (2%)
Hematopoietic cell proliferation	2 (4%)	3 (6%)	4 (8%)	5 (10%)
Hepatodiaphragmatic nodule	3 (6%)	6 (13%)	2 (4%)	3 (6%)
Infiltration cellular, lymphocyte	2 (4%)	3 (6%)	3 (6%)	8 (17%)
Inflammation, focal, granulomatous	18 (38%)	15 (31%)	23 (48%)	19 (40%)
Inflammation, focal, suppurative	1 (2%)	2 (4%)		
Mixed cell focus		2 (4%)		
Mixed cell focus, multiple				1 (2%)
Necrosis		2 (4%)	2 (4%)	1 (2%)
Vacuolization cytoplasmic	4 (8%)	4 (8%)	1 (2%)	6 (13%)
Bile duct, cyst			1 (2%)	
Bile duct, hyperplasia	10 (21%)	10 (21%)	9 (19%)	5 (10%)
Centrilobular, necrosis				4 (8%)
Mesentery	(9)	(5)	(2)	(4)
Fat, necrosis	7 (78%)	4 (80%)	2 (100%)	3 (75%)
Pancreas	(48)	(48)	(48)	(48)
Infiltration cellular, lymphocyte			1 (2%)	2 (4%)
Inflammation				1 (2%)
Acinus, atrophy	13 (27%)	14 (29%)	12 (25%)	7 (15%)
Salivary glands	(48)	(47)	(48)	(48)
Atrophy		2 (4%)	1 (2%)	1 (2%)
Infiltration cellular, lymphocyte	1 (2%)			1 (2%)

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

Table B5a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
Alimentary System (continued				
Stomach, forestomach	(48)	(48)	(48)	(48)
Edema		1 (2%)		
Erosion			1 (2%)	
Inflammation	1 (2%)	2 (4%)	1 (2%)	
Necrosis		1 (2%)		
Epithelium, hyperplasia		1 (2%)		1 (2%)
Cardiovascular System				
Heart	(48)	(48)	(48)	(48)
Cardiomyopathy	13 (27%)	22 (46%)	19 (40%)	14 (29%)
Atrium, thrombosis	1 (2%)			
Endocrine System				
Adrenal cortex	(48)	(48)	(48)	(48)
Accessory adrenal cortical nodule	1 (2%)		1 (2%)	
Angiectasis	10 (21%)	11 (23%)	17 (35%)	16 (33%)
Atrophy				2 (4%)
Hematopoietic cell proliferation				1 (2%)
Hyperplasia, focal		1 (2%)	1 (2%)	1 (2%)
Hypertrophy, focal	1 (2%)	4 (8%)	1 (2%)	4 (8%)
Infiltration cellular, lymphocyte				1 (2%)
Vacuolization cytoplasmic	6 (13%)	5 (10%)	2 (4%)	3 (6%)
Adrenal medulla	(47)	(47)	(48)	(48)
Hyperplasia	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Islets, pancreatic	(48)	(48)	(48)	(48)
Hyperplasia	1 (2%)			
Parathyroid gland	(43)	(43)	(45)	(39)
Hyperplasia, focal	1 (2%)			
Pituitary gland	(48)	(47)	(46)	(45)
Angiectasis	5 (10%)	2 (4%)	2 (4%)	2 (4%)
Pars distalis, cyst	8 (17%)	5 (11%)	9 (20%)	9 (20%)
Pars distalis, hyperplasia	5 (10%)		11 (24%)	6 (13%)
Pars intermedia, cyst				1 (2%)
Thyroid gland	(46)	(48)	(47)	(46)
C-cell, hyperplasia	8 (17%)	5 (10%)	11 (23%)	6 (13%)
Follicle, cyst		1 (2%)	1 (2%)	3 (7%)
Follicular cell, hyperplasia			1 (2%)	2 (4%)
General Body System				
None				
Genital System				
Clitoral gland	(48)	(48)	(47)	(47)
Hyperplasia	5 (10%)	4 (8%)	2 (4%)	6 (13%)
Inflammation	21 (44%)	30 (63%)	23 (49%)	26 (55%)
Metaplasia, squamous				1 (2%)
Duct, dilatation	6 (13%)	5 (10%)	7 (15%)	6 (13%)

Table B5a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
Genital System (continued)				
Ovary	(48)	(48)	(47)	(48)
Atrophy	18 (38%)	14 (29%)	11 (23%)	10 (21%)
Congestion				1 (2%)
Cyst	5 (10%)	4 (8%)	7 (15%)	10 (21%)
Cyst, multiple				1 (2%)
Infiltration cellular, lymphocyte	1 (2%)			
Interstitial cell, hyperplasia		1 (2%)		
Uterus	(48)	(48)	(48)	(48)
Decidual reaction				1 (2%)
Hemorrhage				1 (2%)
Inflammation, suppurative	1 (2%)			
Thrombosis			1 (2%)	1 (2%)
Cervix, hypertrophy	1 (2%)	1 (2%)	1 (2%)	
Cervix, serosa, cyst	1 (2%)			
Endometrium, hyperplasia, cystic	6 (13%)	3 (6%)	11 (23%)	8 (17%)
Vagina	(48)	(48)	(48)	(48)
Inflammation, suppurative	3 (6%)	3 (6%)	5 (10%)	1 (2%)
Prolapse				2 (4%)
Hematopoietic System				
Bone marrow	(48)	(48)	(48)	(48)
Atrophy	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Myelofibrosis			2 (4%)	
Myelostromal proliferation	1 (2%)			
Myeloid cell, hyperplasia	5 (10%)		5 (10%)	4 (8%)
Lymph node	(10)	(7)	(7)	(5)
Pigmentation		1 (14%)		
Deep cervical, hemorrhage	1 (10%)			
Deep cervical, hyperplasia, lymphoid	1 (10%)			1 (20%)
Iliac, hyperplasia, lymphoid	1 (10%)			
Lumbar, ectasia				2 (40%)
Lumbar, hematopoietic cell proliferation		1 (14%)		
Lumbar, hemorrhage		1 (14%)		1 (20%)
Lumbar, hyperplasia, lymphoid	2 (20%)			1 (20%)
Mediastinal, hemorrhage	1 (10%)	1 (14%)	3 (43%)	
Mediastinal, hyperplasia, lymphoid	2 (20%)			
Pancreatic, hematopoietic cell proliferation			1 (14%)	
Pancreatic, hemorrhage	1 (10%)			
Pancreatic, hyperplasia, lymphoid	1 (10%)			1 (20%)
Renal, ectasia	1 (10%)			
Renal, hemorrhage	2 (20%)		1 (14%)	
Renal, hyperplasia, lymphoid		1 (14%)	1 (14%)	
Lymph node, mandibular	(47)	(46)	(47)	(48)
Ectasia		1 (2%)		
Hemorrhage			2 (4%)	
Hyperplasia, lymphoid	1 (2%)	3 (7%)	2 (4%)	5 (10%)
Infiltration cellular, plasma cell	5 (11%)	5 (11%)	5 (11%)	7 (15%)
Lymph node, mesenteric	(47)	(47)	(48)	(48)
Ectasia			1 (2%)	
Hemorrhage		1 (2%)	1 (2%)	
Hyperplasia, lymphoid	2 (4%)			
Infiltration cellular, plasma cell		1 (2%)		1 (2%)

Table B5a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
Hematopoietic System (continued)				
Spleen	(48)	(48)	(48)	(48)
Atrophy				1 (2%)
Fibrosis				1 (2%)
Hematopoietic cell proliferation	6 (13%)	2 (4%)	7 (15%)	12 (25%)
Hyperplasia, focal	1 (2%)			
Pigmentation	1 (2%)	2 (4%)	2 (4%)	3 (6%)
Thrombosis			1 (2%)	
Capsule, hemorrhage		1 (2%)		
Thymus	(40)	(42)	(43)	(40)
Cyst	1 (3%)			
Ectopic parathyroid gland		1 (2%)		
Hemorrhage			1 (2%)	
Hyperplasia, reticulum cell				1 (3%)
Integumentary System				
Mammary gland	(46)	(48)	(48)	(48)
Galactocele	4 (9%)	8 (17%)	2 (4%)	2 (4%)
Hyperplasia	28 (61%)	33 (69%)	29 (60%)	35 (73%)
Inflammation		1 (2%)		
Duct, dilatation	12 (26%)	22 (46%)	18 (38%)	11 (23%)
Skin	(48)	(48)	(48)	(48)
Hyperkeratosis	2 (4%)			
Inflammation		1 (2%)		
Epidermis, hyperplasia		1 (2%)		
Epidermis, necrosis		2 (4%)		
Musculoskeletal System				
Bone	(48)	(48)	(48)	(48)
Femur, fibrous osteodystrophy	1 (2%)	1 (2%)		3 (6%)
Femur, osteopetrosis	8 (17%)	3 (6%)	3 (6%)	3 (6%)
Sternum, fibrous osteodystrophy		1 (2%)		
Sternum, osteopetrosis	8 (17%)	3 (6%)	3 (6%)	3 (6%)
Turbinate, osteopetrosis	7 (15%)	2 (4%)	2 (4%)	2 (4%)
Nervous System				
Brain	(48)	(48)	(48)	(48)
Hemorrhage	1 (2%)	1 (2%)		
Hypothalamus, compression	5 (10%)	14 (29%)	10 (21%)	7 (15%)
Spinal cord	(48)	(48)	(48)	(48)
Hemorrhage	1 (2%)			
Respiratory System				
Lung	(48)	(48)	(48)	(48)
Foreign body			1 (2%)	4 (8%)
Hemorrhage				2 (4%)
Inflammation, chronic, diffuse		1 (2%)		
Inflammation, chronic, focal	1 (2%)	3 (6%)	4 (8%)	8 (17%)
Alveolar epithelium, hyperplasia, focal	1 (2%)	1 (2%)	2 (4%)	
Alveolus, infiltration cellular, histiocyte	1 (2%)	1 (2%)		

TABLE B5a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	300 ppm	600 ppm
Respiratory System (continued)				
Nose	(48)	(48)	(48)	(48)
Foreign body			1 (2%)	2 (4%)
Inflammation, chronic	1 (2%)			
Inflammation, suppurative			1 (2%)	2 (4%)
Nasolacrimal duct, inflammation	19 (40%)	10 (21%)	11 (23%)	13 (27%)
Olfactory epithelium, cytoplasmic alteration	2 (4%)	3 (6%)	6 (13%)	1 (2%)
Olfactory epithelium, respiratory epithelium, cytoplasmic alteration	1 (2%)		2 (4%)	
Respiratory epithelium, cytoplasmic alteration				1 (2%)
Trachea	(48)	(48)	(48)	(48)
Inflammation			3 (6%)	1 (2%)
Epithelium, hyperplasia			1 (2%)	2 (4%)
Special Senses System				
Eye	(47)	(48)	(48)	(48)
Cataract	7 (15%)	2 (4%)		7 (15%)
Hemorrhage			1 (2%)	
Phthisis bulbi			2 (4%)	
Bilateral, cataract	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Bilateral, retina, degeneration	13 (28%)	14 (29%)	20 (42%)	18 (38%)
Cornea, inflammation				1 (2%)
Retina, autolysis	1 (2%)	4 (8%)		2 (4%)
Retina, degeneration	8 (17%)	8 (17%)	9 (19%)	9 (19%)
Sclera, metaplasia, osseous	1 (2%)			
Harderian gland	(47)	(48)	(48)	(48)
Atrophy	1 (2%)			
Infiltration cellular, lymphocyte	34 (72%)	37 (77%)	36 (75%)	32 (67%)
Inflammation	1 (2%)			
Pigmentation				1 (2%)
Lacrimal gland	(18)	(14)	(15)	(9)
Infiltration cellular, lymphocyte	2 (11%)	2 (14%)	2 (13%)	3 (33%)
Metaplasia	18 (100%)	14 (100%)	15 (100%)	9 (100%)
Zymbal's gland	(47)	(48)	(48)	(46)
Hyperplasia	1 (2%)	1 (2%)		
Metaplasia, squamous		1 (2%)		
Urinary System				
Kidney	(48)	(48)	(48)	(48)
Hydronephrosis			1 (2%)	
Mineralization	33 (69%)	31 (65%)	30 (63%)	30 (63%)
Nephropathy	39 (81%)	38 (79%)	41 (85%)	40 (83%)
Pelvis, inflammation		1 (2%)		
Pelvis, transitional epithelium, hyperplasia		1 (2%)		
Renal tubule, necrosis				1 (2%)
Urinary bladder	(47)	(48)	(47)	(47)
Infiltration cellular, lymphocyte	1 (2%)		1 (2%)	1 (2%)

TABLE B5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Leucomalachite Green[a]

	0 ppm	91 ppm	272 ppm	543 ppm
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund	12	11	12	10
Natural deaths	3	1	1	4
Survivors				
Died last week of study				1
Terminal sacrifice	33	36	35	33
Animals examined microscopically	48	48	48	48
Alimentary System				
Intestine large	(48)	(48)	(48)	(48)
Cecum, inflammation			1 (2%)	
Cecum, lymphoid tissue, hyperplasia		1 (2%)		
Colon, lymphoid tissue, hyperplasia		1 (2%)	1 (2%)	1 (2%)
Rectum, inflammation	1 (2%)			
Intestine small	(48)	(48)	(48)	(48)
Ileum, dilatation			1 (2%)	
Ileum, inflammation			1 (2%)	
Ileum, lymphoid tissue, hyperplasia	1 (2%)	2 (4%)	1 (2%)	
Liver	(48)	(48)	(48)	(48)
Angiectasis				1 (2%)
Basophilic focus	3 (6%)	4 (8%)	2 (4%)	1 (2%)
Basophilic focus, multiple	20 (42%)	26 (54%)	2 (4%)	
Degeneration, cystic	3 (6%)	2 (4%)	5 (10%)	3 (6%)
Developmental malformation	7 (15%)	5 (10%)	3 (6%)	5 (10%)
Eosinophilic focus	3 (6%)	4 (8%)	13 (27%)	5 (10%)
Eosinophilic focus, multiple		8 (17%)	7 (15%)	11 (23%)
Hematopoietic cell proliferation	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Hepatodiaphragmatic nodule	4 (8%)	1 (2%)	5 (10%)	1 (2%)
Infiltration cellular, lymphocyte	2 (4%)	3 (6%)	7 (15%)	4 (8%)
Inflammation, focal, granulomatous	10 (21%)	19 (40%)	20 (42%)	13 (27%)
Mixed cell focus	1 (2%)			
Necrosis	4 (8%)	2 (4%)	1 (2%)	3 (6%)
Tension lipidosis	1 (2%)	2 (4%)		
Tension lipidosis, multiple				1 (2%)
Vacuolization cytoplasmic	5 (10%)	5 (10%)	17 (35%)	22 (46%)
Bile duct, cyst			2 (4%)	
Bile duct, hyperplasia	16 (33%)	18 (38%)	19 (40%)	18 (38%)
Centrilobular, necrosis			1 (2%)	2 (4%)
Mesentery	(8)	(7)	(1)	(4)
Accessory spleen		2 (29%)		1 (25%)
Fat, necrosis	6 (75%)	5 (71%)	1 (100%)	3 (75%)
Pancreas	(48)	(48)	(47)	(48)
Infiltration cellular, lymphocyte		2 (4%)	3 (6%)	1 (2%)
Inflammation	1 (2%)		1 (2%)	
Acinus, atrophy	20 (42%)	16 (33%)	17 (36%)	18 (38%)
Salivary glands	(48)	(48)	(48)	(48)
Atrophy	2 (4%)			1 (2%)
Infiltration cellular, lymphocyte			1 (2%)	
Parotid gland, hypertrophy, focal		1 (2%)		

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

Table B5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Alimentary System (continued)				
Stomach	(48)	(48)	(48)	(48)
Forestomach, edema	1 (2%)			
Forestomach, inflammation	2 (4%)		1 (2%)	1 (2%)
Forestomach, epithelium, hyperplasia	2 (4%)		1 (2%)	
Glandular, edema	1 (2%)			
Tongue	(48)	(48)	(48)	(48)
Epithelium, hyperplasia, focal			1 (2%)	
Cardiovascular System				
Heart	(48)	(48)	(48)	(48)
Cardiomyopathy	35 (73%)	35 (73%)	33 (69%)	30 (63%)
Endocrine System				
Adrenal gland	(47)	(48)	(47)	(48)
Cortex, accessory adrenal cortical nodule			3 (6%)	
Cortex, angiectasis	8 (17%)	10 (21%)	2 (4%)	3 (6%)
Cortex, atrophy		1 (2%)		
Cortex, degeneration	1 (2%)			
Cortex, hyperplasia, focal	4 (9%)	2 (4%)	4 (9%)	5 (10%)
Cortex, hypertrophy, focal	2 (4%)		2 (4%)	
Cortex, thrombosis				1 (2%)
Cortex, vacuolization cytoplasmic	2 (4%)			1 (2%)
Medulla, hyperplasia	1 (2%)			
Pituitary gland	(47)	(47)	(45)	(46)
Angiectasis	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Pars distalis, cyst	8 (17%)	4 (9%)	6 (13%)	1 (2%)
Pars distalis, hyperplasia	2 (4%)	4 (9%)	4 (9%)	5 (11%)
Thyroid gland	(46)	(46)	(47)	(48)
C-cell, hyperplasia	13 (28%)	5 (11%)	6 (13%)	1 (2%)
Follicle, cyst		1 (2%)		2 (4%)
Follicular cell, hyperplasia	1 (2%)			3 (6%)
General Body System				
None				
Genital System				
Clitoral gland	(47)	(47)	(46)	(47)
Hyperplasia	4 (9%)	4 (9%)	5 (11%)	3 (6%)
Inflammation	23 (49%)	31 (66%)	22 (48%)	28 (60%)
Duct, dilatation	25 (53%)	24 (51%)	18 (39%)	15 (32%)
Duct, hyperplasia				1 (2%)
Ovary	(48)	(48)	(48)	(48)
Atrophy	6 (13%)	5 (10%)	10 (21%)	7 (15%)
Cyst	8 (17%)	5 (10%)	10 (21%)	4 (8%)
Infiltration cellular, lymphocyte	3 (6%)	3 (6%)	3 (6%)	
Periovarian tissue, hemorrhage		1 (2%)		

Table B5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Genital System (continued)				
Uterus	(48)	(48)	(48)	(48)
Decidual reaction		1 (2%)		1 (2%)
Hemorrhage		1 (2%)		1 (2%)
Inflammation	1 (2%)			
Thrombosis				1 (2%)
Bilateral, horn, dilatation				1 (2%)
Cervix, hypertrophy	5 (10%)	5 (10%)	2 (4%)	5 (10%)
Endometrium, hyperplasia				1 (2%)
Endometrium, hyperplasia, cystic	3 (6%)	3 (6%)	4 (8%)	7 (15%)
Vagina	(48)	(48)	(48)	(48)
Cyst	1 (2%)			
Exudate, mucous	1 (2%)	1 (2%)		
Hemorrhage				1 (2%)
Inflammation, suppurative	3 (6%)	2 (4%)	1 (2%)	2 (4%)
Metaplasia, squamous			1 (2%)	
Prolapse				1 (2%)
Hematopoietic System				
Bone marrow	(48)	(48)	(48)	(48)
Atrophy	4 (8%)	2 (4%)	4 (8%)	3 (6%)
Myelostromal proliferation				1 (2%)
Myeloid cell, hyperplasia		1 (2%)		
Lymph node	(48)	(48)	(48)	(48)
Hyperplasia, lymphoid			1 (2%)	
Bronchial, hyperplasia, lymphoid		1 (2%)		
Lumbar, ectasia	1 (2%)			
Lumbar, hyperplasia, lymphoid	1 (2%)			
Mandibular, ectasia			2 (4%)	2 (4%)
Mandibular, hyperplasia, lymphoid	1 (2%)	1 (2%)	4 (8%)	1 (2%)
Mandibular, infiltration cellular, plasma cell	4 (8%)	8 (17%)	4 (8%)	
Mediastinal, ectasia		1 (2%)		1 (2%)
Mediastinal, hemorrhage	1 (2%)	3 (6%)		1 (2%)
Mediastinal, hyperplasia, lymphoid	1 (2%)	1 (2%)	1 (2%)	
Mediastinal, infiltration cellular, plasma cell	1 (2%)			
Mesenteric, angiectasis			1 (2%)	
Mesenteric, ectasia	2 (4%)	1 (2%)	4 (8%)	2 (4%)
Mesenteric, hemorrhage		1 (2%)		2 (4%)
Mesenteric, hyperplasia, lymphoid	1 (2%)		2 (4%)	2 (4%)
Pancreatic, ectasia				1 (2%)
Pancreatic, hemorrhage		1 (2%)		1 (2%)
Pancreatic, hyperplasia, lymphoid		1 (2%)	2 (4%)	1 (2%)
Renal, ectasia	1 (2%)			
Renal, hemorrhage	1 (2%)			
Renal, hyperplasia, lymphoid	2 (4%)		1 (2%)	
Spleen	(48)	(48)	(48)	(48)
Angiectasis				1 (2%)
Fibrosis				1 (2%)
Hematopoietic cell proliferation	3 (6%)	4 (8%)	6 (13%)	5 (10%)
Hyperplasia, focal		1 (2%)		
Pigmentation	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Stromal hyperplasia			1 (2%)	
Thymus	(41)	(47)	(44)	(42)
Cyst		2 (4%)	1 (2%)	1 (2%)

TABLE B5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Integumentary System				
Mammary gland	(48)	(46)	(48)	(48)
Galactocele	7 (15%)	2 (4%)		
Hyperplasia	25 (52%)	20 (43%)	19 (40%)	22 (46%)
Infiltration cellular, lymphocyte		1 (2%)		
Duct, dilatation	10 (21%)	13 (28%)	7 (15%)	3 (6%)
Skin	(48)	(48)	(48)	(48)
Abscess		1 (2%)		
Hyperkeratosis, multifocal				1 (2%)
Inflammation	2 (4%)		1 (2%)	1 (2%)
Epidermis, hyperplasia	3 (6%)	1 (2%)	3 (6%)	3 (6%)
Epidermis, necrosis	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Musculoskeletal System				
Bone	(48)	(48)	(48)	(48)
Cranium, osteopetrosis				1 (2%)
Femur, fibrous osteodystrophy	2 (4%)			
Femur, fracture healed				1 (2%)
Femur, osteopetrosis	1 (2%)	4 (8%)	3 (6%)	1 (2%)
Mandible, osteopetrosis				1 (2%)
Maxilla, inflammation, chronic		1 (2%)		
Sternum, fibrous osteodystrophy	1 (2%)	1 (2%)		
Sternum, osteopetrosis	1 (2%)	4 (8%)	4 (8%)	1 (2%)
Turbinate, osteopetrosis	1 (2%)	3 (6%)	4 (8%)	1 (2%)
Skeletal muscle	(48)	(48)	(48)	(48)
Mineralization, multifocal	1 (2%)			
Nervous System				
Brain	(48)	(48)	(48)	(48)
Brain stem, hemorrhage	1 (2%)			
Brain stem, hypothalamus, compression	11 (23%)	6 (13%)	5 (10%)	5 (10%)
Cerebrum, hemorrhage	1 (2%)			1 (2%)
Respiratory System				
Lung	(48)	(48)	(48)	(48)
Infiltration cellular, lymphocyte			1 (2%)	
Alveolar epithelium, hyperplasia		3 (6%)	1 (2%)	2 (4%)
Alveolar epithelium, metaplasia, focal, squamous				1 (2%)
Alveolus, infiltration cellular, multifocal				1 (2%)
Alveolus, infiltration cellular, histiocyte	4 (8%)	3 (6%)	10 (21%)	6 (13%)
Alveolus, inflammation, multifocal		1 (2%)		1 (2%)
Nose	(48)	(48)	(48)	(48)
Inflammation, suppurative		1 (2%)		
Nasolacrimal duct, cyst epithelial inclusion		1 (2%)		
Nasolacrimal duct, inflammation	21 (44%)	19 (40%)	18 (38%)	19 (40%)
Respiratory epithelium, concretion				1 (2%)
Trachea	(48)	(48)	(48)	(48)
Inflammation, chronic		1 (2%)		

TABLE B5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	272 ppm	543 ppm
Special Senses System				
Eye	(48)	(48)	(48)	(48)
Cataract	2 (4%)			5 (10%)
Phthisis bulbi		1 (2%)	1 (2%)	2 (4%)
Bilateral, cataract	1 (2%)	1 (2%)		2 (4%)
Bilateral, retina, degeneration	4 (8%)	4 (8%)	5 (10%)	7 (15%)
Conjunctiva, inflammation	1 (2%)			
Cornea, neovascularization				1 (2%)
Retina, autolysis	5 (10%)	1 (2%)	1 (2%)	3 (6%)
Retina, degeneration		3 (6%)	6 (13%)	9 (19%)
Sclera, metaplasia, osseous	1 (2%)			
Harderian gland	(48)	(48)	(48)	(48)
Hyperplasia			1 (2%)	
Infiltration cellular, focal, histiocyte		1 (2%)		
Infiltration cellular, lymphocyte	19 (40%)	26 (54%)	22 (46%)	21 (44%)
Inflammation				1 (2%)
Lacrimal gland	(10)	(6)	(8)	(4)
Infiltration cellular, lymphocyte	3 (30%)	1 (17%)		2 (50%)
Metaplasia	10 (100%)	6 (100%)	8 (100%)	4 (100%)
Urinary System				
Kidney	(48)	(48)	(48)	(48)
Hydronephrosis		1 (2%)	1 (2%)	
Infarct	1 (2%)			
Infiltration cellular, lymphocyte			1 (2%)	
Mineralization	47 (98%)	46 (96%)	47 (98%)	48 (100%)
Necrosis, focal	1 (2%)			
Nephropathy	34 (71%)	31 (65%)	27 (56%)	29 (60%)
Pigmentation	2 (4%)			2 (4%)
Urinary bladder	(47)	(47)	(48)	(48)
Hemorrhage		1 (2%)		
Infiltration cellular, lymphocyte		1 (2%)	1 (2%)	2 (4%)
Inflammation		2 (4%)		

APPENDIX C
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR FEED STUDIES OF MALACHITE GREEN CHLORIDE AND LEUCOMALACHITE GREEN

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Table C1a
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Malachite Green Chloride[a]

	0 ppm	100 ppm	225 ppm	450 ppm
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Accidental death			1	
Moribund	4	3	3	4
Natural deaths	4	1	4	3
Survivors				
Terminal sacrifice	40	44	40	41
Animals examined microscopically	48	48	48	48
Alimentary System				
Gallbladder	(43)	(46)	(44)	(43)
Intestine large, colon	(48)	(46)	(44)	(46)
Intestine large, rectum	(46)	(46)	(46)	(48)
Intestine large, cecum	(46)	(47)	(45)	(46)
Intestine small, duodenum	(46)	(47)	(43)	(47)
Polyp adenomatous			1 (2%)	
Intestine small, jejunum	(46)	(47)	(44)	(47)
Intestine small, ileum	(46)	(47)	(44)	(46)
Liver	(48)	(48)	(46)	(48)
Hemangiosarcoma		1 (2%)		
Hepatocellular carcinoma	1 (2%)	2 (4%)		1 (2%)
Hepatocellular adenoma	3 (6%)	2 (4%)	2 (4%)	2 (4%)
Histiocytic sarcoma	3 (6%)		1 (2%)	1 (2%)
Sarcoma, metastatic, skin		1 (2%)		
Sarcoma, metastatic, uterus	1 (2%)			
Mesentery	(2)	(5)	(1)	
Sarcoma, metastatic, skin		1 (20%)		
Sarcoma, metastatic, uterus	1 (50%)			
Pancreas	(47)	(47)	(44)	(47)
Histiocytic sarcoma	2 (4%)			
Sarcoma, metastatic, ovary	1 (2%)			
Salivary glands	(48)	(48)	(48)	(48)
Stomach, forestomach	(47)	(47)	(46)	(48)
Squamous cell papilloma	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Stomach, glandular	(47)	(47)	(46)	(48)
Cardiovascular System				
Heart	(48)	(48)	(48)	(48)
Endocrine System				
Adrenal cortex	(47)	(47)	(45)	(47)
Sarcoma, metastatic, skin			1 (2%)	
Subcapsular, adenoma				1 (2%)
Adrenal medulla	(47)	(46)	(44)	(46)
Pheochromocytoma benign		2 (4%)	1 (2%)	1 (2%)
Islets, pancreatic	(46)	(46)	(43)	(47)
Adenoma				1 (2%)
Carcinoma	1 (2%)			
Pituitary gland	(48)	(47)	(46)	(45)
Pars distalis, adenoma		2 (4%)	2 (4%)	

Table C1a
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	225 ppm	450 ppm
Endocrine System (continued)				
Thyroid gland	(47)	(46)	(46)	(48)
Follicular cell, adenoma	1 (2%)			
General Body System				
Tissue NOS	(1)		(1)	
Hemangiosarcoma	1 (100%)			
Genital System				
Ovary	(48)	(46)	(46)	(45)
Granulosa cell tumor benign		2 (4%)		
Histiocytic sarcoma	2 (4%)			
Luteoma	1 (2%)			
Sarcoma	1 (2%)			
Sarcoma, metastatic, uterus	1 (2%)			
Yolk sac carcinoma		1 (2%)		
Uterus	(48)	(48)	(47)	(48)
Adenoma	1 (2%)			
Histiocytic sarcoma	2 (4%)		1 (2%)	
Polyp stromal			1 (2%)	
Sarcoma	1 (2%)			
Vagina	(48)	(48)	(46)	(48)
Leiomyoma			1 (2%)	
Sarcoma	1 (2%)			
Squamous cell carcinoma	1 (2%)		1 (2%)	
Hematopoietic System				
Bone marrow	(47)	(47)	(47)	(48)
Hemangiosarcoma	1 (2%)	1 (2%)	1 (2%)	
Lymph node	(13)	(13)	(6)	(9)
Lumbar, histiocytic sarcoma	1 (8%)		1 (17%)	
Pancreatic, sarcoma, metastatic, skin		1 (8%)		
Renal, histiocytic sarcoma	1 (8%)		1 (17%)	
Renal, sarcoma, metastatic, skin		1 (8%)		
Lymph node, mandibular	(48)	(48)	(47)	(48)
Lymph node, mesenteric	(45)	(46)	(44)	(46)
Histiocytic sarcoma	2 (4%)			1 (2%)
Sarcoma, metastatic, ovary	1 (2%)			
Sarcoma, metastatic, uterus	1 (2%)			
Spleen	(47)	(47)	(45)	(47)
Hemangiosarcoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Histiocytic sarcoma	2 (4%)			1 (2%)
Sarcoma, metastatic, ovary	1 (2%)			
Thymus	(43)	(44)	(43)	(47)
Histiocytic sarcoma	2 (5%)			1 (2%)

Table C1a
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	225 ppm	450 ppm
Integumentary System				
Mammary gland	(46)	(48)	(45)	(46)
Carcinoma	2 (4%)	1 (2%)	1 (2%)	4 (9%)
Skin	(48)	(48)	(46)	(48)
Hemangiosarcoma				1 (2%)
Sarcoma			2 (4%)	1 (2%)
Subcutaneous tissue, sarcoma		1 (2%)		
Musculoskeletal System				
Skeletal muscle	(48)	(48)	(48)	(48)
Sarcoma, metastatic, skin		1 (2%)		
Nervous System				
Brain	(48)	(48)	(48)	(48)
Respiratory System				
Lung	(48)	(48)	(48)	(48)
Alveolar/bronchiolar adenoma	2 (4%)	4 (8%)	3 (6%)	
Alveolar/bronchiolar carcinoma		1 (2%)		
Alveolar/bronchiolar carcinoma, multiple			1 (2%)	
Carcinoma, metastatic, mammary gland				1 (2%)
Histiocytic sarcoma	3 (6%)			1 (2%)
Sarcoma, metastatic, skin			1 (2%)	1 (2%)
Nose	(48)	(48)	(48)	(48)
Trachea	(48)	(47)	(46)	(48)
Special Senses System				
Harderian gland	(48)	(48)	(48)	(48)
Adenoma	3 (6%)		2 (4%)	3 (6%)
Urinary System				
Kidney	(48)	(47)	(48)	(47)
Histiocytic sarcoma				1 (2%)
Sarcoma, metastatic, ovary	1 (2%)			
Urinary bladder	(47)	(46)	(45)	(48)
Systemic Lesions				
Multiple organs[b]	(48)	(48)	(48)	(48)
Histiocytic sarcoma	3 (6%)		1 (2%)	1 (2%)
Leukemia granulocytic	1 (2%)			
Lymphoma malignant	7 (15%)	10 (21%)	6 (13%)	13 (27%)

TABLE C1a
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Malachite Green Chloride[a]

	0 ppm	91 ppm	225 ppm	450 ppm
Neoplasm Summary				
Total animals with primary neoplasms[c]	25	23	20	25
Total primary neoplasms	35	32	29	31
Total animals with benign neoplasms	12	11	11	8
Total benign neoplasms	13	13	15	9
Total animals with malignant neoplasms	18	15	12	20
Total malignant neoplasms	22	19	14	22
Total animals with metastatic neoplasms	2	1	1	2
Total metastatic neoplasms	8	5	2	2

[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 C1b
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	204 ppm	408 ppm
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund	4	5	7	4
Natural deaths	7	2	2	5
Survivors				
Terminal sacrifice	37	41	39	39
Animals examined microscopically	47	48	47	47
Alimentary System				
Gallbladder	(44)	(47)	(44)	(46)
Intestine large	(46)	(48)	(47)	(46)
Intestine small	(44)	(47)	(47)	(46)
Ileum, sarcoma, metastatic, ovary	1 (2%)			
Jejunum, adenoma				1 (2%)
Liver	(47)	(48)	(47)	(47)
Hemangiosarcoma	1 (2%)	1 (2%)		
Hepatocellular carcinoma			1 (2%)	2 (4%)
Hepatocellular adenoma	3 (6%)	6 (13%)	4 (9%)	8 (17%)
Hepatocellular adenoma, two			1 (2%)	1 (2%)
Histiocytic sarcoma	1 (2%)	1 (2%)		2 (4%)
Pancreas	(45)	(48)	(47)	(45)
Sarcoma, metastatic, skin				1 (2%)
Sarcoma, metastatic, skeletal muscle		1 (2%)		
Salivary glands	(47)	(48)	(47)	(47)
Stomach	(46)	(48)	(47)	(46)
Forestomach, mast cell tumor malignant	1 (2%)			
Forestomach, squamous cell carcinoma			1 (2%)	
Forestomach, squamous cell papilloma		2 (4%)	1 (2%)	1 (2%)
Glandular, sarcoma, metastatic, skin				1 (2%)
Glandular, sarcoma, metastatic, skeletal muscle		1 (2%)		
Cardiovascular System				
Heart	(47)	(48)	(47)	(47)
Sarcoma, metastatic, skin				1 (2%)
Endocrine System				
Islets, pancreatic	(44)	(48)	(47)	(45)
Adenoma	1 (2%)			
Pituitary gland	(41)	(45)	(44)	(46)
Pars distalis, adenoma	3 (7%)		1 (2%)	2 (4%)
Thyroid gland	(46)	(48)	(47)	(47)
Follicular cell, adenoma	1 (2%)	1 (2%)	1 (2%)	
General Body System				
None				

Table C1b
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	204 ppm	408 ppm
Genital System				
Ovary	(46)	(48)	(47)	(44)
Choriocarcinoma			1 (2%)	
Cystadenocarcinoma			1 (2%)	
Cystadenoma				1 (2%)
Granulosa cell tumor malignant				1 (2%)
Histiocytic sarcoma	1 (2%)			
Sarcoma	1 (2%)			
Teratoma benign	2 (4%)			
Uterus	(46)	(48)	(47)	(46)
Hemangioma		1 (2%)		
Hemangiosarcoma			1 (2%)	
Histiocytic sarcoma	1 (2%)	1 (2%)		
Leiomyoma	1 (2%)	1 (2%)		
Sarcoma stromal	2 (4%)			
Vagina	(45)	(47)	(47)	(46)
Mast cell tumor malignant	1 (2%)			
Sarcoma, metastatic, skin				1 (2%)
Hematopoietic System				
Bone marrow	(46)	(48)	(47)	(46)
Hemangiosarcoma	1 (2%)		1 (2%)	
Histiocytic sarcoma				1 (2%)
Mast cell tumor malignant	1 (2%)			
Lymph node	(47)	(48)	(47)	(47)
Lumbar, histiocytic sarcoma	1 (2%)			
Mandibular, histiocytic sarcoma	1 (2%)			
Mandibular, mast cell tumor malignant	1 (2%)			
Mesenteric, histiocytic sarcoma		1 (2%)		
Mesenteric, sarcoma, metastatic, skin				2 (4%)
Spleen	(45)	(48)	(47)	(45)
Hemangiosarcoma	2 (4%)			
Thymus	(43)	(45)	(45)	(43)
Histiocytic sarcoma				1 (2%)
Mast cell tumor malignant	1 (2%)			
Integumentary System				
Mammary gland	(46)	(48)	(47)	(45)
Adenoacanthoma	2 (4%)		1 (2%)	
Carcinoma	1 (2%)	1 (2%)	1 (2%)	4 (9%)
Carcinoma, two			1 (2%)	
Skin	(46)	(48)	(45)	(45)
Fibrous histiocytoma		1 (2%)		
Hemangioma	1 (2%)			
Hemangiosarcoma	1 (2%)			
Mast cell tumor benign		1 (2%)		
Mast cell tumor malignant	1 (2%)			
Sarcoma		1 (2%)		2 (4%)
Tail, hemangioma			1 (2%)	
Musculoskeletal System				
Skeletal muscle	(47)	(48)	(47)	(46)
Sarcoma		1 (2%)		

TABLE C1b
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	204 ppm	408 ppm
Nervous System				
Brain	(47)	(48)	(47)	(47)
Respiratory System				
Lung	(47)	(48)	(47)	(47)
Adenoacanthoma, metastatic, mammary gland	2 (4%)			
Alveolar/bronchiolar adenoma	3 (6%)	1 (2%)	2 (4%)	1 (2%)
Alveolar/bronchiolar carcinoma	2 (4%)			
Carcinoma, metastatic, mammary gland				1 (2%)
Hepatocellular carcinoma, metastatic, liver				1 (2%)
Histiocytic sarcoma	1 (2%)	1 (2%)		1 (2%)
Sarcoma, metastatic, skin				2 (4%)
Sarcoma, metastatic, skeletal muscle		1 (2%)		
Nose	(47)	(48)	(47)	(47)
Special Senses System				
Harderian gland	(47)	(48)	(47)	(47)
Adenocarcinoma	1 (2%)			1 (2%)
Adenoma	2 (4%)	2 (4%)	4 (9%)	4 (9%)
Bilateral, adenoma				1 (2%)
Zymbal's gland	(47)	(47)	(46)	(47)
Mast cell tumor malignant	1 (2%)			
Urinary System				
Kidney	(47)	(48)	(47)	(47)
Histiocytic sarcoma	1 (2%)			
Sarcoma, metastatic, skin				1 (2%)
Urinary bladder	(46)	(48)	(47)	(44)
Histiocytic sarcoma		1 (2%)		
Sarcoma, metastatic, skin				1 (2%)
Systemic Lesions				
Multiple organs[b]	(47)	(48)	(47)	(47)
Hemangioma	1 (2%)		1 (2%)	
Hemangiosarcoma	2 (4%)	1 (2%)	1 (2%)	
Lymphoma malignant	6 (13%)	5 (10%)	12 (26%)	6 (13%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	28	22	26	32
Total primary neoplasms	48	30	35	41
Total animals with benign neoplasms	16	14	13	19
Total benign neoplasms	17	15	15	20
Total animals with malignant neoplasms	16	11	18	17
Total malignant neoplasms	31	15	20	21
Total animals with metastatic neoplasms	3	1		4
Total metastatic neoplasms	3	3		12

[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 C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 0 ppm

Number of Days on Study	378	562	599	647	673	676	676	694	727	722	727	722	727	722	727	722	727	722	727	722	727	722	727	722	727
Carcass ID Number	009	017	024	027	034	031	035	032	040	041	042	043	044	045	046	047	048	049	073	074	075	076	077	078	079
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Hepatocellular adenoma																						X			
Histiocytic sarcoma		X					X	X																	
Sarcoma, metastatic, uterus																									
Mesentery																									
Sarcoma, metastatic, uterus																									
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X						X																	
Sarcoma, metastatic, ovary				X																					
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma		X		X																					
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
General Body System																									
Tissue NOS																					+				
Hemangiosarcoma																					X				

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 0 ppm

Number of Days on Study	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	
Carcass ID Number	080	105	106	107	108	109	110	111	139	140	141	142	143	144	145	146	147	148	149	175	176	177	178	Total Tissues/ Tumors
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	43
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocellular carcinoma															X									1
Hepatocellular adenoma				X											X									3
Histiocytic sarcoma																								3
Sarcoma, metastatic, uterus															X									1
Mesentery						+									+									2
Sarcoma, metastatic, uterus															X									1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Histiocytic sarcoma																								2
Sarcoma, metastatic, ovary																								1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Squamous cell papilloma																								2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	47
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	47
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	47
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Carcinoma																							X	1
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	47
Follicular cell, adenoma			X																					1
General Body System																									
Tissue NOS																								1
Hemangiosarcoma																								1

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 0 ppm

Number of Days on Study	378	562	599	647	673	676	677	694	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727
Carcass ID Number	009	017	024	027	034	031	035	032	040	041	042	043	044	045	046	047	048	049	073	074	075	076	077	078	079
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X						X																		
Luteoma																										
Sarcoma				X																						
Sarcoma, metastatic, uterus																										
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																										
Histiocytic sarcoma		X						X																		
Sarcoma																										
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																										
Squamous cell carcinoma																										
Hematopoietic System																										
Bone marrow	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																					X					
Lymph node					+	+		+		+			+		+						+	+			+	
Lumbar, histiocytic sarcoma								X																		
Renal, histiocytic sarcoma								X																		
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Histiocytic sarcoma							X	X																		
Sarcoma, metastatic, ovary				X																						
Sarcoma, metastatic, uterus																										
Spleen	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																					X					
Histiocytic sarcoma							X	X																		
Sarcoma, metastatic, ovary				X																						
Thymus	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Histiocytic sarcoma							X	X																		
Integumentary System																										
Mammary gland	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																		X							X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 0 ppm

Number of Days on Study	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	
Carcass ID Number	080	105	106	107	108	109	110	111	139	140	141	142	143	144	145	146	147	148	149	175	176	177	178	Total Tissues/ Tumors
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																								2
Luteoma									X															1
Sarcoma																								1
Sarcoma, metastatic, uterus															X									1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma									X															1
Histiocytic sarcoma																								2
Sarcoma															X									1
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma														X										1
Squamous cell carcinoma																				X				1
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hemangiosarcoma																								1
Lymph node			+		+														+	+				13
Lumbar, histiocytic sarcoma																								1
Renal, histiocytic sarcoma																								1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Histiocytic sarcoma																								2
Sarcoma, metastatic, ovary																								1
Sarcoma, metastatic, uterus															X									1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hemangiosarcoma																								1
Histiocytic sarcoma																								2
Sarcoma, metastatic, ovary																								1
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	43
Histiocytic sarcoma																								2
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Carcinoma																								2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 0 ppm

Number of Days on Study	378	562	599	647	673	676	677	679	722	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727
Carcass ID Number	009	017	024	027	034	031	035	032	040	041	042	043	044	045	046	047	048	049	073	074	075	076	077	078	079
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									X	
Histiocytic sarcoma		X					X	X																		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																							X			
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, ovary				X																						
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X					X	X																		
Leukemia granulocytic	X																									
Lymphoma malignant					X	X									X											

TABLE C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 0 ppm

Number of Days on Study	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	
Carcass ID Number	080	105	106	107	108	109	110	111	139	140	141	142	143	144	145	146	147	148	149	175	176	177	178	Total Tissues/ Tumors
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma		X																						2
Histiocytic sarcoma																								3
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma							X											X						3
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma, metastatic, ovary																								1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																								3
Leukemia granulocytic																								1
Lymphoma malignant		X								X										X		X		7

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 100 ppm

Number of Days on Study	596	644	659	711	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727
Carcass ID Number	023	028	030	038	050	051	052	053	054	055	056	057	081	082	083	084	085	086	087	088	089	090	091	112	113
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Hepatocellular carcinoma		X																							
Hepatocellular adenoma																									
Sarcoma, metastatic, skin																							X		
Mesentery								+															+		
Sarcoma, metastatic, skin																							X		
Pancreas	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																		X							
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																										
Adrenal cortex	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign								X														X			
Islets, pancreatic	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	M	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																							X		
Thyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor benign								X					X												
Yolk sac carcinoma		X																							
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 100 ppm

Number of Days on Study	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	
Carcass ID Number	114	115	116	117	118	119	150	151	152	153	154	155	156	179	180	181	182	183	184	185	186	187	188	Total Tissues/ Tumors
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, colon	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangiosarcoma																			X					1
Hepatocellular carcinoma						X																		2
Hepatocellular adenoma								X					X											2
Sarcoma, metastatic, skin																								1
Mesentery							+			+						+								5
Sarcoma, metastatic, skin																								1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Squamous cell papilloma																								1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	46
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pheochromocytoma benign																								2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Parathyroid gland	+	+	+	+	+	+	M	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	40
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pars distalis, adenoma																							X	2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
General Body System																								
None																								
Genital System																								
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	46
Granulosa cell tumor benign																								2
Yolk sac carcinoma																								1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 100 ppm

Number of Days on Study	596	644	659	711	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727
Carcass ID Number	023	028	030	038	050	051	052	053	054	055	056	057	081	082	083	084	085	086	087	088	089	090	091	112	113
Hematopoietic System																									
Bone marrow	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma											X														
Lymph node	+		+	+							+									+		+	+	+	+
Pancreatic, sarcoma, metastatic, skin																							X		
Renal, sarcoma, metastatic, skin																							X		
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma											X														
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, sarcoma																							X		
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin																							X		
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																						X		X	
Alveolar/bronchiolar carcinoma																				X					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 100 ppm

Number of Days on Study	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	
Carcass ID Number	114	115	116	117	118	119	150	151	152	153	154	155	156	179	180	181	182	183	184	185	186	187	188	Total Tissues/ Tumors
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hemangiosarcoma																								1
Lymph node					+							+			+					+				13
Pancreatic, sarcoma, metastatic, skin																								1
Renal, sarcoma, metastatic, skin																								1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hemangiosarcoma																								1
Thymus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma																				X				1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Subcutaneous tissue, sarcoma																								1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma, metastatic, skin																								1
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Peripheral nerve	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma	X																			X				4
Alveolar/bronchiolar carcinoma																								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 100 ppm

Number of Days on Study	569	644	659	711	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727
Carcass ID Number	023	028	030	038	050	051	052	053	054	055	056	057	081	082	083	084	085	086	087	088	089	090	091	112	113
Urinary System																									
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant	X		X	X							X										X			X	X

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 100 ppm

Number of Days on Study	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	
Carcass ID Number	114	115	116	117	118	119	150	151	152	153	154	155	156	179	180	181	182	183	184	185	186	187	188	Total Tissues/ Tumors
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant												X			X					X				10

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 225 ppm

Number of Days on Study	2	2	4	4	5	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	2	5	4	4	6	7	9	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	6	2	2	4	8	3	4	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
	0	0	1	1	2	2	3	3	5	5	6	6	6	6	9	9	9	9	9	9	9	2	2	2	2
	6	7	6	5	2	9	7	6	8	9	0	1	2	3	2	3	4	5	6	7	8	0	1	2	3
Alimentary System																									
Esophagus	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	A	+	A	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	M	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	M	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	A	+	+	A	A	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																									
Intestine small, jejunum	+	M	+	A	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	M	+	A	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma										X															
Histiocytic sarcoma							X																		
Mesentery																				+					
Pancreas	+	A	+	A	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	M	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Stomach, glandular	+	M	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	M	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin																									
Adrenal medulla	+	M	+	A	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	A	+	A	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	A	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																X									
Thyroid gland	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
Tissue NOS				A				+																	
Genital System																									
Clitoral gland	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	M	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma							X																		
Polyp stromal																									

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 225 ppm

Number of Days on Study	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	
Carcass ID Number	124	125	126	127	128	157	158	159	160	161	162	163	189	190	191	192	193	194	195	196	197	198	199	Total Tissues/ Tumors
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	44
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Polyp adenomatous													X											1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Hepatocellular adenoma								X																2
Histiocytic sarcoma																								1
Mesentery																								1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Squamous cell papilloma											X									X				2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Sarcoma, metastatic, skin		X																						1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Pheochromocytoma benign																X								1
Islets, pancreatic	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	41
Pituitary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pars distalis, adenoma								X																2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
General Body System																									
Tissue NOS																								1
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Histiocytic sarcoma																								1
Polyp stromal																X								1

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 225 ppm

Number of Days on Study	226	252	442	444	568	673	694	707	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727
Carcass ID Number	006	007	016	115	022	029	037	036	058	059	060	061	062	063	092	093	094	095	096	097	098	120	121	122	123
Genital System (continued)																										
Vagina	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																X										
Squamous cell carcinoma																										
Hematopoietic System																										
Bone marrow	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																										
Lymph node						+	+	+									+				+					
Lumbar, histiocytic sarcoma							X																			
Renal, histiocytic sarcoma							X																			
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	A	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	A	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																										
Thymus	+	+	+	A	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																										
Mammary gland	+	A	+	A	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																										
Skin	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma														X												
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																						X				
Alveolar/bronchiolar carcinoma, multiple																										
Sarcoma, metastatic, skin																										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 225 ppm

Number of Days on Study	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	
Carcass ID Number	124	125	126	127	128	157	158	159	160	161	162	163	189	190	191	192	193	194	195	196	197	198	199	Total Tissues/ Tumors
Genital System (continued)																									
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Leiomyoma																								1
Squamous cell carcinoma														X										1
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hemangiosarcoma													X											1
Lymph node																+								6
Lumbar, histiocytic sarcoma																								1
Renal, histiocytic sarcoma																								1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Hemangiosarcoma													X											1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	43
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Carcinoma							X																	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Sarcoma		X																						2
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	47
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma			X																		X			3
Alveolar/bronchiolar carcinoma, multiple		X																						1
Sarcoma, metastatic, skin		X																						1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 225 ppm

Number of Days on Study	2	2	4	4	5	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	2	5	4	4	6	7	9	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	6	2	2	4	8	3	4	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
	0	0	1	1	2	2	3	3	5	5	6	6	6	6	9	9	9	9	9	9	9	9	2	2	2	2
	6	7	6	5	2	9	7	6	8	9	0	1	2	3	2	3	4	5	6	7	8	0	1	2	2	3
Special Senses System																										
Eye	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																										
Zymbal's gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	M	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma							X																			
Lymphoma malignant		X				X		X										X								X

TABLE C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 225 ppm

Number of Days on Study	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	
Carcass ID Number	124	125	126	127	128	157	158	159	160	161	162	163	189	190	191	192	193	194	195	196	197	198	199	Total Tissues/ Tumors
Special Senses System																								
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma		X																			X			2
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Urinary bladder	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																								1
Lymphoma malignant								X																6

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 450 ppm

Number of Days on Study	161	318	626	633	673	704	723	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727
Carcass ID Number	001	008	026	025	033	039	072	064	065	066	067	068	069	070	071	099	100	101	102	103	104	129	130	131	132
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Gallbladder	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																			X						
Hepatocellular adenoma											X														
Histiocytic sarcoma																									
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																				X					
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Subcapsular, adenoma																X									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Clitoral gland	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+
Ovary	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 450 ppm

Number of Days on Study	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	
Carcass ID Number	133	134	135	136	137	138	164	165	166	167	168	169	170	171	172	173	174	200	201	202	203	204	205	Total Tissues/ Tumors
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Gallbladder	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	43
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	46
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocellular carcinoma																								1
Hepatocellular adenoma																				X				2
Histiocytic sarcoma							X																	1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Squamous cell papilloma																								1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Subcapsular, adenoma																								1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	46
Pheochromocytoma benign																	X							1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma			X																					1
Parathyroid gland	+	+	M	+	+	+	M	M	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	42
Pituitary gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	45
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	45
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 450 ppm

Number of Days on Study	161	318	626	633	673	704	723	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727
Carcass ID Number	001	008	026	025	033	039	072	064	065	066	067	068	069	070	071	099	100	101	102	103	104	129	130	131	132
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+			+		+																			
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																										
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																	X									
Histiocytic sarcoma																										
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																										
Integumentary System																										
Mammary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Carcinoma			X	X												X										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																	X									
Sarcoma						X																				
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland				X																						
Histiocytic sarcoma																										
Sarcoma, metastatic, skin						X																				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																										
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 450 ppm

Number of Days on Study	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	
Carcass ID Number	133	134	135	136	137	138	164	165	166	167	168	169	170	171	172	173	174	200	201	202	203	204	205	Total Tissues/ Tumors
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node										+				+		+		+				+		9
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Histiocytic sarcoma							X																	1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hemangiosarcoma																								1
Histiocytic sarcoma							X																	1
Thymus	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	47
Histiocytic sarcoma							X																	1
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Carcinoma																	X							4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangiosarcoma																								1
Sarcoma																								1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma, metastatic, mammary gland																								1
Histiocytic sarcoma							X																	1
Sarcoma, metastatic, skin																								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma									X			X								X				3
Zymbal's gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 450 ppm

Number of Days on Study	161	318	626	633	673	704	723	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727
Carcass ID Number	001	008	026	025	033	039	072	064	065	066	067	068	069	070	071	099	100	101	102	103	104	129	130	131	132
Urinary System																										
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																										
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																										
Lymphoma malignant	X	X			X		X						X						X							

Table C2a
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Malachite Green Chloride: 450 ppm

Number of Days on Study	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	727	
Carcass ID Number	133	134	135	136	137	138	164	165	166	167	168	169	170	171	172	173	174	200	201	202	203	204	205	Total Tissues/ Tumors
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Histiocytic sarcoma							X																	1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma							X																	1
Lymphoma malignant						X				X		X		X				X	X			X		13

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	122	225	518	639	682	682	688	695	700	732	733	733	733	733	733	733	733	733	733	733	734	734	734	734
Carcass ID Number	003	007	015	029	030	033	040	039	041	047	045	046	048	049	050	051	052	053	054	055	075	076	077	078
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	A	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	A	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ileum, sarcoma, metastatic, ovary								X																
Intestine small, duodenum	+	A	A	+	+	M	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma										X														
Hepatocellular adenoma					X															X				
Histiocytic sarcoma									X															
Mesentery																								
Pancreas	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Forestomach, mast cell tumor, malignant																								
Stomach, forestomach	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Adenoma																								
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X									X											
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma								X																
General Body System																								
None																								

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	079	108	109	110	111	112	113	114	138	139	140	141	142	143	144	145	146	174	175	176	177	178	179	Total Tissues/ Tumors
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	46
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Ileum, sarcoma, metastatic, ovary																								1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hemangiosarcoma																								1
Hepatocellular adenoma				X																				3
Histiocytic sarcoma																								1
Mesentery																				+				1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Forestomach, mast cell tumor, malignant																						X		1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	45
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Adenoma					X																			1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	M	42
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	M	+	+	M	M	+	+	+	41
Pars distalis, adenoma																	X							3
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	46
Follicular cell, adenoma																								1
General Body System																								
None																								

Table C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	122	225	518	639	682	682	688	695	700	732	733	733	733	733	733	733	733	733	733	734	734	734	734	734
Carcass ID Number	003	007	015	029	030	033	040	039	041	047	045	046	048	049	050	051	052	053	054	055	075	076	077	078
Genital System																									
Clitoral gland	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X															
Sarcoma								X																
Teratoma benign	X		X																					
Uterus	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X															
Leiomyoma									X															
Sarcoma stromal										X														
Vagina	+	A	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor malignant																								
Hematopoietic System																									
Bone marrow	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma				X																				
Mast cell tumor malignant																								
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lumbar, histiocytic sarcoma									X															
Mandibular, histiocytic sarcoma									X															
Mandibular, mast cell tumor malignant																								
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma				X						X														
Thymus	+	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor malignant																								
Integumentary System																									
Mammary gland	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoacanthoma							X																	
Carcinoma												X												
Skin	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																					X			
Hemangiosarcoma										X														
Mast cell tumor malignant																								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	079	108	109	110	111	112	113	114	138	139	140	141	142	143	144	145	146	174	175	176	177	178	179	Total Tissues/ Tumors
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	45
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Histiocytic sarcoma																								1
Sarcoma																								1
Teratoma benign																								2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Histiocytic sarcoma																								1
Leiomyoma																								1
Sarcoma stromal						X																		2
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Mast cell tumor malignant																						X		1
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Hemangiosarcoma																								1
Mast cell tumor malignant																						X		1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lumbar, histiocytic sarcoma																								1
Mandibular, histiocytic sarcoma																								1
Mandibular, mast cell tumor malignant																						X		1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Hemangiosarcoma																								2
Thymus	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	43
Mast cell tumor malignant																						X		1
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Adenoacanthoma																							X	2
Carcinoma																								1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Hemangioma																								1
Hemangiosarcoma																								1
Mast cell tumor malignant																						X		1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	122	225	518	639	682	682	688	695	700	732	733	733	733	733	733	733	733	733	733	734	734	734	734
Carcass ID Number	003	007	015	029	030	033	040	039	041	047	045	046	048	049	050	051	052	053	054	055	075	076	077	078
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoacanthoma, metastatic, mammary gland							X																	
Alveolar/bronchiolar adenoma																				X				
Alveolar/bronchiolar carcinoma																			X					
Histiocytic sarcoma									X															
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma								X																
Adenoma														X										
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor malignant																								
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X															
Urinary bladder	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																					X			
Hemangiosarcoma				X						X														
Lymphoma malignant		X						X									X				X			

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 0 ppm

Number of Days on Study	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	079	108	109	110	111	112	113	114	138	139	140	141	142	143	144	145	146	174	175	176	177	178	179		Total Tissues/ Tumors
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		46
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Adenoacanthoma, metastatic, mammary gland																								X	2
Alveolar/bronchiolar adenoma												X							X						3
Alveolar/bronchiolar carcinoma									X																2
Histiocytic sarcoma																									1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Adenocarcinoma																									1
Adenoma																					X				2
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Mast cell tumor malignant																						X			1
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Histiocytic sarcoma																									1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		46
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Hemangioma																									1
Hemangiosarcoma																									2
Lymphoma malignant		X						X																	6

Table C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	498	590	675	690	708	709	729	733	733	733	733	734	734	734	734	734	734	734	734	734	734	734	734	734	734
Carcass ID Number	016	024	031	034	036	037	173	056	057	058	059	060	080	081	082	083	084	085	087	088	089	090	104	115	116
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma							X																		
Hepatocellular adenoma	X													X											X
Histiocytic sarcoma				X																					
Mesentery					+							+													
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skeletal muscle									X																
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Forestomach, squamous cell papilloma							X																X		
Glandular, sarcoma, metastatic, skeletal muscle									X																
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	M	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
General Body System																									
None																									

Table C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	117	118	119	120	131	147	148	149	150	151	152	153	154	180	181	182	183	184	185	186	187	188	189	Total Tissues/ Tumors
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangiosarcoma																								1
Hepatocellular adenoma							X									X			X					6
Histiocytic sarcoma																								1
Mesentery																								2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma, metastatic, skeletal muscle																								1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Forestomach, squamous cell papilloma																								2
Glandular, sarcoma, metastatic, skeletal muscle																								1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	M	+	+	+	+	+	+	42
Pituitary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	45
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell, adenoma																X								1
General Body System																								
None																								

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	498	590	675	690	708	709	729	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	734	734	734	734
Carcass ID Number	016	024	031	034	036	037	173	056	057	058	059	060	080	081	082	083	084	085	087	088	089	090	104	115	116
Genital System																										
Clitoral gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																								X	
Histiocytic sarcoma				X																					
Leiomyoma																									
Vagina	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesenteric, histiocytic sarcoma				X																					
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma																									
Mast cell tumor benign																									
Sarcoma					X																				
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma									X																
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	117	118	119	120	131	147	148	149	150	151	152	153	154	180	181	182	183	184	185	186	187	188	189	Total Tissues Tumors
Genital System																								
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	46
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangioma																								1
Histiocytic sarcoma																								1
Leiomyoma													X											1
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Mesenteric, histiocytic sarcoma																								1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	m	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Integumentary System																								
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma											X													1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibrous histiocytoma																			X					1
Mast cell tumor benign									X															1
Sarcoma																								1
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma																								1
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	47
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	498	590	675	690	708	709	729	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	734	734	734	734
Carcass ID Number	016	024	031	034	036	037	173	056	057	058	059	060	080	081	082	083	084	085	087	088	089	090	104	115	116
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																										
Histiocytic sarcoma				X																						
Sarcoma, metastatic, skeletal muscle									X																	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma															X											
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma				X																						
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma							X																			
Lymphoma malignant										X				X							X					

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 91 ppm

Number of Days on Study	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	117	118	119	120	131	147	148	149	150	151	152	153	154	180	181	182	183	184	185	186	187	188	189	Total Tissues Tumors
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma					X																			1
Histiocytic sarcoma																								1
Sarcoma, metastatic, skeletal muscle																								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																				X				2
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	47
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																								1
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangiosarcoma																								1
Lymphoma malignant												X						X						5

Table C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 204 ppm

Number of Days on Study	193	498	620	627	675	690	700	714	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734
Carcass ID Number	006	014	026	028	032	035	038	107	061	062	063	064	065	066	067	068	091	092	093	094	096	098	105	106
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma									X															
Hepatocellular adenoma												X				X								
Hepatocellular adenoma, two																								
Mesentery																								
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Forestomach, squamous cell carcinoma																								
Forestomach, squamous cell papilloma														X										
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Blood vessel	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+
Pituitary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																								
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																								
General Body System																								
None																								

Table C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 204 ppm

Number of Days on Study	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	122	123	124	125	126	127	128	155	156	157	158	159	160	161	190	191	192	193	194	195	196	197	198	Total Tissues/ Tumors
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	44
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	46
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hepatocellular carcinoma																								1
Hepatocellular adenoma				X										X										4
Hepatocellular adenoma, two										X														1
Mesentery																				+				1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Forestomach, squamous cell carcinoma																				X				1
Forestomach, squamous cell papilloma																								1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	41
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	44
Pars distalis, adenoma				X																				1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Follicular cell, adenoma							X																	1
General Body System																									
None																									

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 204 ppm

Number of Days on Study	193	498	620	627	675	690	700	714	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734
Carcass ID Number	006	014	026	028	032	035	038	047	061	062	063	064	065	066	067	068	091	092	093	094	096	098	105	106
Genital System																									
Clitoral gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Choriocarcinoma	X																								
Cystadenocarcinoma						X																			
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoacanthoma		X																							
Carcinoma																									
Carcinoma, two																				X					
Skin		+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	
Tail, hemangioma																							X		
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 204 ppm

Number of Days on Study	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	122	123	124	125	126	127	128	155	156	157	158	159	160	161	190	191	192	193	194	195	196	197	198	Total Tissues/ Tumors
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	44
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Choriocarcinoma																								1
Cystadenocarcinoma																								1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hemangiosarcoma																			X					1
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hemangiosarcoma																			X					1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	45
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	45
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoacanthoma																								1
Carcinoma	X																							1
Carcinoma, two																								1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Tail, hemangioma																								1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 204 ppm

Number of Days on Study	193	498	620	627	675	690	700	714	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734
Carcass ID Number	006	014	026	028	032	035	038	107	061	062	063	064	065	066	067	068	091	092	093	094	096	098	105	106
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma		X														X									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X															X	X				
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																							X		
Hemangiosarcoma																									
Lymphoma malignant			X	X	X		X	X				X			X							X		X

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 204 ppm

Number of Days on Study	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	122	123	124	125	126	127	128	155	156	157	158	159	160	161	190	191	192	193	194	195	196	197	198	Total Tissues/ Tumors
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Alveolar/bronchiolar adenoma																								2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma								X																4
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hemangioma																								1
Hemangiosarcoma																			X					1
Lymphoma malignant	X						X													X				12

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 408 ppm

Number of Days on Study	444	553	584	647	718	718	723	724	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	734
Carcass ID Number	012	023	025	027	042	037	044	043	069	070	071	072	073	099	100	101	102	103	129	130	131	132	133	134
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	M	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Jejunum, adenoma																							X	
Intestine small, duodenum	A	+	M	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																								
Hepatocellular adenoma											X								X					
Hepatocellular adenoma, two														X										
Histiocytic sarcoma	X	X																						
Mesentery															+									
Pancreas	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin								X																
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Forestomach, squamous cell papilloma											X													
Glandular, sarcoma, metastatic, skin								X																
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Blood vessel	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin								X																
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																		X						
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																								
None																								

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 408 ppm

Number of Days on Study	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	135	136	162	163	164	165	166	167	168	169	170	171	172	199	200	201	202	203	204	205	206	207	208	Total Tissues/ Tumors
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Jejunum, adenoma																								1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hepatocellular carcinoma															X	X								2
Hepatocellular adenoma	X	X			X					X							X						X	8
Hepatocellular adenoma, two																								1
Histiocytic sarcoma																								2
Mesentery								+	+															3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Sarcoma, metastatic, skin																								1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Forestomach, squamous cell papilloma																								1
Glandular, sarcoma, metastatic, skin																								1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Sarcoma, metastatic, skin																								1
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Pituitary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	46
Pars distalis, adenoma			X																					2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
General Body System																								
None																								

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 408 ppm

Number of Days on Study	4	5	5	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	4	5	8	4	1	1	2	2	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4
	4	3	4	7	8	8	3	4	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4
Carcass ID Number	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
	1	2	2	2	4	3	4	4	6	7	7	7	7	9	9	0	0	0	0	2	3	3	3	3	3
	2	3	5	7	2	7	4	3	9	0	1	2	3	9	0	1	2	3	9	0	1	2	3	3	4
Genital System																									
Clitoral gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Ovary	M	I	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																									
Granulosa cell tumor malignant												X													
Uterus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vagina	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin					X																				
Hematopoietic System																									
Bone marrow	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesenteric, sarcoma, metastatic, skin					X			X																	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Integumentary System																									
Mammary gland	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma					X				X													X			
Skin	A	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma					X			X																	
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 408 ppm

Number of Days on Study	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	135	136	162	163	164	165	166	167	168	169	170	171	172	199	200	201	202	203	204	205	206	207	208	Total Tissues/ Tumors
Genital System																								
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Cystadenoma																				X				1
Granulosa cell tumor malignant																								1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Vagina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Sarcoma, metastatic, skin																								1
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Histiocytic sarcoma																								1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Mesenteric, sarcoma, metastatic, skin																								2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	43
Histiocytic sarcoma																								1
Integumentary System																								
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Carcinoma										X														4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Sarcoma																								2
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Bone, femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Brain, brain stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Brain, cerebellum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Brain, cerebrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spinal cord, thoracic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 408 ppm

Number of Days on Study	444	553	584	647	718	718	723	724	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	734	734
Carcass ID Number	012	023	025	027	042	037	044	043	069	070	071	072	073	099	100	101	102	103	129	130	131	132	133	134
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma									X																
Carcinoma, metastatic, mammary gland																					X				
Hepatocellular carcinoma, metastatic, liver																									
Histiocytic sarcoma	X																								
Sarcoma, metastatic, skin					X			X																	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma														X											
Adenoma															X	X									
Bilateral, adenoma																									
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin								X																	
Urinary bladder	A	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin					X																				
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant			X			X	X						X					X							

TABLE C2b
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Leucomalachite Green: 408 ppm

Number of Days on Study	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	135	136	162	163	164	165	166	167	168	169	170	171	172	199	200	201	202	203	204	205	206	207	208		Total Tissues/ Tumors
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Alveolar/bronchiolar adenoma																									1
Carcinoma, metastatic, mammary gland																									1
Hepatocellular carcinoma, metastatic, liver															X										1
Histiocytic sarcoma																									1
Sarcoma, metastatic, skin																									2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Adenocarcinoma																									1
Adenoma							X		X																4
Bilateral, adenoma														X											1
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Sarcoma, metastatic, skin																									1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		44
Sarcoma, metastatic, skin																									1
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Lymphoma malignant																		X							6

TABLE C3a
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	225 ppm	450 ppm
Harderian Gland: Adenoma				
Overall rate[a]	3/48 (6%)	0/48 (0%)	2/48 (4%)	3/48 (6%)
Adjusted rate[b]	6.7%	0.0%	4.6%	6.7%
Terminal rate[c]	3/40 (8%)	0/44 (0%)	2/40 (5%)	3/41 (7%)
First incidence (days)	727 (T)	—[e]	727 (T)	727 (T)
Poly-3 test[d]	P=0.370	P=0.112N	P=0.516N	P=0.662
Liver: Hepatocellular Adenoma				
Overall rate	3/48 (6%)	2/48 (4%)	2/46 (4%)	2/48 (4%)
Adjusted rate	6.7%	4.3%	4.6%	4.4%
Terminal rate	3/40 (8%)	2/44 (5%)	2/40 (5%)	2/41 (5%)
First incidence (days)	727 (T)	727 (T)	727 (T)	727 (T)
Poly-3 test	P=0.446N	P=0.482N	P=0.519N	P=0.501N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	3/48 (6%)	4/48 (8%)	2/46 (4%)	3/48 (6%)
Adjusted rate	6.7%	8.5%	4.6%	6.7%
Terminal rate	3/40 (8%)	3/44 (7%)	2/40 (5%)	3/41 (7%)
First incidence (days)	727 (T)	644	727 (T)	727 (T)
Poly-3 test	P=0.501N	P=0.525	P=0.519N	P=0.662
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	2/48 (4%)	4/48 (8%)	3/48 (6%)	0/48 (0%)
Adjusted rate	4.4%	8.5%	6.9%	0.0%
Terminal rate	2/40 (5%)	4/44 (9%)	3/40 (8%)	0/41 (0%)
First incidence (days)	727 (T)	727 (T)	727 (T)	—
Poly-3 test	P=0.132N	P=0.355	P=0.484	P=0.237N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	2/48 (4%)	5/48 (10%)	4/48 (8%)	0/48 (0%)
Adjusted rate	4.4%	10.7%	9.2%	0.0%
Terminal rate	2/40 (5%)	5/44 (11%)	4/40 (10%)	0/41 (0%)
First incidence (days)	727 (T)	727 (T)	727 (T)	—
Poly-3 test	P=0.128N	P=0.232	P=0.321	P=0.237N
Mammary Gland: Carcinoma				
Overall rate	2/48 (4%)	1/48 (2%)	1/48 (2%)	4/48 (8%)
Adjusted rate	4.4%	2.1%	2.3%	8.7%
Terminal rate	2/40 (5%)	1/44 (2%)	1/40 (3%)	2/41 (5%)
First incidence (days)	727 (T)	727 (T)	727 (T)	626
Poly-3 test	P=0.157	P=0.486N	P=0.512N	P=0.344
All Organs: Histiocytic Sarcoma				
Overall rate	3/48 (6%)	0/48 (0%)	1/48 (2%)	1/48 (2%)
Adjusted rate	6.5%	0.0%	2.3%	2.2%
Terminal rate	0/40 (0%)	0/44 (0%)	0/40 (0%)	1/41 (2%)
First incidence (days)	562	—	694	727 (T)
Poly-3 test	P=0.323N	P=0.115N	P=0.324N	P=0.313N
All Organs: Malignant Lymphoma				
Overall rate	7/48 (15%)	10/48 (21%)	6/48 (13%)	13/48 (27%)
Adjusted rate	15.4%	21.0%	13.4%	27.5%
Terminal rate	5/40 (13%)	7/44 (16%)	3/40 (8%)	9/41 (22%)
First incidence (days)	673	596	252	161
Poly-3 test	P=0.117	P=0.334	P=0.512N	P=0.120

TABLE C3a
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	225 ppm	450 ppm
All Organs: Benign Neoplasms				
Overall rate	12/48 (25%)	11/48 (23%)	11/48 (23%)	8/48 (17%)
Adjusted rate	26.1%	23.4%	25.2%	17.8%
Terminal rate	10/40 (25%)	11/44 (25%)	11/40 (28%)	8/41 (20%)
First incidence (days)	562	727 (T)	727 (T)	727 (T)
Poly-3 test	P=0.215N	P=0.477N	P=0.559N	P=0.240N
All Organs: Malignant Neoplasms				
Overall rate	18/48 (38%)	15/48 (31%)	12/48 (25%)	20/48 (42%)
Adjusted rate	37.9%	31.3%	26.7%	41.7%
Terminal rate	11/40 (28%)	11/44 (25%)	8/40 (20%)	13/41 (32%)
First incidence (days)	378	596	252	161
Poly-3 test	P=0.322	P=0.323N	P=0.178N	P=0.432
All Organs: Benign or Malignant Neoplasms				
Overall rate	25/48 (52%)	23/48 (48%)	20/48 (42%)	25/48 (52%)
Adjusted rate	52.6%	47.9%	44.5%	52.1%
Terminal rate	18/40 (45%)	19/44 (43%)	16/40 (40%)	18/41 (44%)
First incidence (days)	378	596	252	161
Poly-3 test	P=0.524	P=0.402N	P=0.285N	P=0.562N

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

TABLE C3b
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	204 ppm	408 ppm
Harderian Gland: Adenoma				
Overall rate[a]	2/48 (4%)	2/48 (4%)	4/48 (8%)	5/48 (10%)
Adjusted rate[b]	4.6%	4.3%	9.0%	11.1%
Terminal rate[c]	2/37 (5%)	2/41 (5%)	3/39 (8%)	5/39 (13%)
First incidence (days)	733 (T)	733 (T)	675	733 (T)
Poly-3 test[d]	P=0.114	P=0.672N	P=0.348	P=0.231
Harderian Gland: Adenoma or Carcinoma				
Overall rate	3/48 (6%)	2/48 (4%)	4/48 (8%)	6/48 (13%)
Adjusted rate	6.9%	4.3%	9.0%	13.3%
Terminal rate	2/37 (5%)	2/41 (5%)	3/39 (8%)	6/39 (15%)
First incidence (days)	695	733 (T)	675	733 (T)
Poly-3 test	P=0.107	P=0.474N	P=0.511	P=0.258
Liver: Hepatocellular Adenoma				
Overall rate	3/47 (6%)	6/48 (13%)	5/47 (11%)	9/47 (19%)
Adjusted rate	6.9%	12.8%	11.4%	20.2%
Terminal rate	2/37 (5%)	5/41 (12%)	5/39 (13%)	9/39 (23%)
First incidence (days)	682	498	733 (T)	733 (T)
Poly-3 test	P=0.055	P=0.282	P=0.363	P=0.065
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	3/47 (6%)	6/48 (13%)	6/47 (13%)	11/47 (23%)
Adjusted rate	6.9%	12.8%	13.6%	24.6%
Terminal rate	2/37 (5%)	5/41 (12%)	6/39 (15%)	11/39 (28%)
First incidence (days)	682	498	733 (T)	733 (T)
Poly-3 test	P=0.013	P=0.282	P=0.248	P=0.022
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	3/47 (6%)	1/48 (2%)	2/47 (4%)	1/47 (2%)
Adjusted rate	6.9%	2.2%	4.5%	2.2%
Terminal rate	3/37 (8%)	1/41 (2%)	1/39 (3%)	1/39 (3%)
First incidence (days)	733 (T)	733 (T)	498	733 (T)
Poly-3 test	P=0.290N	P=0.281N	P=0.485N	P=0.294N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	5/47 (11%)	1/48 (2%)	2/47 (4%)	1/47 (2%)
Adjusted rate	11.6%	2.2%	4.5%	2.2%
Terminal rate	5/37 (14%)	1/41 (2%)	1/39 (3%)	1/39 (3%)
First incidence (days)	733 (T)	733 (T)	498	733 (T)
Poly-3 test	P=0.100N	P=0.086N	P=0.203N	P=0.094N
Mammary Gland: Carcinoma				
Overall rate	1/48 (2%)	1/48 (2%)	2/48 (4%)	4/48 (8%)
Adjusted rate	2.3%	2.2%	4.5%	8.9%
Terminal rate	1/37 (3%)	1/41 (2%)	2/39 (5%)	3/39 (8%)
First incidence (days)	733 (T)	733 (T)	733 (T)	718
Poly-3 test	P=0.072	P=0.747N	P=0.507	P=0.190
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	3/41 (7%)	0/45 (0%)	1/44 (2%)	2/46 (4%)
Adjusted rate	7.8%	0.0%	2.4%	4.6%
Terminal rate	2/32 (6%)	0/38 (0%)	1/37 (3%)	2/38 (5%)
First incidence (days)	639	—[e]	733 (T)	733 (T)
Poly-3 test	P=0.545N	P=0.098N	P=0.281N	P=0.443N

Table C3b
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	204 ppm	408 ppm
All Organs: Malignant Lymphoma				
Overall rate	6/48 (13%)	5/48 (10%)	12/48 (25%)	6/48 (13%)
Adjusted rate	13.4%	10.8%	26.4%	13.1%
Terminal rate	4/37 (11%)	5/41 (12%)	7/39 (18%)	3/39 (8%)
First incidence (days)	225	733 (T)	620	584
Poly-3 test	P=0.431	P=0.475N	P=0.101	P=0.604N
All Organs: Benign Neoplasms				
Overall rate	16/48 (33%)	14/48 (29%)	13/48 (27%)	19/48 (40%)
Adjusted rate	34.8%	29.8%	28.7%	42.1%
Terminal rate	10/37 (27%)	12/41 (29%)	11/39 (28%)	19/39 (49%)
First incidence (days)	122	498	498	733 (T)
Poly-3 test	P=0.212	P=0.386N	P=0.346N	P=0.308
All Organs: Malignant Neoplasms				
Overall rate	16/48 (33%)	11/48 (23%)	18/48 (38%)	17/48 (35%)
Adjusted rate	35.3%	23.7%	38.0%	36.1%
Terminal rate	10/37 (27%)	8/41 (20%)	10/39 (26%)	10/39 (26%)
First incidence (days)	225	690	193	444
Poly-3 test	P=0.308	P=0.159N	P=0.480	P=0.557
All Organs: Benign or Malignant Neoplasms				
Overall rate	28/48 (58%)	22/48 (46%)	26/48 (54%)	32/48 (67%)
Adjusted rate	59.4%	46.6%	54.9%	67.9%
Terminal rate	19/37 (51%)	18/41 (44%)	18/39 (46%)	25/39 (64%)
First incidence (days)	122	498	193	444
Poly-3 test	P=0.107	P=0.148N	P=0.407N	P=0.261

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

TABLE C4
Historical Incidence of Hepatocellular Neoplasms in Control Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Chloral hydrate	5/144	4/144	9/144
Doxylamine	0/46	0/46	0/46
Fumonisin B$_1$	5/47	0/47	5/47
Pyrilamine	1/47	0/47	1/47
Sulfamethazine	8/184	2/184	10/184
Triprolidine	2/47	2/47	4/47
Urethane/ethanol	5/48	0/48	5/48
Total (%)	26/563 (4.6%)	8/563 (1.4%)	34/563 (6.0%)
Range	0%-11%	0%-4%	0%-11%

[a] Data as of April 30, 2003. Studies were performed at the National Center for Toxicological Research in animals given NIH-31 feed.

Table C5a
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Malachite Green Chloride[a]

	0 ppm	100 ppm	225 ppm	450 ppm
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Accidental death			1	
Moribund	4	3	3	4
Natural deaths	4	1	4	3
Survivors				
Terminal sacrifice	40	44	40	41
Animals examined microscopically	48	48	48	48
Alimentary System				
Intestine large, cecum	(46)	(47)	(45)	(46)
Hyperplasia, lymphoid				1 (2%)
Intestine small, jejunum	(46)	(47)	(44)	(47)
Hyperplasia, lymphoid				1 (2%)
Liver	(48)	(48)	(46)	(48)
Basophilic focus	1 (2%)	1 (2%)	3 (7%)	
Fatty change, focal			1 (2%)	
Hematopoietic cell proliferation	2 (4%)	1 (2%)		2 (4%)
Infiltration cellular, lymphocyte	4 (8%)	6 (13%)	8 (17%)	12 (25%)
Infiltration cellular, histiocyte		1 (2%)		
Inflammation, chronic active, focal	5 (10%)	9 (19%)	8 (17%)	4 (8%)
Necrosis				2 (4%)
Tension lipidosis	10 (21%)	7 (15%)	8 (17%)	6 (13%)
Bile duct, cyst				1 (2%)
Mesentery	(2)	(5)	(1)	
Infiltration cellular, lymphocyte		1 (20%)		
Fat, necrosis, focal	2 (100%)	3 (60%)	1 (100%)	
Pancreas	(47)	(47)	(44)	(47)
Cyst				1 (2%)
Cytoplasmic alteration		1 (2%)	1 (2%)	1 (2%)
Infiltration cellular, lymphocyte	6 (13%)	4 (9%)	2 (5%)	3 (6%)
Necrosis, focal		1 (2%)		
Acinus, atrophy	2 (4%)			
Salivary glands	(48)	(48)	(48)	(48)
Infiltration cellular, mononuclear cell		1 (2%)		
Parotid gland, infiltration cellular, lymphocyte	5 (10%)	12 (25%)	4 (8%)	5 (10%)
Sublingual gland, infiltration cellular, lymphocyte		1 (2%)		
Submandibular gland, infiltration cellular, lymphocyte	35 (73%)	28 (58%)	28 (58%)	28 (58%)
Stomach, forestomach	(47)	(47)	(46)	(48)
Hyperkeratosis, focal	1 (2%)			
Infiltration cellular, mast cell				1 (2%)
Inflammation, chronic active	2 (4%)	3 (6%)	2 (4%)	1 (2%)
Ulcer				1 (2%)
Epithelium, hyperplasia, diffuse	2 (4%)	2 (4%)	1 (2%)	
Epithelium, hyperplasia, focal	11 (23%)	8 (17%)	4 (9%)	7 (15%)
Stomach, glandular	(47)	(47)	(46)	(48)
Erosion		1 (2%)		1 (2%)
Ulcer	1 (2%)		1 (2%)	1 (2%)
Tongue	(48)	(48)	(48)	(48)
Artery, inflammation, chronic	2 (4%)			

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

Table C5a
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	225 ppm	450 ppm
Cardiovascular System				
Heart	(48)	(48)	(48)	(48)
Infiltration cellular, lymphocyte			2 (4%)	
Artery, inflammation, chronic	1 (2%)			1 (2%)
Endocrine System				
Adrenal cortex	(47)	(47)	(45)	(47)
Accessory adrenal cortical nodule		1 (2%)	1 (2%)	1 (2%)
Hematopoietic cell proliferation	1 (2%)			
Hyperplasia, focal	1 (2%)		1 (2%)	
Hypertrophy, focal			1 (2%)	2 (4%)
Mineralization, focal			1 (2%)	
Vacuolization cytoplasmic	1 (2%)			
Bilateral, hypertrophy, focal				1 (2%)
Subcapsular, hyperplasia	46 (98%)	46 (98%)	44 (98%)	45 (96%)
Adrenal medulla	(47)	(46)	(44)	(46)
Hyperplasia, focal		1 (2%)	1 (2%)	
Pituitary gland	(48)	(47)	(46)	(45)
Pars distalis, hyperplasia, focal	1 (2%)	2 (4%)	5 (11%)	
Pars intermedia, hyperplasia, focal		1 (2%)		
Thyroid gland	(47)	(46)	(46)	(48)
Hyperplasia, focal			1 (2%)	
Infiltration cellular, lymphocyte	1 (2%)		1 (2%)	
Ultimobranchial cyst			2 (4%)	2 (4%)
Follicular cell, cyst	2 (4%)	2 (4%)	3 (7%)	1 (2%)
General Body System				
Tissue NOS	(1)		(1)	
Hemorrhage			1 (100%)	
Genital System				
Clitoral gland	(48)	(47)	(46)	(44)
Inflammation, chronic			1 (2%)	
Duct, cyst	44 (92%)	47 (100%)	45 (98%)	44 (100%)
Ovary	(48)	(46)	(46)	(45)
Angiectasis	1 (2%)	1 (2%)	1 (2%)	
Atrophy	28 (58%)	33 (72%)	36 (78%)	36 (80%)
Cyst	15 (31%)	18 (39%)	11 (24%)	11 (24%)
Hemorrhage	3 (6%)		1 (2%)	1 (2%)
Inflammation, acute		1 (2%)		
Inflammation, chronic active			1 (2%)	
Mineralization	1 (2%)			
Thrombosis		1 (2%)		
Bilateral, cyst	4 (8%)	3 (7%)	3 (7%)	2 (4%)
Germinal epithelium, hyperplasia			2 (4%)	
Uterus	(48)	(48)	(47)	(48)
Angiectasis	1 (2%)			1 (2%)
Hyperplasia, cystic	42 (88%)	46 (96%)	44 (94%)	42 (88%)
Inflammation, acute			1 (2%)	
Thrombosis	1 (2%)			

Table C5a
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	225 ppm	450 ppm
Genital System (continued)				
Vagina	(48)	(48)	(46)	(48)
Inflammation, chronic	1 (2%)			
Artery, inflammation, chronic			1 (2%)	
Epithelium, hyperplasia			1 (2%)	
Hematopoietic System				
Bone marrow	(47)	(47)	(47)	(48)
Fibrosis			1 (2%)	
Lymph node	(13)	(13)	(6)	(9)
Inguinal, hyperplasia, lymphoid			1 (17%)	
Lumbar, hyperplasia, lymphoid	4 (31%)	4 (31%)	2 (33%)	1 (11%)
Mediastinal, hemorrhage		2 (15%)		
Mediastinal, infiltration cellular, mononuclear cell		1 (8%)		
Pancreatic, infiltration cellular, mononuclear cell		1 (8%)		
Renal, hemorrhage	1 (8%)			
Renal, hyperplasia, lymphoid	2 (15%)	1 (8%)	2 (33%)	1 (11%)
Lymph node, mandibular	(48)	(48)	(47)	(48)
Hemorrhage			1 (2%)	
Hyperplasia, lymphoid	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Infiltration cellular, mast cell				1 (2%)
Lymph node, mesenteric	(45)	(46)	(44)	(46)
Hemorrhage		1 (2%)		
Hyperplasia, lymphoid	8 (18%)	8 (17%)	5 (11%)	5 (11%)
Spleen	(47)	(47)	(45)	(47)
Angiectasis	1 (2%)			1 (2%)
Depletion cellular			1 (2%)	
Hematopoietic cell proliferation	9 (19%)	8 (17%)	3 (7%)	5 (11%)
Hyperplasia, lymphoid	6 (13%)	6 (13%)	7 (16%)	2 (4%)
Infiltration cellular, mononuclear cell		1 (2%)		
Necrosis, focal		1 (2%)		
Thymus	(43)	(44)	(43)	(47)
Angiectasis				1 (2%)
Cyst	4 (9%)	2 (5%)	3 (7%)	3 (6%)
Ectopic parathyroid gland	3 (7%)	1 (2%)	2 (5%)	3 (6%)
Hyperplasia, lymphoid	3 (7%)	5 (11%)	6 (14%)	2 (4%)
Infiltration cellular, mononuclear cell		1 (2%)		
Integumentary System				
Mammary gland	(46)	(48)	(45)	(46)
Infiltration cellular, lymphocyte		1 (2%)		
Skin	(48)	(48)	(46)	(48)
Fibrosis		1 (2%)		
Hyperplasia				1 (2%)
Musculoskeletal System				
Bone	(48)	(48)	(48)	(48)
Hyperostosis	1 (2%)			
Cranium, fibrous osteodystrophy	1 (2%)			
Femur, fibrous osteodystrophy	4 (8%)	5 (10%)	2 (4%)	2 (4%)
Sternum, degeneration, cystic		2 (4%)	4 (8%)	3 (6%)
Sternum, fibrous osteodystrophy	12 (25%)	14 (29%)	5 (10%)	5 (10%)

Table C5a
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Malachite Green Chloride

	0 ppm	100 ppm	225 ppm	450 ppm
Nervous System				
Brain	(48)	(48)	(48)	(48)
Infiltration cellular, lymphocyte	1 (2%)			
Cerebrum, mineralization	25 (52%)	18 (38%)	17 (35%)	17 (35%)
Spinal cord	(48)	(48)	(48)	(48)
Thoracic, axon, degeneration	13 (27%)	14 (29%)	19 (40%)	19 (40%)
Respiratory System				
Lung	(48)	(48)	(48)	(48)
Hemorrhage, focal				1 (2%)
Infiltration cellular, lymphocyte	6 (13%)	6 (13%)	6 (13%)	2 (4%)
Infiltration cellular, histiocyte	2 (4%)	3 (6%)	2 (4%)	
Inflammation, chronic, focal	1 (2%)			
Pigmentation		1 (2%)		
Proteinosis	3 (6%)			
Thrombosis			1 (2%)	
Alveolar epithelium, hyperplasia	1 (2%)	3 (6%)		1 (2%)
Special Senses System				
Eye	(48)	(48)	(47)	(48)
Bilateral, lens, cataract	4 (8%)	2 (4%)	5 (11%)	2 (4%)
Cornea, mineralization	1 (2%)			
Lens, cataract	1 (2%)	2 (4%)	1 (2%)	6 (13%)
Harderian gland	(48)	(48)	(48)	(48)
Atrophy		1 (2%)		
Hyperplasia			1 (2%)	1 (2%)
Infiltration cellular, lymphocyte	4 (8%)	5 (10%)	5 (10%)	4 (8%)
Zymbal's gland	(48)	(48)	(47)	(47)
Atrophy		1 (2%)		1 (2%)
Inflammation, chronic			1 (2%)	
Urinary System				
Kidney	(48)	(47)	(48)	(47)
Infiltration cellular, lymphocyte	2 (4%)	7 (15%)	3 (6%)	2 (4%)
Metaplasia, focal		1 (2%)		
Nephropathy			1 (2%)	1 (2%)
Glomerulus, amyloid deposition				2 (4%)
Glomerulus, casts protein	1 (2%)	1 (2%)		
Glomerulus, hypertrophy				1 (2%)
Glomerulus, inflammation, acute				1 (2%)
Pelvis, dilatation	2 (4%)			
Renal tubule, accumulation, hyaline droplet	4 (8%)		1 (2%)	1 (2%)
Renal tubule, hyperplasia	1 (2%)			
Urinary bladder	(47)	(46)	(45)	(48)
Inclusion body intracytoplasmic	7 (15%)	15 (33%)	34 (76%)	39 (81%)
Infiltration cellular, lymphocyte	36 (77%)	34 (74%)	33 (73%)	25 (52%)

TABLE C5b
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Leucomalachite Green[a]

	0 ppm	91 ppm	204 ppm	408 ppm
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund	4	5	7	4
Natural deaths	7	2	2	5
Survivors				
Terminal sacrifice	37	41	39	39
Animals examined microscopically	47	48	47	47
Alimentary System				
Gallbladder	(44)	(47)	(44)	(46)
Infiltration cellular, lymphocytic		1 (2%)		
Inflammation, acute				1 (2%)
Polyarteritis	1 (2%)			
Mucosa, hyperplasia, focal				2 (4%)
Intestine large	(46)	(48)	(47)	(46)
Cecum, polyarteritis			1 (2%)	
Colon, angiectasis			1 (2%)	
Colon, proliferation connective tissue				1 (2%)
Rectum, inflammation, chronic	1 (2%)	1 (2%)		
Rectum, polyarteritis			1 (2%)	
Intestine small	(44)	(47)	(47)	(46)
Ileum, hyperplasia, lymphoid	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Jejunum, hyperplasia, lymphoid			2 (4%)	1 (2%)
Liver	(47)	(48)	(47)	(47)
Basophilic focus	1 (2%)	2 (4%)		2 (4%)
Basophilic focus, two				1 (2%)
Clear cell focus		1 (2%)	1 (2%)	
Eosinophilic focus, single			1 (2%)	1 (2%)
Hematopoietic cell proliferation	2 (4%)	2 (4%)	3 (6%)	3 (6%)
Infarct			1 (2%)	
Infiltration cellular, lymphocytic	8 (17%)	2 (4%)	2 (4%)	8 (17%)
Inflammation, chronic active, focal	15 (32%)	13 (27%)	16 (34%)	11 (23%)
Leukocytosis				1 (2%)
Mineralization, focal	1 (2%)			
Necrosis	1 (2%)			
Tension lipidosis	10 (21%)	12 (25%)	9 (19%)	9 (19%)
Vacuolization cytoplasmic	2 (4%)	1 (2%)		
Bile duct, cyst	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Oval cell, hyperplasia	1 (2%)			
Serosa, fibrosis				1 (2%)
Mesentery	(1)	(2)	(1)	(3)
Fat, necrosis, focal	1 (100%)	2 (100%)	1 (100%)	3 (100%)
Pancreas	(45)	(48)	(47)	(45)
Cyst		2 (4%)		
Cytoplasmic alteration	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Infiltration cellular, lymphocytic	7 (16%)	9 (19%)	4 (9%)	6 (13%)
Polyarteritis			1 (2%)	
Acinus, atrophy	1 (2%)	2 (4%)	1 (2%)	2 (4%)

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

Table C5b
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	204 ppm	408 ppm
Alimentary System (continued)				
Salivary glands	(47)	(48)	(47)	(47)
Acinus, parotid gland, atrophy	1 (2%)	3 (6%)		1 (2%)
Acinus, parotid gland, necrosis, focal			3 (6%)	2 (4%)
Acinus, submandibular gland, atrophy	1 (2%)	1 (2%)		1 (2%)
Parotid gland, infiltration cellular, lymphocytic	9 (19%)	9 (19%)	8 (17%)	11 (23%)
Parotid gland, vacuolization cytoplasmic	2 (4%)		1 (2%)	
Sublingual gland, infiltration cellular, lymphocytic			2 (4%)	1 (2%)
Submandibular gland, infiltration cellular, lymphocytic	33 (70%)	30 (63%)	35 (74%)	31 (66%)
Stomach	(46)	(48)	(47)	(46)
Forestomach, foreign body			1 (2%)	
Forestomach, hyperkeratosis, focal	1 (2%)			
Forestomach, infiltration cellular, lymphocytic	1 (2%)			
Forestomach, inflammation, chronic active			2 (4%)	
Forestomach, epithelium, hyperplasia, diffuse	1 (2%)			
Forestomach, epithelium, hyperplasia, focal	3 (7%)	4 (8%)	8 (17%)	6 (13%)
Glandular, infiltration cellular, lymphocytic	1 (2%)	2 (4%)	1 (2%)	
Glandular, polyarteritis			1 (2%)	
Glandular, ulcer	1 (2%)			
Tongue	(47)	(48)	(47)	(47)
Foreign body		3 (6%)	1 (2%)	3 (6%)
Artery, inflammation, chronic	1 (2%)	2 (4%)	2 (4%)	
Cardiovascular System				
Blood vessel	(47)	(48)	(46)	(46)
Aorta, embolus bacterial		1 (2%)		
Heart	(47)	(48)	(47)	(47)
Cardiomyopathy		1 (2%)		
Inflammation, acute, focal		1 (2%)		1 (2%)
Mineralization				1 (2%)
Polyarteritis	1 (2%)			
Atrium left, thrombosis	1 (2%)			
Endocrine System				
Adrenal gland	(46)	(48)	(47)	(47)
Bilateral, cortex, hypertrophy, focal			1 (2%)	
Cortex, accessory adrenal cortical nodule	3 (7%)	1 (2%)	1 (2%)	1 (2%)
Cortex, cyst	1 (2%)			
Cortex, hematopoietic cell proliferation			1 (2%)	1 (2%)
Cortex, hyperplasia, focal	2 (4%)		2 (4%)	
Cortex, hypertrophy, focal	2 (4%)	2 (4%)	1 (2%)	
Cortex, polyarteritis			1 (2%)	
Cortex, subcapsular, hyperplasia	42 (91%)	47 (98%)	46 (98%)	47 (100%)
Medulla, hyperplasia, focal		1 (2%)		
Parathyroid gland	(42)	(42)	(41)	(44)
Infiltration cellular, lymphocytic		1 (2%)		
Pituitary gland	(41)	(45)	(44)	(46)
Pars distalis, hyperplasia, focal	3 (7%)	1 (2%)	3 (7%)	2 (4%)
Thyroid gland	(46)	(48)	(47)	(47)
Ectopic thymus				1 (2%)
Polyarteritis			1 (2%)	
Ultimobranchial cyst		2 (4%)	2 (4%)	
Follicular cell, cyst	7 (15%)	10 (21%)	6 (13%)	9 (19%)

Table C5b
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	204 ppm	408 ppm
General Body System				
None				
Genital System				
Clitoral gland	(45)	(46)	(44)	(45)
Inflammation, chronic		1 (2%)		
Inflammation, subacute			1 (2%)	
Duct, cyst	44 (98%)	46 (100%)	41 (93%)	45 (100%)
Ovary	(46)	(48)	(47)	(44)
Abscess		1 (2%)		
Angiectasis	1 (2%)	1 (2%)	2 (4%)	
Atrophy	42 (91%)	45 (94%)	44 (94%)	41 (93%)
Cyst	14 (30%)	16 (33%)	17 (36%)	10 (23%)
Hemorrhage	4 (9%)	2 (4%)	2 (4%)	2 (5%)
Mineralization			1 (2%)	
Thrombosis	3 (7%)			
Bilateral, cyst	7 (15%)	3 (6%)	2 (4%)	3 (7%)
Uterus	(46)	(48)	(47)	(46)
Adenomyosis, focal	1 (2%)			
Angiectasis		4 (8%)	3 (6%)	1 (2%)
Hyperplasia, cystic	42 (91%)	46 (96%)	47 (100%)	43 (93%)
Inflammation, acute				1 (2%)
Polyarteritis			1 (2%)	
Thrombosis		1 (2%)	3 (6%)	
Endometrium, metaplasia		1 (2%)		
Stroma, fibrosis			1 (2%)	
Stroma, hyperplasia			1 (2%)	
Vagina	(45)	(47)	(47)	(46)
Epithelium, hyperplasia	1 (2%)			3 (7%)
Hematopoietic System				
Bone marrow	(46)	(48)	(47)	(46)
Angiectasis, focal	1 (2%)			
Fibrosis		1 (2%)	1 (2%)	
Femoral, infarct	1 (2%)			
Myeloid cell, hyperplasia	4 (9%)	1 (2%)	4 (9%)	6 (13%)
Lymph node	(47)	(48)	(47)	(47)
Inguinal, hyperplasia, lymphoid		1 (2%)		
Lumbar, cyst	1 (2%)			
Lumbar, hematopoietic cell proliferation	1 (2%)			
Lumbar, hyperplasia, lymphoid	1 (2%)			
Lumbar, hyperplasia, plasma cell				1 (2%)
Mandibular, hyperplasia, lymphoid	1 (2%)	3 (6%)	8 (17%)	2 (4%)
Mediastinal, hyperplasia, lymphoid	1 (2%)			
Mesenteric, hematopoietic cell proliferation	1 (2%)		1 (2%)	
Mesenteric, hemorrhage	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Mesenteric, hyperplasia, lymphoid	5 (11%)	2 (4%)	3 (6%)	6 (13%)
Mesenteric, infiltration cellular, plasma cell			2 (4%)	1 (2%)
Renal, hyperplasia, lymphoid	2 (4%)			
Thoracic, hyperplasia, plasma cell				1 (2%)

Table C5b
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	204 ppm	408 ppm
Hematopoietic System (continued)				
Spleen	(45)	(48)	(47)	(45)
Angiectasis		1 (2%)		
Hematopoietic cell proliferation	11 (24%)	9 (19%)	17 (36%)	17 (38%)
Hyperplasia, lymphoid	14 (31%)	4 (8%)	8 (17%)	9 (20%)
Infiltration cellular, plasma cell		1 (2%)		1 (2%)
Proliferation connective tissue, focal	1 (2%)			
Thymus	(43)	(45)	(45)	(43)
Atrophy	2 (5%)	2 (4%)	1 (2%)	
Cyst	2 (5%)	1 (2%)	2 (4%)	2 (5%)
Ectopic parathyroid gland	4 (9%)	7 (16%)	4 (9%)	7 (16%)
Hyperplasia, lymphoid	2 (5%)	3 (7%)	5 (11%)	6 (14%)
Integumentary System				
Mammary gland	(46)	(48)	(47)	(45)
Infiltration cellular, lymphocytic				1 (2%)
Skin	(46)	(48)	(45)	(45)
Face, ulcer	1 (2%)			
Musculoskeletal System				
Bone	(47)	(48)	(47)	(47)
Femur, fibrous osteodystrophy	8 (17%)	2 (4%)	5 (11%)	3 (6%)
Sternum, degeneration, cystic	1 (2%)	3 (6%)	1 (2%)	5 (11%)
Sternum, fibrous osteodystrophy	26 (55%)	21 (44%)	19 (40%)	21 (45%)
Skeletal muscle	(47)	(48)	(47)	(46)
Mineralization				2 (4%)
Nervous System				
Brain	(47)	(48)	(47)	(47)
Cerebellum, infiltration cellular, lymphocytic				1 (2%)
Cerebrum, mineralization	15 (32%)	18 (38%)	21 (45%)	21 (45%)
Cerebrum, polyarteritis			1 (2%)	
Spinal cord	(47)	(48)	(47)	(47)
Thoracic, infiltration cellular, lymphocytic				1 (2%)
Thoracic, axon, degeneration	12 (26%)	11 (23%)	9 (19%)	14 (30%)
Respiratory System				
Lung	(47)	(48)	(47)	(47)
Crystals		1 (2%)		1 (2%)
Hemorrhage, focal	1 (2%)			
Infarct				1 (2%)
Infiltration cellular, histiocytic	1 (2%)	2 (4%)		2 (4%)
Infiltration cellular, lymphocytic	13 (28%)	7 (15%)	9 (19%)	12 (26%)
Inflammation, chronic, focal	1 (2%)	1 (2%)		1 (2%)
Inflammation, granulomatous				1 (2%)
Leukocytosis		2 (4%)		1 (2%)
Alveolar epithelium, hyperplasia	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Artery, mineralization				1 (2%)
Vein, thrombosis	1 (2%)			

TABLE C5b
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Leucomalachite Green

	0 ppm	91 ppm	204 ppm	408 ppm
Special Senses System				
Eye	(47)	(48)	(47)	(47)
Phthisis bulbi				1 (2%)
Bilateral, lens, cataract	4 (9%)	5 (10%)	3 (6%)	7 (15%)
Conjunctiva, polyarteritis				1 (2%)
Cornea, inflammation, chronic	1 (2%)			
Lens, cataract	5 (11%)	4 (8%)	4 (9%)	4 (9%)
Harderian gland	(47)	(48)	(47)	(47)
Atrophy		1 (2%)		
Hyperplasia		1 (2%)	1 (2%)	3 (6%)
Infiltration cellular, lymphocytic	4 (9%)	9 (19%)	8 (17%)	5 (11%)
Acinus, dilatation		1 (2%)		
Zymbal's gland	(47)	(47)	(46)	(47)
Atrophy		1 (2%)		1 (2%)
Urinary System				
Kidney	(47)	(48)	(47)	(47)
Glomerulosclerosis	1 (2%)			1 (2%)
Hematopoietic cell proliferation		1 (2%)		
Infarct	2 (4%)		1 (2%)	1 (2%)
Infiltration cellular, lymphocytic	9 (19%)	5 (10%)	15 (32%)	11 (23%)
Nephropathy	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Polyarteritis			1 (2%)	
Glomerulus, casts protein		1 (2%)		1 (2%)
Papilla, casts protein	1 (2%)			
Pelvis, dilatation	2 (4%)	1 (2%)		
Renal tubule, accumulation, hyaline droplet	1 (2%)	2 (4%)	1 (2%)	3 (6%)
Renal tubule, hyperplasia		1 (2%)		
Renal tubule, hypertrophy		1 (2%)		
Renal tubule, necrosis			1 (2%)	
Renal tubule, pigmentation			1 (2%)	
Urinary bladder	(46)	(48)	(47)	(44)
Inclusion body intracytoplasmic	14 (30%)	33 (69%)	44 (94%)	44 (100%)
Infiltration cellular, lymphocytic	29 (63%)	22 (46%)	24 (51%)	26 (59%)
Polyarteritis			1 (2%)	
Submucosa, edema			1 (2%)	

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

TABLE D1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats in the 2-Year Feed Study of Malachite Green Chloride 262
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TABLE D1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats in the 2-Year Feed Study of Malachite Green Chloride[a]

	0 ppm	100 ppm	300 ppm	600 ppm
n	28	23	32	25
Necropsy body wt	315 ± 7	312 ± 10	287 ± 9**	277 ± 10***
R. Kidney				
Absolute	1.215 ± 0.023	1.195 ± 0.034	1.133 ± 0.031*	1.155 ± 0.033
Relative	3.90 ± 0.10	3.86 ± 0.14	3.99 ± 0.13	4.20 ± 0.14
L. Kidney				
Absolute	1.214 ± 0.019	1.225 ± 0.033	1.168 ± 0.030	1.170 ± 0.032
Relative	3.91 ± 0.10	3.96 ± 0.14	4.11 ± 0.13	4.25 ± 0.14*
Liver				
Absolute	11.087 ± 0.346	10.732 ± 0.544	11.002 ± 0.501	11.352 ± 0.532
Relative	35.70 ± 1.48	34.72 ± 2.21	38.90 ± 2.03	41.06 ± 2.16*
Thyroid gland				
Absolute	0.036 ± 0.002	0.035 ± 0.005	0.037 ± 0.004	0.035 ± 0.004
Relative	0.11 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.13 ± 0.01

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** $P \leq 0.01$
*** $P \leq 0.001$
[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). Statistical tests were performed on unrounded data.

TABLE D2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Year Feed Study of Leucomalachite Green[a]

	0 ppm	91 ppm	272 ppm	543 ppm
Males				
n	23	29	34	29
Necropsy body wt	432 ± 8	424 ± 10	397 ± 10**	377 ± 10***
R. Kidney				
Absolute	1.651 ± 0.037	1.625 ± 0.057	1.692 ± 0.055	1.706 ± 0.057
Relative	3.84 ± 0.10	3.84 ± 0.16	4.30 ± 0.16*	4.57 ± 0.16***
L. Kidney				
Absolute	1.634 ± 0.033	1.604 ± 0.056	1.648 ± 0.054	1.669 ± 0.056
Relative	3.80 ± 0.09	3.79 ± 0.16	4.18 ± 0.16*	4.48 ± 0.16***
Liver				
Absolute	14.727 ± 0.523	14.815 ± 0.691	17.182 ± 0.668**	19.288 ± 0.691***
Relative	34.30 ± 1.38	34.99 ± 1.86	43.55 ± 1.80***	51.69 ± 1.86***
Thyroid gland				
Absolute	0.038 ± 0.002	0.042 ± 0.003	0.036 ± 0.002[b]	0.042 ± 0.003[c]
Relative	0.10 ± 0.00	0.10 ± 0.01	0.09 ± 0.01[b]	0.11 ± 0.01**[c]
Females				
n	33	36	35	33
Necropsy body wt	312 ± 7	299 ± 7	277 ± 7***	240 ± 7***
R. Kidney				
Absolute	1.138 ± 0.026	1.052 ± 0.028**	1.067 ± 0.028*	1.022 ± 0.029***
Relative	3.72 ± 0.15	3.54 ± 0.14	3.88 ± 0.14	4.26 ± 0.14***
L. Kidney				
Absolute	1.154 ± 0.025[d]	1.068 ± 0.026**	1.064 ± 0.026**	1.029 ± 0.027***
Relative	3.73 ± 0.14[d]	3.59 ± 0.13	3.87 ± 0.13	4.29 ± 0.13***
Liver				
Absolute	10.344 ± 0.368	9.865 ± 0.365	10.433 ± 0.367	11.162 ± 0.373
Relative	33.76 ± 1.58	33.09 ± 1.45	37.87 ± 1.46*	46.57 ± 1.48***
Thyroid gland				
Absolute	0.033 ± 0.002	0.032 ± 0.002[e]	0.032 ± 0.002[d]	0.033 ± 0.002[d]
Relative	0.11 ± 0.01	0.11 ± 0.01[e]	0.12 ± 0.01[d]	0.14 ± 0.01***[d]

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
*** P≤0.001
[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). Statistical tests were performed on unrounded data.
[b] n=33
[c] n=28
[d] n=32
[e] n=35

TABLE D3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Mice in the 2-Year Feed Study of Malachite Green Chloride[a]

	0 ppm	100 ppm	225 ppm	450 ppm
n	40	44	40	41
Necropsy body wt	33.8 ± 0.9	35.8 ± 1.0	34.7 ± 1.0	32.4 ± 1.0
R. Kidney				
Absolute	0.224 ± 0.004	0.217 ± 0.005	0.210 ± 0.005**	0.206 ± 0.005***
Relative	6.74 ± 0.14	6.12 ± 0.18**	6.08 ± 0.18**	6.42 ± 0.18
L. Kidney				
Absolute	0.209 ± 0.003[b]	0.208 ± 0.004	0.201 ± 0.005	0.188 ± 0.005***
Relative	6.26 ± 0.15[b]	5.87 ± 0.17	5.83 ± 0.18*	5.86 ± 0.18
Liver				
Absolute	1.403 ± 0.042	1.403 ± 0.054[c]	1.336 ± 0.055	1.393 ± 0.055[d]
Relative	42.17 ± 1.38	39.31 ± 1.91[c]	38.70 ± 1.94	43.16 ± 1.94[d]

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
*** P≤0.001
[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). Statistical tests were performed on unrounded data.
[b] n=39
[c] n=43
[d] n=40

TABLE D4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Mice in the 2-Year Feed Study of Leucomalachite Green[a]

	0 ppm	91 ppm	204 ppm	408 ppm
n	37	41	39	39
Necropsy body wt	32.7 ± 0.6	34.0 ± 1.0	37.6 ± 1.0***	34.5 ± 1.0
R. Kidney				
Absolute	0.227 ± 0.005	0.216 ± 0.005	0.227 ± 0.005	0.217 ± 0.005
Relative	7.03 ± 0.21	6.43 ± 0.20*	6.12 ± 0.21***	6.34 ± 0.21**
L. Kidney				
Absolute	0.216 ± 0.004	0.202 ± 0.005*	0.216 ± 0.005	0.204 ± 0.005
Relative	6.69 ± 0.20	6.00 ± 0.20**	5.83 ± 0.20***	5.96 ± 0.20**
Liver				
Absolute	1.412 ± 0.084	1.365 ± 0.106	1.579 ± 0.108	1.542 ± 0.108
Relative	43.75 ± 2.81	40.28 ± 3.25	42.37 ± 3.29	45.25 ± 3.29

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
*** P≤0.001
[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). Statistical tests were performed on unrounded data.

APPENDIX E
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Malachite Green Chloride

Malachite green chloride was obtained from Chemsyn Science Laboratories (Lenexa, KS) in one lot (CSL-98-808-08-04). Identity, purity, and stability analyses were conducted by the study laboratory. Reports on analyses performed in support of the malachite green chloride studies are on file at the National Center for Toxicological Research (NCTR).

Lot CSL-98-808-08-04, a dark green solid, was identified as malachite green chloride by the study laboratory using ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectroscopy, direct exposure probe electron impact mass spectrometry (DEP/EI/MS), and high-performance liquid chromatography (HPLC)/EI/MS by system A (Table E1) All spectra were consistent with the structure of malachite green chloride. The ^{1}H- and ^{13}C- NMR spectra are presented in Figures E1 and E2.

The purity of malachite green chloride was determined by the study laboratory using HPLC by system A, gas chromatography (GC), and inductively coupled plasma (ICP) spectrometry. Gas chromatography was performed with a Hewlett-Packard gas chromatograph using a flame ionization detector with a helium carrier gas at 24 psi. A Carbopak B packed column with 5% Carbowax 20M (6 ft × 2 mm, Supelco) was used with an isocratic oven temperature of 80° C.

HPLC analysis by system A indicated a purity of approximately 88% and four impurity peaks with a combined area of approximately 12.1% of the total peak area. GC indicated that malachite green chloride contained 1.4% methanol by weight. Heavy metal analysis by ICP spectrometry indicated the presence of less than 4 ppm lead. The overall purity of lot CSL-98-808-08-04 was determined to be approximately 87%.

The bulk chemical was stored in the original amber bottle inside a plastic bag in the original cardboard box at –70° C. The stability of bulk lot CSL-98-808-08-04 was monitored at 0, 12, 18, 21, and 24 months during the studies with HPLC by system A. Analysis indicated a 3.1% degradation of the compound during the course of the 2-year studies.

Leucomalachite Green

Leucomalachite green was obtained from Chemsyn Science Laboratories in one lot (CSL-97-718-77-10). Identity, purity, and stability analyses were conducted by the study laboratory. Reports on analyses performed in support of the leucomalachite green studies are on file at the NCTR.

Lot CSL-97-718-77-10, a faint green solid, was identified as leucomalachite green by the supplier using ^{1}H-NMR and infrared spectroscopy and by the study laboratory using DEP/EI/MS and ^{1}H- and ^{13}C-NMR. All spectra were consistent with the structure of leucomalachite green. The infrared, ^{1}H-, and ^{13}C-NMR spectra are presented in Figures E3, E4, and E5, respectively.

The purity of leucomalachite green was determined by elemental and heavy metal analysis (performed by Galbraith Laboratories, Inc., Knoxville, TN), HPLC performed by the chemical supplier (system B) and the study laboratory (using systems similar to systems C and D), ^{1}H-NMR and gas chromatography coupled with mass spectrometry (performed by the study laboratory).

Elemental analysis for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for leucomalachite green. Heavy metal analysis indicated 0.4 ppm lead. Consideration of all analyses indicated a leucomalachite green purity of approximately 99%.

The bulk chemical was stored at –70° C in the original amber glass container. The stability of leucomalachite green was monitored at 0, 13, 19, and 24 months during the studies using HPLC by systems similar to system C. Analysis indicated a 0.6% degradation of the compound during the course of the 2-year studies the impurity was identified as malachite green.

Preparation and Analysis of Dose Formulations

The dose formulations for malachite green chloride were prepared approximately every 2 months by dissolving the chemical in water and then mixing it with feed (Table E2). The solution was blended with feed in a Patterson-Kelly V-shell blender using an intensifier bar and a heater under a vacuum of at least 15 mm mercury for approximately 20 minutes. The dose formulations for leucomalachite green, were prepared approximately every 2 months by mixing the chemical with feed (Table E2). A premix was prepared by hand then blended with additional feed in a Patterson-Kelly V-shell blender using an intensifier bar for approximately 20 minutes. Dose formulations were stored in stainless steel feed cans at 4° ± 2° C for up to 92 days (malachite green chloride) or 95 days (leucomalachite green).

For malachite green chloride, a homogeneity study of a 100 ppm dose formulation and a stability study of a 25 ppm dose formulation were performed by the study laboratory using HPLC by system C. Homogeneity was confirmed, and stability was confirmed for at least 10 days for dose formulations stored at room temperature exposed to light and for at least 92 days for formulations stored at up to 6° C protected from light. For leucomalachite green, homogeneity studies of 96 and 91 ppm dose formulations were performed by the study laboratory using HPLC by system D. Stability studies of a 96 ppm dose formulation were also performed by the study laboratory using HPLC by system C. Homogeneity was confirmed, and stability was confirmed for at least 32 days for dose formulations stored at room temperature exposed to light and for at least 95 days for formulations stored at up to 6° C protected from light.

For malachite green chloride, periodic analyses of the dose formulations were conducted by the study laboratory using HPLC by system C. The dose formulations were analyzed approximately every 7 weeks (Table E3). Of the dose formulations analyzed and used, 96% (65/68) of the dose formulations for rats and 97% (33/34) of the dose formulations for mice were within 10% of the target concentrations. For leucomalachite green, periodic analyses of the dose formulations were conducted by the study laboratory using HPLC by system D. The dose formulations were analyzed approximately every 7 weeks; animal room samples were also analyzed (Table E4). Of the dose formulations analyzed and used, all of the dose formulations for rats (90/90) and mice (50/50) were within 10% of the target concentrations. Of the animal room samples analyzed, 56% (5/9) of the samples for rats and 78% (7/9) of the samples for mice were within 10% of the target concentrations.

Table E1
High-Performance Liquid Chromatography Systems Used in the 2-Year Feed Studies of Malachite Green Chloride and Leucomalachite Green[a]

Detection System	Column	Solvent System
System A		
Ultraviolet/visible photodiode array (scanning from 220 to 800 nm) with monitoring at 618 nm	Spherisorb Cyano, 250 mm × 4.6 mm, 5-µm particle size (Waters, Corp., Milford, MA) with 20 mm × 2.0 mm PbO_2 post column	A) 20% acetonitrile and 80% 0.05 M ammonium acetate, pH 4.5 and B) 80% acetonitrile and 20% 0.05 M ammonium acetate, pH 4.5; 100% A for 5 minutes then 10 minutes to 100% B, flow rate 1.5 mL/minute
System B		
Ultraviolet (254 nm)	Interstil 5 ODS 2, 250 mm × 4.6 mm, 5-µm particle size (Varian, Palo Alto, CA)	A) 95% acetonitrile and B) 20 mM H_3PO_4, pH 3.0, flow rate 1 mL/minute
System C		
Ultraviolet/visible photodiode array (scanning from 220 nm to 800 nm) with monitoring at 267 and 618 nm	Cyano, 250 mm × 4.6 mm, 5-µm particle size (Supelco, Inc. Bellefonte, PA)	A) 60% acetonitrile and 40% 0.05 M ammonium acetate buffer, pH 4.5, flow rate 1.0 mL/minute
System D		
Evaporative light scattering with polychromatic source in visible range	Prodigy ODS, 250 mm × 4.6 mm, 5-µm particle size (Phenomenex, Torrance, CA)	A) 50% acetonitrile and 50% 0.05 M ammonium acetate buffer, pH 4.5 and B) acetonitrile; 100% A to 100% B in 10 minutes, flow rate 1.0 mL/minute

[a] High-performance liquid chromatographs were manufactured by Waters Corp. (Milford, MA) (systems A and C) and Varian, Inc. (Palo Alto, CA) (systems B and D).

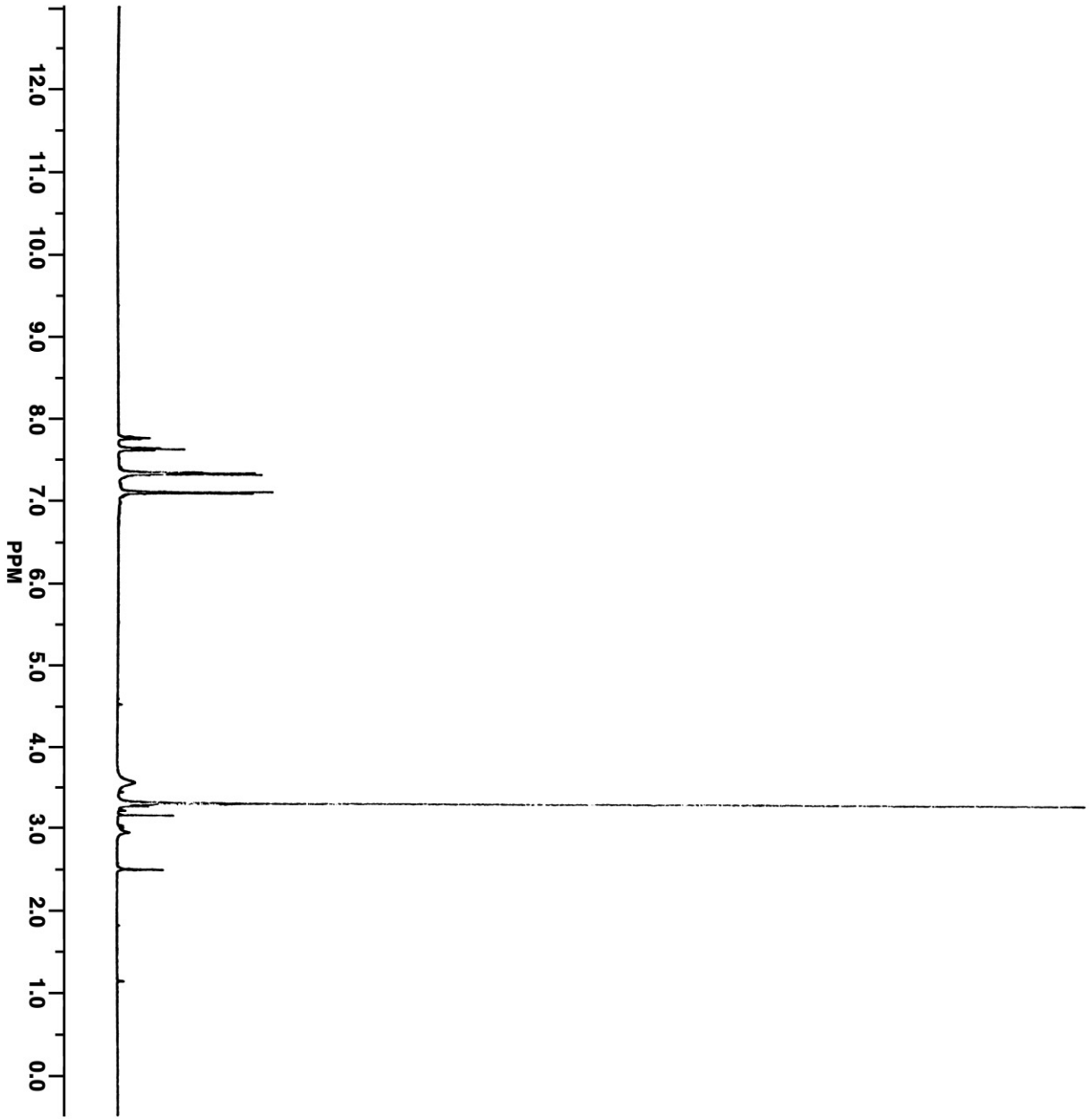

FIGURE E1
^{1}H-Nuclear Magnetic Resonance Spectrum of Malachite Green Chloride

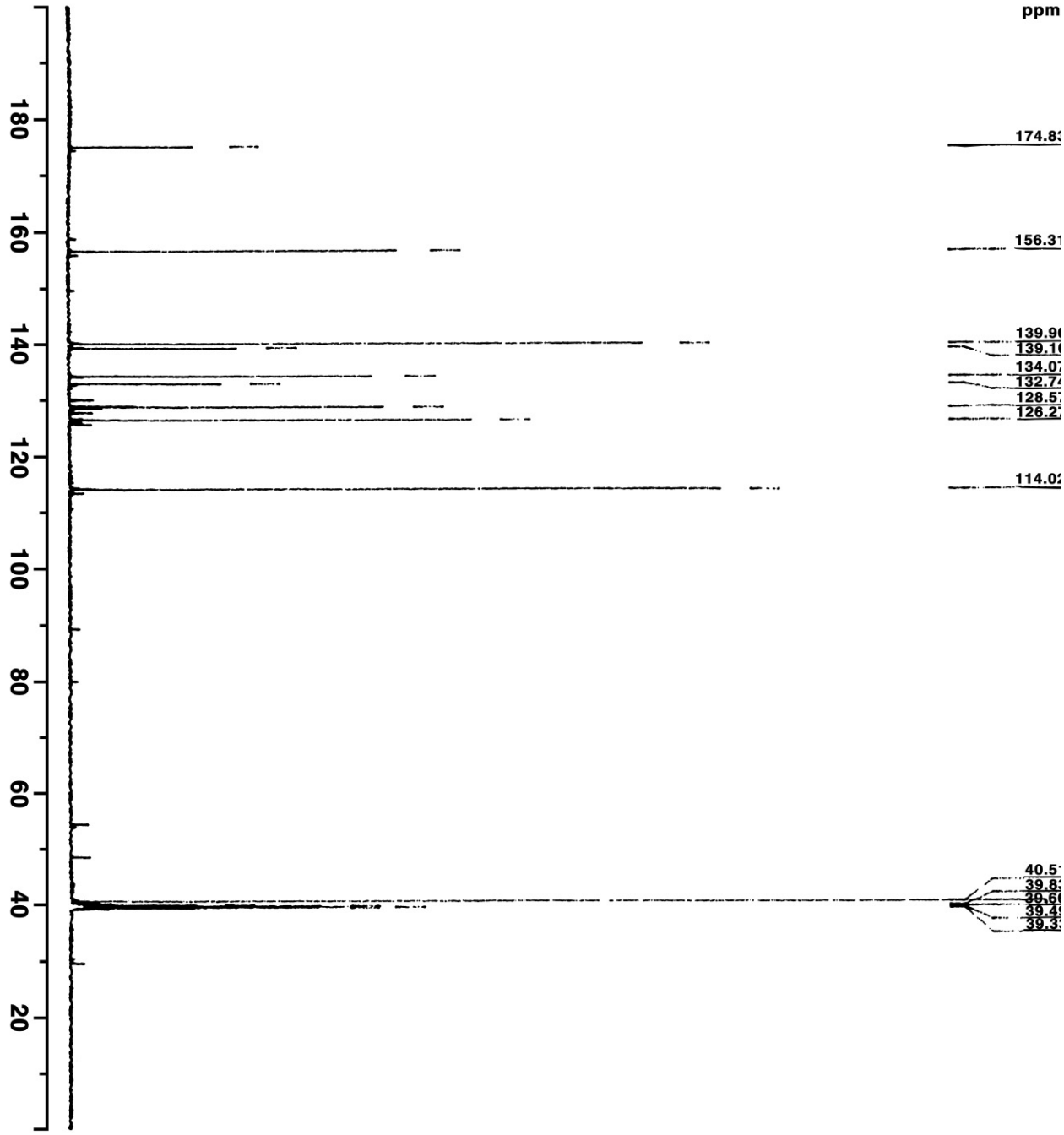

FIGURE E2
^{13}C-Nuclear Magnetic Resonance Spectrum of Malachite Green Chloride

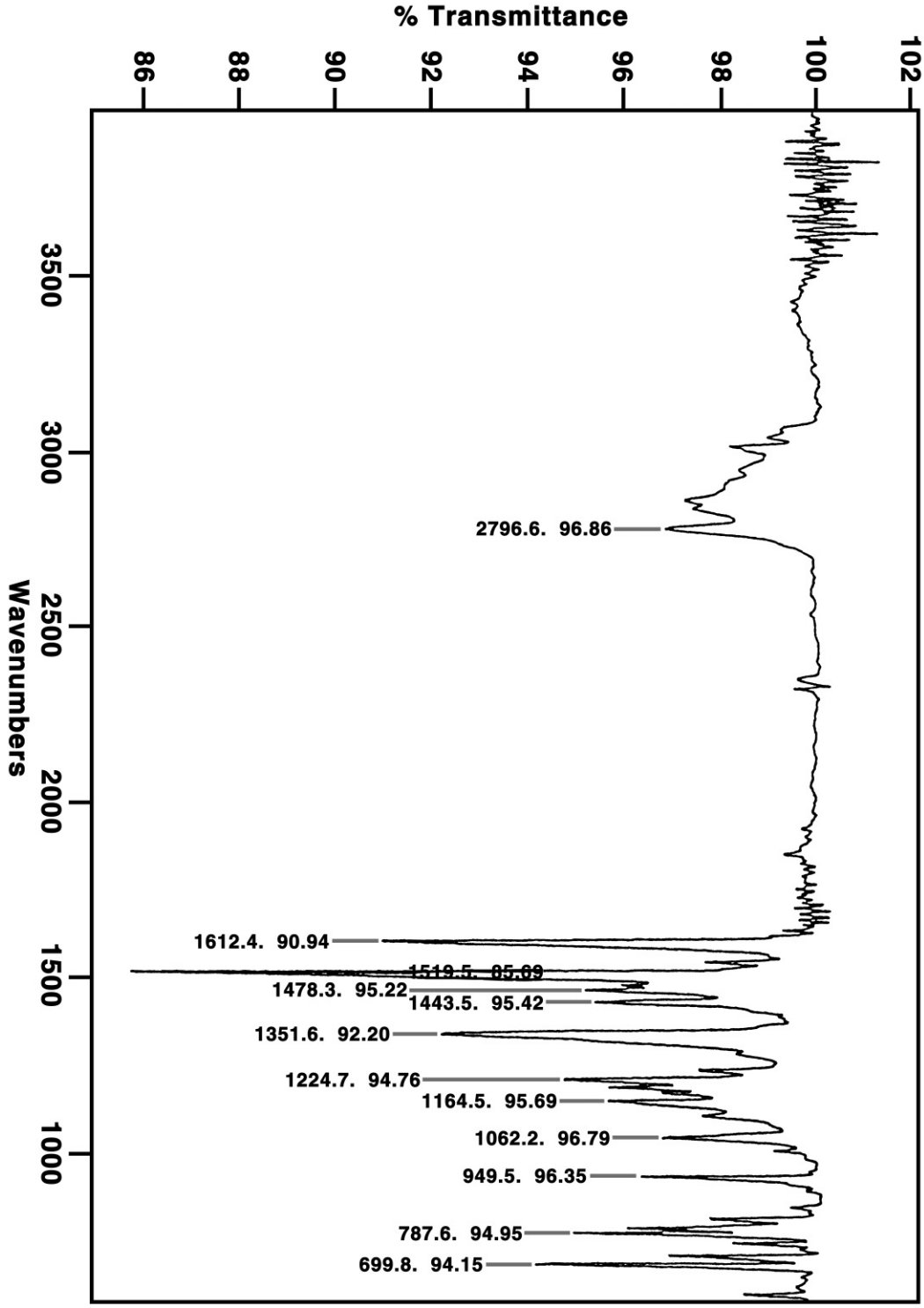

FIGURE E3
Infrared Spectrum of Leucomalachite Green

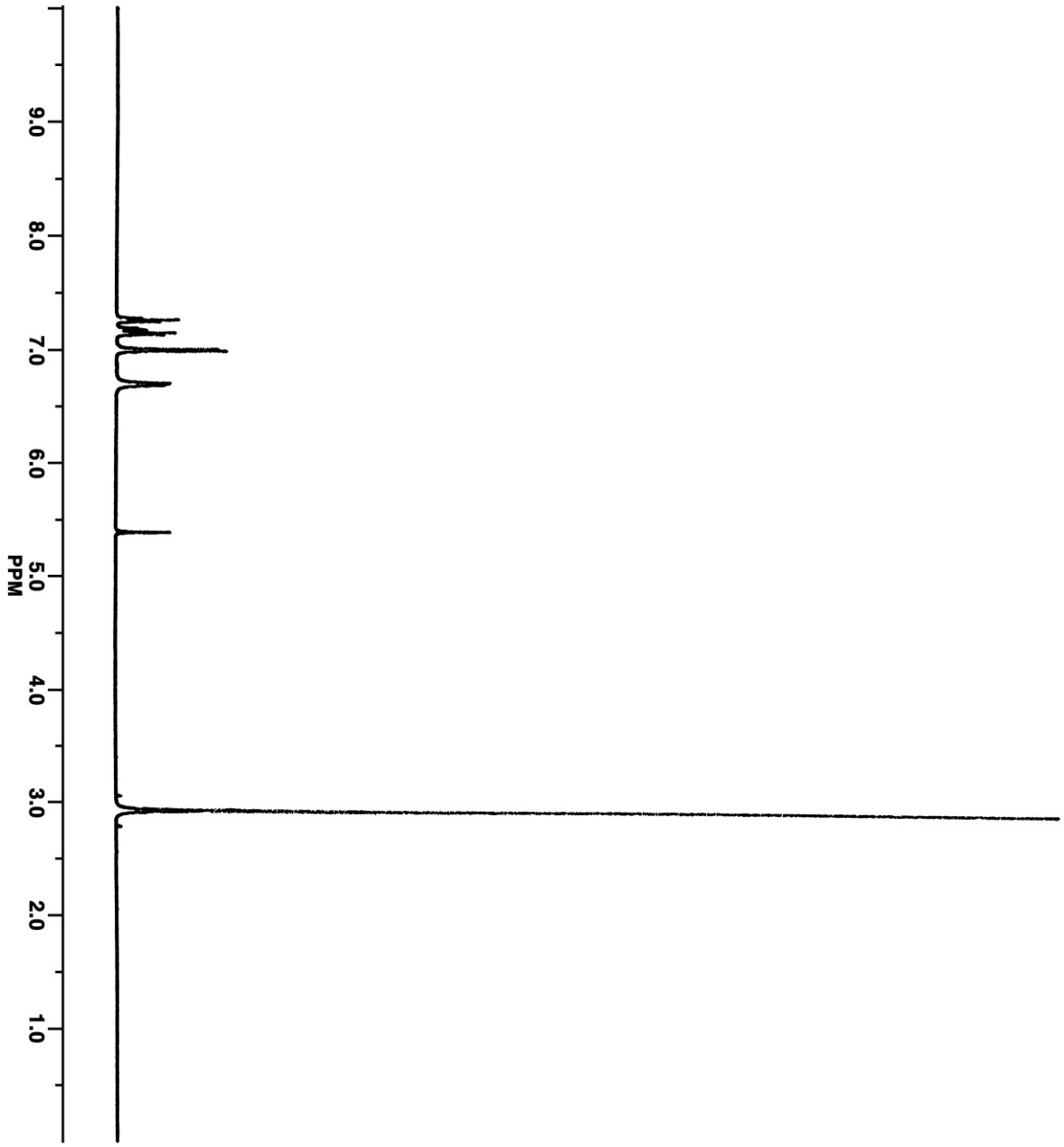

FIGURE E4
^{1}H-Nuclear Magnetic Resonance Spectrum of Leucomalachite Green

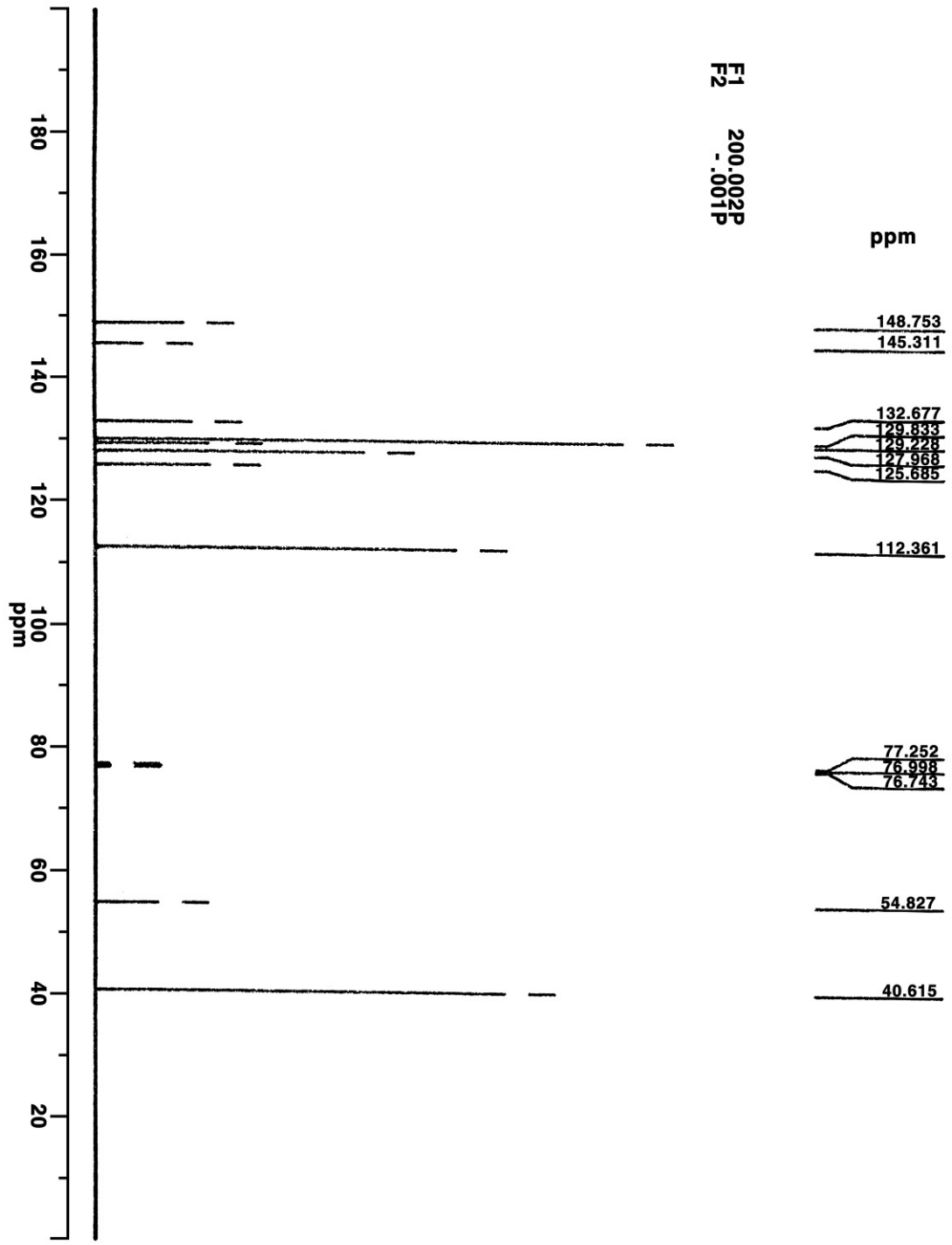

FIGURE E5
^{13}C-Nuclear Magnetic Resonance Spectrum of Leucomalachite Green

TABLE E2
Preparation and Storage of Dose Formulations in the 2-Year Feed Studies of Malachite Green Chloride and Leucomalachite Green

Malachite Green Chloride	Leucomalachite Green
Preparation	
A solution of malachite green chloride in deionized distilled water was prepared by stirring and sonication. Malachite green chloride solution was then directly injected into a Patterson-Kelly V-shell blender mixing with the intensifier bar and heater on under a vacuum of at least 15 mm of mercury. Mixing was continued for approximately 20 minutes, with the use of the intensifier bar. Dose formulations were prepared approximately every 2 months.	A premix was prepared by hand and then blended with additional feed in a Patterson-Kelly V-shell blender for approximately 20 minutes with the intensifier bar on. Dose formulations were prepared approximately every 2 months.
Chemical Lot Number	
CSL-98-808-08-04	CSL-97-718-77-10
Maximum Storage Time	
92 days	95 days
Storage Conditions	
Stored at 4° ± 2° C in stainless steel feed cans with lids secured by tie-downs.	Stored at 4° ± 2° C in stainless steel feed cans with lids secured by tie-downs.
Study Laboratory	
National Center for Toxicological Research (Jefferson, AR)	National Center for Toxicological Research (Jefferson, AR)

TABLE E3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Malachite Green Chloride

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats				
March 1, 1999	March 5, 1999	100	98	–2
		300	296	–1
		600	616	+3
May 28, 1999	June 3, 1999	100	87	–13
July 9, 1999	July 20, 1999	100	99	–1
August 4, 2000	August 5, 2000	100	101	+1
August 18, 1999	August 19, 1999	100	102	+2
		100	103	+3
		300	334	+11
		300	302	+1
		600	666	+11
		600	605	+1
October 20, 1999	October 22, 1999	100	97	–3
		100	102	+2
		300	296	–1
		300	309	+3
		600	611	+2
		600	624	+4
December 29, 1999	January 11, 2000	100	93	–7
		100	99	–1
		100	102	+2
		300	297	–1
		300	305	+2
		600	608	+1
		600	620	+3
March 1, 2000	March 2, 2000	100	105	+5
		300	322	+7
		600	650	+8
April 19, 2000	April 21, 2000	100	98	–2
		100	106	+6
		300	308	+3
		300	317	+6
		600	655	+9
		600	640	+7
May 17, 2000	May 24, 2000	100	96	–4
June 28, 2000	June 30, 2000	100	106	+6
		100	101	+1
		300	299	0
		300	303	+1
		600	618	+3
		600	615	+3

TABLE E3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Malachite Green Chloride

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
August 16, 2000	August 19, 2000	100	100	0
		100	104	+4
		300	305	+2
		300	309	+3
		600	624	+4
		600	630	+5
October 11, 2000	October 20, 2000	100	85[b]	–15
		100	83[c]	–17
October 18, 2000	October 20, 2000	100	99	–1
		100	98	–2
		300	627[b]	+109
		300	631[b]	+110
		300	294[d]	–2
		300	300	0
		600	622	+4
		600	628	+5
October 26, 2000	November 2, 2000	100	119[e]	+19
November 2, 2000	November 3, 2000	100	99	–1
December 12, 2000	December 20, 2000	100	90	–10
December 20, 2000	December 29, 2000 and January 3, 2001	100	101	+1
		100	96	–4
		300	284	–5
		300	288	–4
		600	555	–8
		600	632	+5
February 28, 2001	March 7, 2001	100	107	+7
March 7, 2001	March 12, 2001	100	101	+1
		100	100	0
		300	319	+6
		300	321	+7
		600	627	+5
		600	645	+8

TABLE E3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Malachite Green Chloride

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice				
March 1, 1999	March 5, 1999	100	98	–2
		225	223	–1
		450	456	+1
May 28, 1999	June 3, 1999	100	87	–13
August 4, 1999	August 5, 1999	100	101	+1
		225	233	+4
		450	471	+5
August 18, 1999	August 19, 1999	100	102	+2
October 13, 1999	October 14, 1999	100	102	+2
		225	231	+3
		450	474	+5
December 29, 1999	January 11, 2000	100	93	–7
		100	99	–1
		100	102	+2
		225	225	0
		450	492	+9
March 8, 2000	March 9, 2000	100	101	+1
		225	227	+1
		450	488	+8
May 17, 2000	May 24, 2000	100	96	–4
		225	239	+6
		450	446	–1
August 2, 2000	August 4, 2000	100	109	+9
		225	230	+2
		450	472	+5
October 11, 2000	October 20, 2000	100	85[b]	–15
		100	83[c]	–17
		225	229	+2
		450	453	+1
October 18, 2000	October 20, 2000	100	98	–2
October 26, 2000	November 3, 2000	100	119[e]	+19
December 12, 2000	December 20, 2000	225	220	–2
		450	448	0
December 20, 2000	December 29, 2000	100	96	–4

TABLE E3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Malachite Green Chloride

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
February 28, 2001	March 7, 2001	100	107	+7
		225	249[b]	+11
		225	287[b]	+28
		450	493	+10
March 8, 2001	March 12, 2001	225	224[f]	–1

[a] Results of triplicate analyses
[b] Reassayed, not used in study
[c] Results of reassay, not used in study; remixed
[d] Results of reassay
[e] Results of remix, not used in study
[f] Results of remix

TABLE E4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Leucomalachite Green

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats				
September 30, 1998	October 5, 1998	91	89	–2
		543	505	–7
October 6, 1998	October 7, 1998	272	271	0
December 3, 1998	December 8, 1998	91	87	–4
December 18, 1998	December 18, 1998	91	87	–4
		272	261	–4
		543	503	–7
January 22, 1999	January 22, 1999	272	257	–6
		543	504	–7
March 2, 1999	March 3, 1999	91	86	–5
March 26, 1999	March 29, 1999	272	257	–6
		543	500	–8
May 4, 1999	May 4, 1999	91	87	–4
		272	260	–4
		543	522	–4
July 7, 1999	July 13, 1999	91	91	0
August 2, 1999	August 2, 1999	272	262	–4
		272	251	–8
		272	255	–6
		543	512	–6
		543	517	–5
		543	517	–5
August 30, 1999	August 30, 1999	91	86	–5
		91	90	–1
		91	87	–4
October 4, 1999	October 4, 1999	272	259	–5
		272	259	–5
		272	262	–4
		543	527	–3
		543	516	–5
		543	538	–1
October 15, 1999	October 26-November 1, 1999	543	348[b]	–36
		543	524[c]	–3
October 25, 1999	October 26, 1999	91	68[b]	–25
		91	78[b]	–14
		91	85	–7

TABLE E4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Leucomalachite Green

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
October 29, 1999	November 1, 1999	91	92[c]	+1
		91	90[c]	–1
December 6, 1999	December 7, 1999	272	280	+3
		272	275	+1
		272	276	+1
		272	270	–1
		543	545	0
		543	523	–4
		543	529	–3
		543	536	–1
December 20, 1999	December 20, 1999-January 13, 2000	91	95	+4
		91	90	–1
		91	90	–1
		91	89[d]	–2
February 14, 2000	February 14, 2000	91	88	–3
		91	88	–3
February 22, 2000	February 22, 2000	272	260	–4
		543	520	–4
March 13, 2000	March 13, 2000	272	266	–2
		272	270	–1
		543	546	+1
		543	541	0
March 20, 2000	March 20, 2000	91	91	0
April 10, 2000	April 10, 2000	91	88	–3
		91	89	–2
		91	92	+1
April 24, 2000	April 25, 2000	272	266	–2
		272	256	–6
		272	264	–3
		543	538	–1
		543	540	–1
		543	522	–4
June 5, 2000	June 5, 2000	91	91	0
		91	91	0
		91	90	–1
June 26, 2000	June 26, 2000	272	259	–5
		272	268	–1
		543	531	–2
		543	533	–2

TABLE E4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Leucomalachite Green

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
July 31, 2000	August 1, 2000	91	88	–3
		91	89	–2
		91	90	–1
August 7, 2000	August 8, 2000	272	257	–6
		272	257	–6
		272	256	–6
		543	515	–5
		543	508	–6
		543	515	–5
September 25, 2000	September 25, 2000	91	88	–3
		91	88	–3
		91	89	–2
October 10, 2000	October 12, 2000	272	263	–3
		272	275	+1
		272	268	–1
		543	540	–1
		543	526	–3
		543	549	+1
Mice				
September 30, 1998	October 5-7, 1998	91	89	–2
		408	377	–8
October 6, 1998	October 7, 1998	204	206	+1
December 3, 1998	December 8, 1998	91	87	–4
December 18, 1998	December 18, 1998	91	87	–4
March 2, 1999	March 3, 1999	91	86	–5
March 3, 1999	March 3, 1999	204	197	–3
		408	393	–4
May 4, 1999	May 4, 1999	91	87	–4
July 7, 1999	July 13, 1999	91	91	0
		204	205	0
		408	393	–4
August 30, 1999	August 30, 1999	91	86	–5
		91	90	–1
		91	87	–4

TABLE E4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Leucomalachite Green

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice (continued)				
September 20, 1999	September 21, 1999	204	192	–6
		408	396	–3
October 25, 1999	October 26, 1999	91	68[b]	–25
		91	78[b]	–14
		91	85	–7
October 29, 1999	November 1, 1999	91	92[c]	+1
		91	90[c]	–1
November 29, 1999	November 29, 1999	204	194	–5
		408	389	–5
December 20, 1999	December 20, 1999	91	95	+4
		91	90	–1
		91	90	–1
		91	89[d]	–2
February 7, 2000	February 7, 2000	204	193	–5
		408	394	–3
February 14, 2000	February 14, 2000	91	88	–3
		91	88	–3
March 20, 2000	March 20, 2000	91	91	0
		204	200	–2
		408	404	–1
April 10, 2000	April 10, 2000	91	88	–3
		91	89	–2
		91	92	
May 30, 2000	May 30, 2000	204	205	0
		408	402	–1
June 5, 2000	June 5, 2000	91	91	0
		91	91	0
		91	90	–1
July 31, 2000	August 1, 2000	91	88	–3
		91	89	–2
		91	90	–1
August 7, 2000	August 8, 2000	204	201	–1
		408	394	–3
September 25, 2000	September 25, 2000	91	88	–3
		91	88	–3
		91	89	–2

TABLE E4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Leucomalachite Green

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice (continued)				
October 16, 2000	October 23, 2000	204	196	–4
		408	401	–2

[a] Results of triplicate analyses
[b] Remixed, not used in study
[c] Results of remix
[d] Results of reassay

APPENDIX F
FEED AND COMPOUND CONSUMPTION IN THE 2-YEAR FEED STUDIES OF MALACHITE GREEN CHLORIDE AND LEUCOMALACHITE GREEN

TABLE F1 Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of Malachite Green Chloride 286

TABLE F2 Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of Leucomalachite Green 287

TABLE F3 Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of Leucomalachite Green 288

TABLE F4 Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of Malachite Green Chloride 289

TABLE F5 Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of Leucomalachite Green 290

TABLE F1
Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of Malachite Green Chloride

Weeks on Study	0 ppm		100 ppm			300 ppm			600 ppm		
	Feed (g)[a]	Body Weight (g)	Feed (g)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
1	16.3	105	16.3	105	15.5	15.7	103	45.5	15.9	103	92.0
2	15.8	123	16.0	122	13.1	15.5	121	38.5	14.8	121	73.2
3	15.5	138	15.5	137	11.3	14.6	135	32.6	14.4	135	64.0
4	15.1	149	15.1	148	10.2	13.9	144	29.1	14.0	145	58.0
5	14.3	157	14.8	154	9.6	14.0	152	27.5	13.5	152	53.2
6	13.6	163	15.2	163	9.4	13.8	159	26.1	13.4	158	50.9
7	13.2	170	14.7	170	8.7	12.9	165	23.4	12.7	163	46.6
8	13.3	175	15.1	175	8.7	13.5	170	23.7	12.4	168	44.1
9	13.1	180	14.4	179	8.0	13.1	175	22.4	11.9	172	41.5
10	12.8	184	14.4	184	7.8	12.8	179	21.4	12.3	176	41.8
11	12.9	189	13.8	188	7.3	12.7	183	20.8	12.0	179	40.1
12	12.6	193	13.1	192	6.8	12.2	187	19.6	11.3	182	37.3
16	13.3	206	12.3	203	6.1	11.8	196	18.1	11.1	191	35.0
20	13.8	217	13.1	211	6.2	12.4	203	18.4	11.8	199	35.6
24	13.8	224	13.2	219	6.0	13.1	213	18.5	12.4	205	36.3
28	13.9	230	13.6	225	6.0	13.5	218	18.6	13.8	211	39.1
32	14.4	233	14.9	233	6.4	14.4	225	19.3	14.5	216	40.2
36	14.8	241	14.9	240	6.2	14.2	230	18.5	14.6	222	39.4
40	15.1	249	15.8	248	6.4	14.9	237	18.9	14.2	226	37.8
44	15.7	261	17.3	258	6.7	14.5	244	17.8	15.7	231	40.7
48	15.4	272	16.6	269	6.2	14.8	250	17.8	16.9	238	42.6
52	16.1	282	16.6	277	6.0	15.8	260	18.2	16.5	244	40.5
56	16.6	292	15.7	284	5.5	16.3	267	18.4	17.5	251	41.7
60	17.9	305	15.9	292	5.5	15.6	276	16.9	16.9	259	39.2
64	16.3	315	18.4	302	6.1	17.0	283	18.1	18.4	266	41.6
68	15.9	320	20.4	310	6.6	18.1	288	18.9	21.5	276	46.8
72	15.1	325	17.2	315	5.5	15.3	295	15.5	17.7	279	37.9
76	16.7	330	18.3	317	5.8	16.9	301	16.9	16.9	281	36.0
80	18.2	335	18.9	326	5.8	16.9	303	16.8	16.5	281	35.1
84	18.5	337	17.3	322	5.4	16.7	301	16.6	17.7	288	36.8
88	16.9	335	16.8	328	5.1	16.9	302	16.8	18.1	291	37.3
92	16.1	340	17.8	326	5.5	15.5	298	15.6	19.0	295	38.7
96	17.5	343	19.8	327	6.1	16.0	297	16.2	17.3	298	34.8
100	18.8	335	17.5	322	5.4	18.1	298	18.2	19.7	295	40.1
103	—[c]	332	—	326	—[e]	—	300	—	—	293	—
104	18.6	—[d]	17.8	—	—	18.6	—	—	18.3	—	—
Mean for weeks											
1-13	14.0	160	14.9	160	9.3	13.7	156	26.4	13.2	155	51.3
14-52	14.6	241	14.8	238	6.2	14.0	228	18.4	14.1	218	38.9
53-104	17.1	329	17.8	318	5.6	16.8	295	17.1	18.1	284	38.2

[a] Grams of feed consumed per animal per day
[b] Milligrams of malachite green chloride consumed per kilogram body weight per day
[c] Feed consumption was not measured during this week
[d] Animals were not weighed during this week
[e] Value of statistic cannot be computed

TABLE F2
Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of Leucomalachite Green

Weeks on Study	0 ppm		91 ppm			272 ppm			543 ppm		
	Feed (g)[a]	Body Weight (g)	Feed (g)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
1	17.8	131	17.0	127	12.2	16.9	129	35.5	17.1	126	73.5
2	16.1	162	16.2	153	9.6	15.9	156	27.6	16.0	152	57.0
3	17.4	190	17.4	182	8.7	17.1	186	25.0	17.0	180	51.1
4	17.9	214	17.8	208	7.8	17.7	211	22.9	17.1	205	45.4
5	17.4	235	17.2	228	6.9	16.9	231	19.9	16.6	223	40.4
6	17.5	253	17.6	246	6.5	17.4	248	19.1	17.4	241	39.2
7	17.9	268	17.6	260	6.2	17.1	261	17.8	16.6	254	35.6
8	17.5	282	17.5	275	5.8	17.3	273	17.2	16.6	267	33.8
9	18.3	296	18.1	287	5.7	18.4	287	17.5	17.5	280	33.9
10	18.6	305	19.0	298	5.8	17.6	297	16.2	17.8	288	33.6
11	19.3	316	19.3	309	5.7	18.1	307	16.1	18.1	299	33.0
12	20.5	343	19.6	333	5.3	18.0	312	15.7	18.6	316	32.0
16	19.9	362	19.8	351	5.1	18.8	348	14.7	18.4	339	29.6
20	19.8	386	19.0	376	4.6	19.1	371	14.0	18.2	360	27.4
24	19.6	408	20.3	400	4.6	19.3	392	13.4	18.3	378	26.2
28	18.9	423	18.8	413	4.2	18.4	405	12.4	17.4	389	24.2
32	18.6	433	18.1	425	3.9	18.2	417	11.9	17.4	397	23.8
36	16.9	440	18.8	436	3.9	17.2	422	11.1	16.5	404	22.1
40	18.1	452	18.7	446	3.8	17.4	432	10.9	17.8	412	23.4
44	18.5	458	17.7	446	3.6	18.4	439	11.4	17.6	417	22.9
48	19.0	462	18.3	454	3.7	18.7	443	11.5	18.2	422	23.5
52	18.6	467	19.1	456	3.8	19.3	449	11.7	18.1	424	23.2
56	20.2	476	19.7	462	3.9	19.1	452	11.5	17.9	432	22.5
60	19.1	476	20.7	472	4.0	18.1	449	11.0	17.6	430	22.2
64	19.2	476	21.3	475	4.1	18.9	452	11.4	18.9	428	24.0
68	20.1	485	18.5	477	3.5	17.9	453	10.7	16.3	428	20.7
72	18.5	489	19.6	478	3.7	18.3	452	11.0	18.6	431	23.5
76	18.1	486	19.0	474	3.6	17.5	447	10.6	16.3	428	20.7
80	18.5	488	19.2	475	3.7	17.2	446	10.5	15.6	425	19.9
84	18.8	479	19.2	465	3.8	19.0	440	11.7	18.3	422	23.5
88	21.2	479	20.6	462	4.1	19.5	436	12.1	20.0	419	25.9
92	20.0	475	19.6	458	3.9	19.1	434	11.9	18.4	411	24.3
96	19.1	475	20.5	453	4.1	20.2	431	12.7	17.8	403	24.0
100	21.3	459	22.6	445	4.6	22.4	427	14.3	20.6	399	28.0
103	—[c]	446	—	440	—[e]	—	416	—	—	395	—
104	19.3	—[d]	18.2	—	—	19.2	—	—	18.5	—	—
Mean for weeks											
1-13	18.0	243	17.9	235	6.9	17.4	236	20.0	17.2	229	40.7
14-52	18.8	429	18.9	420	4.1	18.5	412	12.2	17.8	394	24.5
53-104	19.5	476	19.9	460	3.9	18.9	437	11.8	18.0	415	23.6

[a] Grams of feed consumed per animal per day
[b] Milligrams of leucomalachite green consumed per kilogram body weight per day
[c] Feed consumption was not measured during this week
[d] Animals were not weighed during this week
[e] Value of statistic cannot be computed

TABLE F3
Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of Leucomalachite Green

Weeks on Study	0 ppm		91 ppm			272 ppm			543 ppm		
	Feed (g)[a]	Body Weight (g)	Feed (g)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
1	13.8	110	13.6	107	11.5	13.7	106	35.3	13.6	107	68.7
2	11.3	124	12.1	121	9.1	13.1	122	29.0	11.0	119	50.1
3	12.4	136	12.9	133	8.8	13.4	134	27.2	11.4	130	47.7
4	12.1	145	12.2	142	7.8	12.5	142	23.9	11.3	137	44.7
5	12.1	155	12.4	151	7.5	11.9	151	21.5	10.7	143	40.4
6	11.8	161	12.3	156	7.2	11.5	156	20.1	10.8	148	39.7
7	12.3	167	12.5	162	7.0	10.7	160	18.3	10.8	152	38.5
8	12.0	171	12.8	168	6.9	11.4	164	18.9	10.8	156	37.7
9	12.8	176	13.0	172	6.9	11.6	167	18.8	10.8	159	36.8
10	12.4	179	13.1	175	6.8	12.2	170	19.4	11.1	161	37.5
11	12.9	182	13.5	179	6.9	12.0	172	18.9	11.0	164	36.4
12	13.3	186	14.2	178	7.3	13.0	173	20.4	11.4	164	37.7
16	12.9	196	13.2	189	6.4	12.4	183	18.4	13.4	173	42.2
20	13.2	205	13.5	198	6.2	12.3	190	17.7	11.6	178	35.2
24	12.3	214	12.8	207	5.6	12.1	198	16.6	11.1	185	32.4
28	12.0	220	12.6	212	5.4	11.6	202	15.6	10.7	190	30.6
32	12.6	227	12.3	219	5.1	11.8	208	15.5	10.3	192	29.0
36	11.8	234	12.5	225	5.0	11.2	212	14.4	10.4	197	28.8
40	12.1	242	12.4	233	4.8	11.6	218	14.5	11.4	201	30.8
44	13.4	250	12.6	241	4.8	12.5	223	15.2	11.8	205	31.2
48	13.9	257	13.4	245	5.0	13.2	229	15.7	12.6	208	32.8
52	13.7	268	13.3	253	4.8	13.1	234	15.2	11.9	214	30.2
56	14.2	277	14.1	258	5.0	13.1	240	14.8	12.8	217	32.2
60	12.8	284	13.7	271	4.6	12.2	244	13.6	12.0	219	29.6
64	14.8	291	14.6	278	4.8	14.2	250	15.4	13.9	223	33.8
68	15.4	302	14.7	287	4.7	13.6	259	14.3	13.0	230	30.7
72	15.0	311	15.2	294	4.7	14.6	264	15.0	14.7	235	33.9
76	15.5	319	14.3	299	4.4	14.1	269	14.2	12.9	239	29.2
80	15.4	326	15.1	306	4.5	14.3	276	14.1	13.1	243	29.3
84	15.0	328	14.8	308	4.4	14.7	279	14.4	14.8	244	33.0
88	15.3	327	15.9	312	4.6	15.0	281	14.5	15.2	247	33.3
92	16.4	334	15.0	314	4.4	14.5	284	13.8	15.6	252	33.6
96	16.7	332	14.7	312	4.3	14.2	286	13.5	14.4	249	31.4
100	17.3	326	15.4	312	4.5	16.5	288	15.6	15.8	251	34.0
103	—[c]	326	—	310	—[e]	—	294	—	—	251	—
104	14.2	—[d]	14.1	—	—	14.6	—	—	13.8	—	—
Mean for weeks											
1-13	12.4	156	12.9	152	7.7	12.2	150	22.2	11.2	144	41.9
14-52	12.8	231	12.9	222	5.3	12.2	210	15.8	11.5	194	32.2
53-100	15.2	320	14.7	303	4.4	14.3	275	14.1	14.0	242	31.3

[a] Grams of feed consumed per animal per day
[b] Milligrams of leucomalachite green consumed per kilogram body weight per day
[c] Feed consumption was not measured during this week
[d] Animals were not weighed during this week
[e] Value of statistic cannot be computed

TABLE F4
Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of Malachite Green Chloride

Weeks on Study	0 ppm		100 ppm			225 ppm			450 ppm		
	Feed (g)[a]	Body Weight (g)	Feed (g)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
2	3.6	17.2	3.8	17.3	22.0	4.3	17.1	56.6	4.0	16.9	106.5
3	3.6	18.0	3.7	17.6	21.0	3.8	17.8	48.0	4.0	17.5	102.9
4	3.5	18.5	3.6	18.3	19.7	3.7	18.3	45.5	3.8	18.0	95.0
5	3.5	19.1	3.7	18.6	19.9	3.8	19.0	45.0	3.8	18.4	92.9
6	3.4	19.5	3.7	19.0	19.5	3.5	19.3	40.8	3.8	19.0	90.0
7	3.4	19.8	4.0	19.7	20.3	3.7	20.2	41.2	3.8	19.5	87.7
8	3.3	20.8	3.9	20.3	19.2	3.3	20.5	36.2	3.7	20.0	83.3
9	3.2	20.9	4.1	20.9	19.6	3.4	21.0	36.4	3.7	20.6	80.8
10	3.2	21.1	4.0	21.1	19.0	3.5	21.3	37.0	3.5	21.0	75.0
11	3.3	21.6	3.9	21.5	18.1	3.5	21.6	36.5	3.7	21.4	77.8
12	4.5	22.1	3.7	21.5	17.2	3.4	21.7	35.3	3.7	21.7	76.7
13	3.4	22.4	3.8	21.9	17.4	3.5	22.3	35.3	3.6	22.2	73.0
17	3.4	24.6	3.8	24.1	15.8	3.1	23.6	29.6	3.0	23.2	58.2
21	3.5	25.4	3.9	24.6	15.9	3.5	24.4	32.3	3.4	23.5	65.1
25	3.7	27.6	3.5	26.0	13.5	3.2	26.2	27.5	3.6	25.9	62.5
29	3.6	28.0	3.5	26.6	13.2	3.3	26.5	28.0	3.7	26.1	63.8
33	3.9	28.4	3.5	26.5	13.2	3.9	27.3	32.1	3.6	26.4	61.4
37	4.0	29.0	3.8	27.7	13.7	4.2	28.7	32.9	3.9	27.3	64.3
41	3.6	29.9	3.7	28.8	12.8	4.0	29.8	30.2	4.0	28.5	63.2
45	4.2	31.2	4.2	30.3	13.9	4.3	31.2	31.0	4.1	30.0	61.5
49	4.0	32.2	4.0	31.0	12.9	4.6	32.3	32.0	4.8	31.1	69.5
53	3.9	33.5	4.1	32.1	12.8	4.4	32.8	30.2	4.2	32.0	59.1
57	3.7	34.3	3.9	32.6	12.0	4.2	33.7	28.0	4.5	33.3	60.8
61	3.9	35.7	3.8	33.5	11.3	4.0	34.4	26.2	3.6	33.2	48.8
65	4.1	37.1	4.3	35.2	12.2	4.8	36.6	29.5	3.5	32.8	48.0
69	3.5	37.0	3.9	36.4	10.7	4.5	37.4	27.1	3.5	33.4	47.2
73	3.7	36.5	4.0	36.6	10.9	3.9	37.3	23.5	3.9	33.7	52.1
77	3.7	37.0	4.0	37.7	10.6	4.3	37.9	25.5	4.0	34.7	51.9
81	4.3	37.7	4.0	37.4	10.7	4.6	38.2	27.1	4.6	35.0	59.1
85	4.2	38.2	3.6	36.4	9.9	4.4	37.6	26.3	4.5	35.5	57.0
89	3.5	37.1	3.6	35.7	10.1	4.6	37.9	27.3	3.5	34.9	45.1
93	3.4	35.7	3.8	35.4	10.7	4.7	38.2	27.7	3.6	34.3	47.2
97	3.6	35.3	4.1	35.7	11.5	4.6	38.8	26.7	4.0	34.1	52.8
101	4.0	35.4	4.0	36.6	10.9	3.9	37.5	23.4	3.7	34.5	48.3
104	4.2	36.1	4.3	37.7	11.4	3.8	37.1	23.0	3.8	34.7	49.3
Mean for weeks											
1-13	3.5	20.1	3.8	19.8	19.4	3.6	20.0	41.2	3.8	19.7	86.8
14-52	3.8	28.5	3.8	27.3	13.9	3.8	27.8	30.6	3.8	26.9	63.3
53-104	3.8	36.2	4.0	35.6	11.1	4.3	36.8	26.5	3.9	34.0	51.9

[a] Grams of feed consumed per animal per day
[b] Milligrams of malachite green chloride consumed per kilogram body weight per day

TABLE F5
Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of Leucomalachite Green

Weeks on Study	0 ppm		91 ppm			204 ppm			408 ppm		
	Feed (g)[a]	Body Weight (g)	Feed (g)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
1	3.5	16.5	3.1	16.5	17.1	4.4	16.8	53.4	3.5	17.0	84.0
2	3.4	16.9	3.5	17.0	18.7	3.6	17.3	42.5	3.7	17.3	87.3
3	3.3	17.7	3.1	17.3	16.3	3.5	18.0	39.7	3.7	18.1	83.4
4	3.3	18.2	3.0	17.7	15.4	3.8	18.3	42.4	3.6	18.4	79.8
5	3.4	18.5	3.2	18.3	15.9	3.4	18.6	37.3	3.6	18.5	79.4
6	3.4	18.7	3.1	18.7	15.1	3.5	18.8	38.0	3.6	19.1	76.9
7	3.4	19.4	3.0	19.2	14.2	4.1	19.4	43.1	3.7	19.6	77.0
8	3.6	19.8	3.0	19.4	14.1	3.2	19.7	33.1	3.2	19.4	67.3
9	3.7	20.2	3.1	19.6	14.4	3.3	19.9	33.8	3.5	19.7	72.5
10	3.6	20.2	3.3	20.1	14.9	3.4	20.0	34.7	3.8	19.9	77.9
11	3.7	20.4	3.6	20.5	16.0	3.4	20.2	34.3	3.5	20.2	70.7
16	3.6	22.0	3.6	21.5	15.2	4.1	21.5	38.9	4.0	21.4	76.3
20	3.6	22.1	3.9	22.2	16.0	3.7	22.3	33.8	3.6	21.9	67.1
24	3.6	23.8	3.5	23.1	13.8	3.6	23.2	31.7	3.8	23.3	66.5
28	3.7	23.8	3.7	24.1	14.0	3.7	24.2	31.2	4.0	24.2	67.4
32	3.8	25.4	3.8	25.4	13.6	3.5	25.0	28.6	4.2	25.7	66.7
36	3.3	25.6	3.4	25.6	12.1	3.2	25.4	25.7	3.8	25.8	60.1
40	3.8	26.5	3.7	26.5	12.7	3.4	26.2	26.5	3.7	26.6	56.8
44	3.7	27.7	3.5	27.5	11.6	3.5	27.3	26.2	3.6	27.5	53.4
48	3.5	28.6	3.5	28.8	11.1	3.3	28.0	24.0	3.6	27.8	52.8
52	3.7	28.5	3.2	29.3	9.9	3.5	27.2	26.3	3.9	28.3	56.2
56	3.3	28.8	3.1	28.6	9.9	3.4	29.0	23.9	4.0	29.6	55.1
60	3.5	29.8	3.4	29.8	10.4	3.8	29.8	26.0	3.5	30.6	46.7
64	4.0	30.9	3.7	30.6	11.0	4.3	30.7	28.6	3.8	30.6	50.7
68	3.8	31.0	3.9	31.9	11.1	4.2	32.1	26.7	3.5	30.9	46.2
72	3.9	32.2	4.1	33.4	11.2	4.2	33.6	25.5	3.6	31.5	46.6
76	4.1	34.1	4.3	34.7	11.3	4.2	35.0	24.5	3.7	31.7	47.6
80	4.2	34.4	3.9	34.7	10.2	4.3	34.9	25.1	4.1	32.2	52.0
84	3.7	34.5	3.5	34.7	9.2	3.8	35.2	22.0	3.8	32.8	47.3
88	3.7	35.2	3.9	35.4	10.0	4.0	35.9	22.7	3.7	33.0	45.7
92	3.8	35.1	3.7	36.2	9.3	4.6	37.1	25.3	3.9	33.7	47.2
96	3.8	34.6	3.8	35.3	9.8	4.5	37.5	24.5	4.1	34.2	48.9
100	3.9	35.1	3.7	35.5	9.5	4.8	39.3	24.9	4.4	35.5	50.6
104	4.7	34.3	4.4	35.6	11.2	5.0	39.4	25.9	5.0	36.2	56.4
Mean for weeks											
1-13	3.5	18.8	3.2	18.6	15.6	3.6	18.8	39.3	3.6	18.8	77.8
14-52	3.6	25.4	3.6	25.4	13.0	3.6	25.0	29.3	3.8	25.3	62.3
53-104	3.9	33.1	3.8	33.6	10.3	4.2	34.6	25.0	3.9	32.5	49.3

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

APPENDIX G
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-31 RAT AND MOUSE RATION

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TABLE G1
Ingredients of NIH-31 Rat and Mouse Ration

Ingredients[a]	Percent by Weight
Ground #2 yellow shelled corn	21.0
Ground whole hard wheat	35.5
Ground whole oats	10.0
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	9.0
Wheat middlings	10.0
Alfalfa meal (17% protein)	2.0
Corn gluten meal (60% protein)	2.0
Soy oil	1.5
Dried brewer's yeast	1.0
Dicalcium phosphate (food grade)[b]	1.5
Ground limestone[b]	0.5
Salt	0.5
Premixes (vitamin and mineral)	0.5

[a] Ingredients were ground to pass through a U.S. Standard Screen No. 16 before being mixed.
[b] Specific ingredient requirement for cadmium content not to exceed 1 mg/kg

TABLE G2
Vitamins and Minerals in NIH-31 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	22,000,000 IU	Vitamin A palmitate or acetate
D_3	3,800,000 IU	D-activated animal sterol
K_3	20 g	Menadione activity
E	15 g	*dl*-α-Tocopheryl acetate
Choline	700 g	Choline chloride
Folic acid	1 g	
Niacin	20 g	
d-Pantothenic acid	25 g	*d*-Calcium pantothenate
Riboflavin	5 g	
Thiamine	65 g	Thiamine mononitrate
B_{12}	14 g	
Pyridoxine	2 g	Pyridoxine hydrochloride
Biotin	0.12 g	*d*-Biotin
Minerals		
Iron	60 g	Iron sulfate
Magnesium	400 g	Magnesium oxide
Manganese	100 g	Manganous oxide
Zinc	10 g	Zinc oxide
Copper	4 g	Copper sulfate
Iodine	1.5 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2,000 lb) of finished product; after autoclaving

TABLE G3
Nutrient Composition of NIH-31 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation[a]
Crude protein (% by weight)	18.8 ± 0.9
Crude fat (% by weight)	5.13 ± 0.92
Vitamins	
Vitamin A (µg/g)	10.7 ± 1.4
Vitamin E (µg/g)	58.4 ± 5.6
Vitamin B_1 (mg/g)	0.092 ± 0.018
Minerals	
Selenium (µg/g)	0.37 ± 0.08

[a] Average of 12 diet production lots; prior to autoclaving

TABLE G4
Contaminant Levels in NIH-31 Rat and Mouse Ration

	Mean ± Standard Deviation[a]
Contaminants	
Arsenic (µg/g)	0.16 ± 0.05
Cadmium (µg/g)	0.10 ± 0.03
Lead (µg/g)	0.48 ± 0.21
Aflatoxin B_1 (ppb)	<0.25
Aflatoxin B_2 (ppb)	<0.25
Aflatoxin G_1 (ppb)	<0.25
Aflatoxin G_2 (ppb)	<0.10
Fumonisin B_1 (ppb)	35.4 ± 11.0
Total fumonisin (ppb)	52.6 ± 15.1
Volatiles (%)	7.4 ± 1.8
Pesticides (ppb)	
Heptachlor	<10
DDT, total[b]	<5
Dieldrin	<5
PCB	19 ± 2
Malathion	227 ± 23
Lindane	<1

[a] Average of 12 diet production lots; for values less than the limit of detection, the detection limit is given as the mean.
[b] DDE+DDT+DDD

APPENDIX H
SENTINEL ANIMAL PROGRAM

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SENTINEL ANIMAL PROGRAM

METHODS

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

Serum samples were collected from randomly selected rats and mice during the 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated. Samples were processed appropriately at the National Center for Toxicological Research Division of Microbiology (Jefferson, AR), for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

MALACHITE GREEN CHLORIDE

Method and Test	Time of Analysis
RATS	
ELISA	
H-1 (Toolan's H-1 virus)	6, 12, and 18 months, study termination
KRV (Kilham Rat Virus)	6, 12, and 18 months, study termination
Mycoplasma arthritidis	6, 12, and 18 months, study termination
Mycoplasma pulmonis	6, 12, and 18 months, study termination
PVM (pneumonia virus of mice)	6, 12, and 18 months, study termination
RCV/SDA (rat coronavirus/sialodacryoadentis virus)	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
MICE	
ELISA	
Ectromelia virus	6, 12, and 18 months, study termination
GDVII (mouse encephalomyelitis)	6, 12, and 18 months, study termination
LCM (lymphocytic choriomeningitis virus)	6, 12, and 18 months, study termination
MVM (minute virus of mice)	6, 12, and 18 months, study termination
MHV (mouse hepatitis virus)	6, 12, and 18 months, study termination
M. arthritidis	6, 12, and 18 months, study termination
PVM	6, 12, and 18 months, study termination
Polyoma virus	6, 12, and 18 months, study termination
Reovirus 3	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination

LEUCOMALACHITE GREEN

Method and Test	Time of Analysis
RATS	
ELISA	
H-1	6, 12, and 18 months, study termination
KRV	6, 12, and 18 months, study termination
M. arthritidis	6, 12, and 18 months, study termination
M. pulmonis	6, 12, and 18 months, study termination
PVM	6, 12, and 18 months, study termination
RCV/SDA	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
MICE	
ELISA	
Ectromelia virus	6, 12, and 18 months, study termination
GDVII	6, 12, and 18 months, study termination
LCM	6, 12, and 18 months, study termination
MVM	6, 12, and 18 months, study termination
MHV	6, 12, and 18 months, study termination
M. arthritidis	6, 12, and 18 months, study termination
M. pulmonis	6, 12, and 18 months, study termination
PVM	6, 12, and 18 months, study termination
Polyoma virus	6, 12, and 18 months, study termination
Reovirus 3	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination

RESULTS

For the 2-year studies in rats and mice, all serology tests were negative.

APPENDIX I
GENOTOXICITY OF MALACHITE GREEN CHLORIDE AND LEUCOMALACHITE GREEN IN FEMALE BIG BLUE B6C3F$_1$ MICE

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INTRODUCTION

Malachite green is a triphenylmethane dye that is rapidly reduced *in vivo* to leucomalachite green (Culp and Beland, 1996). It is used commercially in dyestuffs and inks, and in aquaculture to control fungal infections. Besides the potential for worker exposure, chemical analyses found detectable levels of malachite green and leucomalachite green in the flesh of fish sold in Great Britain (VMD, 1996, 1999; Doerge *et al*., 1998), indicating a potential for consumer exposure. Similar surveys have not been reported for fish sold in the United States. Malachite green presently is not authorized for use in aquaculture in the U.S.; however, given the ready availability of malachite green, its low cost, and its acknowledged efficacy in aquaculture, there is a strong suspicion that it is currently used in this country.

The structural similarity of malachite green to carcinogenic arylamine dyes (IARC, 1978; Littlefield *et al.*, 1985; Littlefield, 1988) and experimental evidence for tumor promoting activity (Fernandes *et al.*, 1991) suggest a potential for adverse health effects from exposure to malachite green and leucomalachite green. The possibility of consumer exposure, suggestive evidence for carcinogenic activity, and a lack of adequate toxicological data for risk assessment prompted the U.S. FDA Center for Veterinary Medicine to nominate malachite green for carcinogenicity evaluation by the National Toxicology Program (NTP). Leucomalachite green was also evaluated because it is the principal residue of malachite green found in edible tissue. As described in this Technical Report, there was equivocal evidence of carcinogenicity for malachite green chloride and leucomalachite green in F344/N rats and some evidence of carcinogenicity for leucomalachite green in $B6C3F_1$ mice.

The mechanism for this tumorigenicity is unclear. Although there is some evidence for malachite green damaging DNA *in vitro* (Fessard *et al.*, 1999), the overwhelming majority of *in vitro* genotoxicity assays on malachite green and leucomalachite green have been negative (Culp and Beland, 1996; Fessard *et al.*, 1999; NTP, 2004), and efforts to metabolize these compounds to DNA binding species in cell-free systems have been unsuccessful (unpublished observations). A somewhat better case can be made for malachite green and leucomalachite green as *in vivo* genotoxins. Feeding leucomalachite green to mice for 28 days increased the frequency of micronucleated normochromogenic erythrocytes (NTP, 2004), and feeding both malachite green and leucomalachite green to rats and mice for 28 days resulted in dose-responsive increases in apparently identical DNA adducts (Culp *et al.*, 1999).

The significance of these DNA adducts is uncertain, however, since they have been detected only by ^{32}P-postlabeling and their structural identity is unknown. Also, their formation is negatively correlated to carcinogenicity. As described in this Technical Report, liver tumors were produced in female mice fed leucomalachite green; however, no increased liver tumor incidence was found in female mice fed malachite green chloride, an equivocal response was observed in female F344 rats fed malachite green chloride or leucomalachite green, and no increase was found in male F344 rats fed leucomalachite green. In contrast, liver DNA adducts were formed in both mice and rats fed malachite green chloride or leucomalachite green for 28 days, and adduct formation was lowest in female mice fed leucomalachite green (Culp *et al.*, 1999). The mutagenic activity associated with the adducts was examined in female transgenic Big Blue rats fed leucomalachite green for up to 32 weeks. Although dose responsive levels of liver DNA adducts were detected, there was no increase in bone marrow micronuclei, *Hprt* lymphocyte mutant frequency, or liver *lacI* mutation frequency (Culp *et al.*, 2002; Manjanatha *et al.*, 2003).

In order to further investigate whether or not leucomalachite green is acting as a genotoxic carcinogen, female Big Blue $B6C3F_1$ mice were fed malachite green chloride, a treatment that results in relatively high levels of adducts but not tumors, or leucomalachite green, a treatment that results in relatively low levels of DNA adducts but produces liver tumors. DNA adducts were measured by ^{32}P-postlabeling after 28 days and peripheral blood micronuclei, *Hprt* lymphocyte mutant frequency, and liver *cII* mutant frequency were measured at 28 days and 16 weeks. The results indicate that leucomalachite green is an *in vivo* mutagen, and that the *in vivo* mutagenicities of leucomalachite green and malachite green correlate with their tumorigenicities.

Materials and Methods

Animal Treatments

Female Big Blue B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, PA). Groups of 12, approximately 6-week-old mice were fed control diet (NIH-31) or diet containing 450 ppm malachite green chloride or 204 or 408 ppm leucomalachite green. Six mice from each group were killed after 28 days, with the remaining mice killed after 16 weeks of exposure. Approximately 0.1 mL of blood was removed from each mouse by cardiac puncture for micronucleus analysis, the spleens were aseptically removed for the lymphocyte *Hprt* assay, and the livers were removed and quickly frozen on dry ice for use in the *cII* mutant assay. At the 28-day sacrifice, the livers were divided approximately in half; one portion was used for the mutant assay and the other for the measurement of DNA adducts.

DNA Adduct Analysis

Liver DNA was extracted using slight modifications of the method described by Beland *et al.* (1984). Approximately 10 µg of DNA from each liver were ^{32}P-postlabeled, with the resulting adducts resolved by thin layer chromatography and quantified as described by Culp *et al.* (2002).

Micronucleus Analysis

Peripheral blood was fixed, washed, and stained for flow cytometric analysis using components from the µFlow Micronucleus Analysis Kit and the manufacturer's protocol (Litron Laboratories, Rochester, NY). Flow analysis was performed using a FACSort flow cytometer (Becton-Dickinson, San Jose, CA), with a total of 2×10^4 polychromatic erythrocytes (reticulocytes) being evaluated. Positive and negative controls supplied by the manufacturer, as well as blood from male and female 9-day-old neonatal B6C3F$_1$ mice that had been treated on days 1 to 8 with 5 µL of dimethyl sulfoxide (DMSO) or 5 µL of DMSO containing 200 mg/kg dideoxycytidine (Von Tungeln *et al.*, 2003), were analyzed for micronucleus frequency at the same time as the test samples.

Lymphocyte Hprt Mutant Analysis

The methodology employed for the lymphocyte *Hprt* mutant analysis is described by Meng *et al.* (1998) and Dobrovolsky *et al.* (2003). In brief, lymphocytes were harvested from crushed spleens, stimulated by treatment for approximately 40 hours with concanavalin A, and processed for limiting-dilution cloning in 96-well dishes. Cloning efficiencies in the presence and absence of the mutant selective agent 6-thioguanine were estimated by Poisson statistics, and mutant frequencies were calculated.

Liver cII Mutant Frequency Analysis

High-molecular-weight DNA was extracted from the mouse livers using the RecoverEase DNA Extraction Kit (Stratagene, La Jolla, CA). Approximately 10 µg of DNA were packaged and processed for determining *cII* mutant frequency using the reagents and protocol supplied with the λSelect-*cII* Mutation Detection System (Stratagene). Assays were repeated until a minimum of approximately 2×10^5 plaque-forming units from each liver were examined for mutation. For comparison, liver *cII* mutant frequencies were also determined for Big Blue rats fed control diet or 543 ppm leucomalachite green for 16 weeks (Culp *et al.*, 2002; Manjanatha *et al.*, 2004).

Evaluation of Mutations in Liver cII Mutants

cII Mutants were cored from the mutant frequency assay plates and the mutant phenotype confirmed by replating under selective conditions. A 432-bp fragment containing the *cII* gene was amplified by PCR from well-isolated, confirmed mutant plaques as modified from the protocol for the λSelect-*cII* Mutation Detection System. Briefly, 5 µL plaque supernatant were combined with 15 µL 2X Ampligold Master Mix (Applied Biosystems, Foster City, CA) and 0.1 µM of each primer (Stratagene) in a 30 µL reaction. After 3 minutes at 95° C to activate the polymerase, the reactions progressed through 30 cycles of 95° C for 30 seconds, 60° C for 1 minute, 72° C for 1 minute, and ended with 72° C for 10 minutes in an ABI 9700 Thermal Cycler (Applied Biosystems). The PCR products were purified using QiaQuick columns (Qiagen, Carlsbad, CA) and used as the template in sequencing

reactions with ET-terminator chemistry (Pharmacia, Piscataway, NJ) and the primer, 5′-CCACACCTATGGTGTATG. Sequencing products were analyzed on an ABI 377 DNA sequencer (Applied Biosystems).

RESULTS

Female Big Blue B6C3F$_1$ mice were fed leucomalachite green and malachite green chloride for up to 16 weeks. ^{32}P-postlabeled DNA adduct analysis performed on DNA from livers of mice treated for 28 days indicated the formation of a single adduct or co-eluting adducts in animals treated with malachite green (Figure I1). Adduct formation in mice treated with leucomalachite green was similar to that in control animals (Figure I1; Table I1).

Feeding malachite green chloride or leucomalachite green did not increase the peripheral blood micronucleus frequency (Table I2a). Positive controls for the micronucleus assay were significantly elevated (Table I2b), and their frequencies consistent with previous observations (Von Tungeln *et al.*, 2003). Analysis of variance (ANOVA) on the lymphocyte *Hprt* mutant frequency data for mice treated for 28 days indicated a significant variation among the groups (Table I3). However, this appeared to be due to a relatively low mutant frequency in mice treated with 204 ppm leucomalachite green, and Dunnett's test indicated that there were no significant differences between the mutant frequencies in any of the treated groups and the control. *Hprt* lymphocyte mutant frequencies were not significantly different from the controls after 16 weeks of treatment with malachite green chloride or leucomalachite green (Table I3).

cII mutant frequency was determined in the livers of mice fed 450 ppm malachite green chloride or 408 ppm leucomalachite green for 16 weeks (Table I4). Statistical analysis by ANOVA, followed by Dunnett's test indicated that leucomalachite green increased the liver mutant frequency while malachite green chloride did not. Sequencing of mutant clones (see below) indicated that the degree of mutant independence for the control and treated mice was similar. In this analysis, different mutations isolated from the same mouse were assumed to be induced independently; if the same mutation was isolated more than once from the same mouse, it was assumed to be derived from an expanded mutant clone, and therefore not independent. The correction of *cII* mutant frequencies for independence to derive a mutation frequency indicated that leucomalachite green also increased liver *cII* mutation frequency, while malachite green did not (Table I4). Female Big Blue rats fed 543 ppm leucomalachite green for 16 weeks were also evaluated for liver *cII* mutant frequency. There was no increase in *cII* mutant or mutation frequency in the treated rats (Table I5). This result was consistent with previous results on *lacI* mutant and mutation frequency performed with these same rat tissues (Culp *et al.*, 2002; Manjanatha *et al.*, 2004).

The liver *cII* mutants isolated from the mutant frequency assays were further analyzed for mutations in the *cII* gene (Tables I6 and I7). A high proportion of mutations from control mice (49%) contained transitions, with the most common mutation being G→A. The spectrum of mutations from mice treated with malachite green chloride was similar to that for control mice, while the mutations from mice fed leucomalachite green had increased frequencies of G→T and A→T transversions. Statistical evaluation of these spectra using the test devised by Adams and Skopek (1987) indicated that the spectrum of mutations from leucomalachite green treated mice was significantly different from the control (P = 0.04), while the spectrum for malachite green chloride treated mice was not (P = 0.47). The spectrum of mutations from rats fed 543 ppm leucomalachite green did not differ from that of control rats (P = 0.54).

DISCUSSION

Previous observations on the genotoxicity and carcinogenicity of malachite green and leucomalachite green contain a number of contradictions. Results of the carcinogenicity bioassays performed on these compounds indicate the strongest tumorigenicity response is a dose-dependent induction of liver tumors in female mice fed leucomalachite green (this Technical Report). Culp *et al.* (1999) also found that leucomalachite green forms a relatively low level of DNA adducts in mouse liver. In the present study, adduct formation in mice fed leucomalachite green was similar to that in control mice; however, the doses of leucomalachite green used in the present study were lower than those used by Culp *et al.* (1999). We interpret these results to indicate that leucomalachite green may form DNA adducts in female mice at the doses administered in the present study, but the concentration of adducts formed is close to the limit of detection of the ^{32}P-postlabeling assay.

The observation of dose-responsive liver tumors and liver DNA adducts suggests that leucomalachite green is acting as a genotoxic carcinogen in mice. However, leucomalachite green is not genotoxic *in vitro* (Culp and Beland, 1996; Fessard *et al.*, 1999; NTP, 2004), and the most compelling result indicating that it is an *in vivo* genotoxin, a weak induction of micronucleated normochromogenic erythrocytes in female mice fed leucomalachite green for one month (NTP, 2004), was not replicated in the present study. Also, *in vivo* DNA adduct formation has been observed for malachite green and leucomalachite green in the absence of tumor induction. Liver DNA adduct formation for leucomalachite green is lower in mice than in rats and lower than that of malachite green, all of which are treatments that did not result in tumors. Previous efforts to demonstrate a mutagenic response by these adducts using *in vivo* mutagenicity assays were unsuccessful (Manjanatha *et al.*, 2004). Finally, it is not clear why the tumorigenicity responses for malachite green are dissimilar to those of leucomalachite green, since they are known to be readily interconvertible *in vivo* (Culp and Beland, 1996).

The results from the present study indicate that treatment of female mice with leucomalachite green, a treatment that results in liver tumors, also results in a significant increase in *cII* liver mutant frequency and a unique mutation spectrum. The mutation spectrum in leucomalachite green treated mice involves an increase of G→T and A→T mutations, the types of mutations typical of those produced by bulky arylamine carcinogens (Heflich and Neft, 1994; Chen *et al.*, 2002). Treatments that did not result in tumors or gave equivocal results did not produce increases in *cII* liver mutant frequencies and resulted in mutation spectra similar to that found in control animals. Thus, liver *cII* mutant induction is positively correlated with liver tumor induction, which is consistent with leucomalachite green acting as a genotoxic carcinogen in the liver of female mice. The lack of lymphocyte *Hprt* mutants and micronuclei in female mice treated with leucomalachite green indicates that the genotoxicity of leucomalachite green is targeted to the tissue at risk for tumor induction.

The lack of a correlation between the concentration of DNA adducts produced by leucomalachite green and malachite green chloride and the tumorigenicity and mutagenicity of these compounds remains an unsolved problem. As previously indicated (Manjanatha *et al.*, 2004), this result may indicate that these adducts have nothing to do with the genotoxicity and tumorigenicity of these compounds. Then the question becomes, where are the adducts responsible for the mutagenicity and carcinogenicity of leucomalachite green in mouse liver? Perhaps leucomalachite green produces additional DNA adducts that are not resolved by the ^{32}P-postlabeling method used in this study (best suited for detecting bulky, aromatic adducts). Alternatively, it may be that mutations and tumors are produced as a result of a processing step that occurs only in leucomalachite green treated mouse liver. Since the evaluation of *cII* mutant independence indicates that there is no excess clonal expansion of mutations in leucomalachite green treated mice, it does not appear that this treatment produces increased cell proliferation and consequent fixation of mutations. It may be significant that the leucomalachite green treatment of female mice resulted in the lowest adduct concentration among the treatments evaluated. This may indicate that an error-prone DNA-repair process is responsible for the mutations and liver tumors produced by leucomalachite green. This hypothesis could be tested by measuring DNA adduct formation in various strains of DNA repair-deficient mice.

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A. 0 ppm
B. 408 ppm Leucomalachite Green
C. 450 ppm Malachite Green Chloride

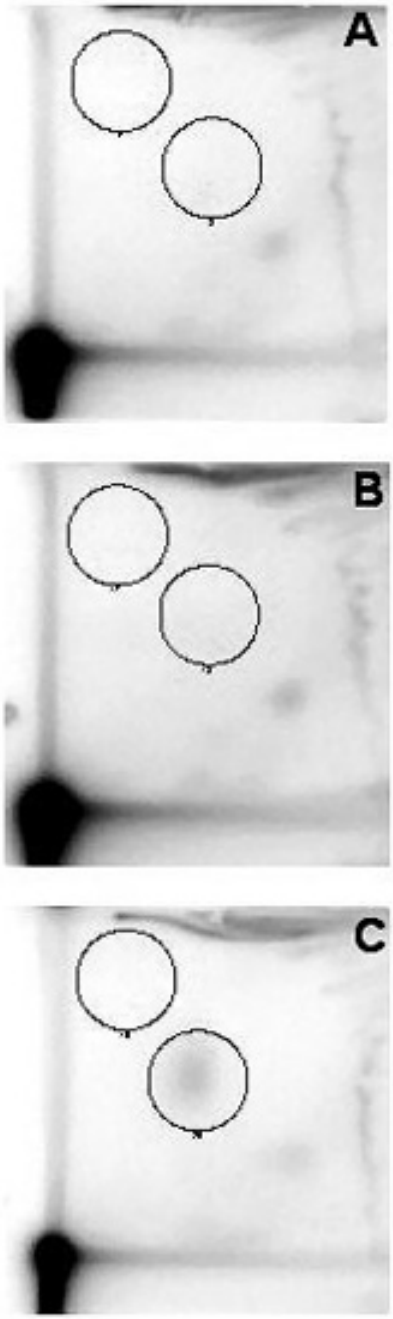

FIGURE I1
^{32}P-Phosphorimages of DNA from Female Big Blue B6C3F$_1$ Mice Exposed to Malachite Green Chloride or Leucomalachite Green in Feed for 28 Days

TABLE I1
^{32}P-Postlabeled DNA Adduct Formation in Female Big Blue B6C3F$_1$ Mice Exposed to Malachite Green Chloride or Leucomalachite Green in Feed for 28 Days[a]

	Malachite Green Chloride	Leucomalachite Green	
Control	450 ppm	204 ppm	408 ppm
0.5	2.0	0.6	0.5
0.7	2.4	0.4	0.9

[a] Data are given as DNA adducts/10^8 nucleotides, n = 1

TABLE I2a
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Female Big Blue B6C3F$_1$ Mice Exposed to Malachite Green Chloride or Leucomalachite Green in Feed for 28 Days or 16 Weeks[a]

	Control	Malachite Green Chloride 450 ppm	Leucomalachite Green 204 ppm	Leucomalachite Green 408 ppm
28-Day Exposure				
RETs (%)[b]	3.23 ± 0.17[c]	3.67 ± 0.20	3.22 ± 0.25	3.15 ± 0.12
Micronucleated RETs (%)	0.32 ± 0.03	0.24 ± 0.01	0.23 ± 0.02	0.25 ± 0.03
Micronucleated NCEs (%)	0.11 ± 0.01	0.13 ± 0.00	0.11 ± 0.01	0.11 ± 0.00
16-Week Exposure				
RETs (%)	2.87 ± 0.23	3.31 ± 0.24	3.19 ± 0.20	3.25 ± 0.13
Micronucleated RETs (%)	0.33 ± 0.02	0.29 ± 0.02	0.29 ± 0.03	0.28 ± 0.04
Micronucleated NCEs (%)	0.11 ± 0.00	0.12 ± 0.00	0.12 ± 0.01	0.11 ± 0.00

[a] n = 5 or 6, differences from the control group were not significant by ANOVA
[b] NCE=normochromogenic erythrocyte; RET=reticulocyte
[c] Mean ± standard error

TABLE I2b
Controls for Micronucleus Assay. Measurement of Micronuclei in Peripheral Blood Erythrocytes of 9-Day-Old B6C3F$_1$ Mice Treated with DMSO or Dideoxycytidine on Days 1-8[a]

	DMSO	DMSO/ Dideoxycytidine
n	5	5
Male		
RETs (%)	35.7 ± 2.20	39.3 ± 2.92
Micronucleated RETs (%)	0.34 ± 0.06	0.94 ± 0.14**
Micronucleated NCEs (%)	0.28 ± 0.03	1.65 ± 0.22***
Female		
RETs (%)	37.9 ± 3.57	37.7 ± 3.00
Micronucleated RETs (%)	0.26 ± 0.05	0.66 ± 0.14
Micronucleated NCEs (%)	0.22 ± 0.02	0.85 ± 0.14*

* Significantly different ($P \leq 0.05$) from the DMSO group by Bonferroni's t-test
** $P \leq 0.01$
*** $P \leq 0.001$
[a] NCE=normochromogenic erythrocyte, RET=reticulocyte, DMSO =dimethyl sulfoxide; data are presented as mean ± standard error. The detailed protocol for the animal treatment is presented in Von Tungeln *et al*., 2003.

TABLE I3
***Hprt* Lymphocyte Mutant Frequency in Female Big Blue B6C3F$_1$ Mice Exposed to Malachite Green Chloride or Leucomalachite Green in Feed for 28 Days or 16 Weeks[a]**

	Control	Malachite Green Chloride 450 ppm	Leucomalachite Green 204 ppm	Leucomalachite Green 408 ppm
28-Day Exposure*	0.9 ± 0.2	1.1 ± 0.3	0.3 ± 0.1	1.1 ± 0.2
16-Week Exposure	1.0 ± 0.3	1.7 ± 0.6	1.3 ± 0.4	1.2 ± 0.4

* Significant difference (P≤0.05) by ANOVA. No differences between treated groups and control by Dunnett's test.
[a] Data are given as mutant frequency × 10^{-6} (mean ± standard error); differences from the control group were not significant by Dunnett's test

TABLE I4
Liver *cII* Mutant and Mutation Frequencies in Female Big Blue B6C3F$_1$ Mice Exposed to Malachite Green Chloride or Leucomalachite Green in Feed for 16 Weeks

	Control	Malachite Green Chloride 450 ppm	Leucomalachite Green 408 ppm
n	6	6	6
Mutant frequency (× 10^{-6})	50.1 ± 4.9	66.1 ± 5.9	86.2 ± 14.1*
Independent mutations (%)	87.0 ± 2.1	82.7 ± 3.1	88.4 ± 1.9
Mutation frequency (× 10^{-6})	44.3 ± 3.8	55.0 ± 6.2	76.0 ± 12.4*

* Significantly different (P≤0.05) from the control group by Dunnett's test
[a] Data are given as mean ± standard error

TABLE I5
Liver *cII* Mutant and Mutation Frequencies in Female Big Blue F344 Rats Exposed to Leucomalachite Green in Feed for 16 Weeks[a]

	Control	543 ppm
n	6	6
Mutant frequency ($\times 10^{-6}$)	55.1 ± 11.0	55.0 ± 6.8
Independent mutations (%)	87.9 ± 7.0	85.0 ± 3.7
Mutation frequency ($\times 10^{-6}$)	45.0 ± 4.5	46.7 ± 5.9

[a] Data are given as mean ± standard error. The detailed protocol for the animal treatment is presented by Culp *et al.* (2002) and Manjanatha *et al.* (2004).

TABLE I6
Spectrum of Mutations in the *cII* Gene of Female Big Blue B6C3F$_1$ Mice Exposed to Malachite Green Chloride or Leucomalachite Green in Feed for 16 Weeks[a]

Sequence Alterations	Control	Malachite Green Chloride 450 ppm	Leucomalachite Green 408 ppm
Transitions			
G→A	26 (39%)	35 (43%)	32 (33%)
A→G	7 (10%)	3 (4%)	3 (3%)
Transversions			
G→T	12 (17%)	19 (23%)	28 (29%)
G→C	7 (10%)	10 (12%)	11 (11%)
A→T	1 (1%)	0	7 (7%)
A→C	4 (6%)	2 (2%)	2 (2%)
± G Frameshifts	8 (12%)	7 (9%)	9 (9%)
Others	2 (3%)	5 (6%)	4 (4%)
Total	67	81	97
		P=0.47[b]	P=0.04

[a] Data presented as number of mutations (%)
[b] Comparison (Adams and Skopek, 1987) of the control group spectrum to the exposed group spectrum

TABLE I7
Spectrum of Mutations in the *cII* Gene of Female Big Blue F344 Rats Exposed to Leucomalachite Green in Feed for 16 Weeks[a]

	Control	543 ppm
Transitions		
G→A	26 (49%)	38 (51%)
A→G	1 (2%)	6 (8%)
Transversions		
G→T	9 (17%)	10 (14%)
G→C	4 (8%)	6 (8%)
A→T	2 (4%)	3 (4%)
A→C	1 (2%)	3 (4%)
± G Frameshifts	8 (15%)	5 (7%)
Others	0	3 (4%)
Total	53	74
		P=0.54[b]

[a] Data are presented as number of mutations (%). The detailed protocol for the animal treatments is presented by Culp *et al.* (2002) and Manjanatha *et al.* (2004).
[b] Comparison (Adams and Skopek, 1987) of the exposed group spectrum and the control group spectrum

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Chemical	TR No.
Acetaminophen	394
Acetonitrile	447
Acrylonitrile	506
Agar	230
Allyl Glycidyl Ether	376
Allyl Isothiocyanate	234
Allyl Isovalerate	253
1-Amino-2,4-Dibromoanthraquinone	383
2-Amino-4-Nitrophenol	339
2-Amino-5-Nitrophenol	334
11-Aminoundecanoic Acid	216
dl-Amphetamine Sulfate	387
Ampicillin Trihydrate	318
Asbestos, Amosite (Hamsters)	249
Asbestos, Amosite (Rats)	279
Asbestos, Chrysotile (Hamsters)	246
Asbestos, Chrysotile (Rats)	295
Asbestos, Crocidolite	280
Asbestos, Tremolite	277
L-Ascorbic Acid	247
AZT and AZT/α-Interferon A/D	469
Barium Chloride Dihydrate	432
Benzaldehyde	378
Benzene	289
Benzethonium Chloride	438
Benzofuran	370
Benzyl Acetate (Gavage)	250
Benzyl Acetate (Feed)	431
Benzyl Alcohol	343
o-Benzyl-*p*-Chlorophenol (Gavage)	424
o-Benzyl-*p*-Chlorophenol (Mouse Skin)	444
2-Biphenylamine Hydrochloride	233
2,2-Bis(Bromomethyl)-1,3-Propanediol	452
Bis(2-Chloro-1-Methylethyl) Ether	239
Bisphenol A	215
Boric Acid	324
Bromodichloromethane	321
Bromoethane	363
1,3-Butadiene	288
1,3-Butadiene	434
t-Butyl Alcohol	436
Butyl Benzyl Phthalate	213
Butyl Benzyl Phthalate	458
n-Butyl Chloride	312
t-Butylhydroquinone	459
γ-Butyrolactone	406
Caprolactam	214
d-Carvone	381
Chloral Hydrate	502
Chloral Hydrate	503
Chlorinated and Chloraminated Water	392
Chlorendic Acid	304
Chlorinated Paraffins: C_{23}, 43% Chlorine	305
Chlorinated Paraffins: C_{12}, 60% Chlorine	308
Chlorinated Trisodium Phosphate	294
2-Chloroacetophenone	379
p-Chloroaniline Hydrochloride	351
Chlorobenzene	261
Chlorodibromomethane	282
Chloroethane	346
2-Chloroethanol	275
3-Chloro-2-Methylpropene	300
Chloroprene	467
1-Chloro-2-Propanol	477
Chlorpheniramine Maleate	317
C.I. Acid Orange 3	335
C.I. Acid Orange 10	211
C.I. Acid Red 14	220
C.I. Acid Red 114	405
C.I. Basic Red 9 Monohydrochloride	285
C.I. Direct Blue 15	397
C.I. Direct Blue 218	430
C.I. Disperse Blue 1	299
C.I. Disperse Yellow 3	222
C.I. Pigment Red 3	407
C.I. Pigment Red 23	411
C.I. Solvent Yellow 14	226
trans-Cinnamaldehyde	514
Citral	505
Cobalt Sulfate Heptahydrate	471
Coconut Oil Acid Diethanolamine Condensate	479
Codeine	455
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Corn Oil, Safflower Oil, and Tricaprylin	426
Coumarin	422
CS2	377
Cytembena	207
D&C Red No. 9	225
D&C Yellow No. 11	463
Decalin	513
Decabromodiphenyl Oxide	309
Diallyl Phthalate (Mice)	242
Diallyl Phthalate (Rats)	284
4,4′-Diamino-2,2′-Stilbenedisulfonic Acid, Disodium Salt	412
2,4-Diaminophenol Dihydrochloride	401
1,2-Dibromo-3-Chloropropane	206
1,2-Dibromoethane	210
2,3-Dibromo-1-Propanol	400
1,2-Dichlorobenzene (*o*-Dichlorobenzene)	255
1,4-Dichlorobenzene (*p*-Dichlorobenzene)	319
p,p′-Dichlorodiphenyl sulfone	501
2,4-Dichlorophenol	353
2,6-Dichloro-*p*-Phenylenediamine	219
1,2-Dichloropropane	263
1,3-Dichloropropene (Telone II)	269
Dichlorvos	342
Dietary Restriction	460
Diethanolamine	478
Di(2-Ethylhexyl) Adipate	212
Di(2-Ethylhexyl) Phthalate	217
Diethyl Phthalate	429
Diglycidyl Resorcinol Ether	257
3,4-Dihydrocoumarin	423
1,2-Dihydro-2,2,4-Trimethylquinoline (Monomer)	456
Dimethoxane	354
3,3′-Dimethoxybenzidine Dihydrochloride	372
N,N-Dimethylaniline	360
3,3′-Dimethylbenzidine Dihydrochloride	390
Dimethyl Hydrogen Phosphite	287
Dimethyl Methylphosphonate	323
Dimethyl Morpholinophosphoramidate	298
Dimethylvinyl Chloride	316
Diphenhydramine Hydrochloride	355
5,5-Diphenylhydantoin	404
Dipropylene Glycol	511
Elmiron®	512
Emodin	493
Ephedrine Sulfate	307
Epinephrine Hydrochloride	380
1,2-Epoxybutane	329
Erythromycin Stearate	338
Ethyl Acrylate	259

Chemical	TR No.
Ethylbenzene	466
Ethylene Glycol	413
Ethylene Glycol Monobutyl Ether	484
Ethylene Oxide	326
Ethylene Thiourea	388
Eugenol	223
FD&C Yellow No. 6	208
Fumonisin B_1	496
Furan	402
Furfural	382
Furfuryl Alcohol	482
Furosemide	356
Gallium Arsenide	492
Geranyl Acetate	252
Glutaraldehyde	490
Glycidol	374
Guar Gum	229
Gum Arabic	227
HC Blue 1	271
HC Blue 2	293
HC Red 3	281
HC Yellow 4	419
Hexachlorocyclopentadiene	437
Hexachloroethane	361
2,4-Hexadienal	509
4-Hexylresorcinol	330
Hydrochlorothiazide	357
Hydroquinone	366
8-Hydroxyquinoline	276
Indium Phosphide	499
Iodinated Glycerol	340
Isobutene	487
Isobutyl Nitrite	448
Isobutyraldehyde	472
Isophorone	291
Isoprene	486
Lauric Acid Diethanolamine Condensate	480
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Magnetic Field Promotion	489
Malachite Green Chloride and Leucomalachite Green	527
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Manganese Sulfate Monohydrate	428
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Marine Diesel Fuel and JP-5 Navy Fuel	310
Melamine	245
2-Mercaptobenzothiazole	332
Mercuric Chloride	408
Methacrylonitrile	497
8-Methoxypsoralen	359
α-Methylbenzyl Alcohol	369
Methyl Bromide	385
Methyl Carbamate	328
Methyldopa Sesquihydrate	348
Methylene Chloride	306
4,4′-Methylenedianiline Dihydrochloride	248
Methyleugenol	491
2-Methylimidazole	516
Methyl Methacrylate	314
N-Methylolacrylamide	352
Methylphenidate Hydrochloride	439
Mirex	313
Molybdenum Trioxide	462
Monochloroacetic Acid	396
Monuron	266
Nalidixic Acid	368
Naphthalene (Mice)	410
Naphthalene (Rats)	500
Nickel (II) Oxide	451
Nickel Sulfate Hexahydrate	454
Nickel Subsulfide	453

Chemical	TR No.
p-Nitroaniline	418
o-Nitroanisole	416
p-Nitrobenzoic Acid	442
Nitrofurantoin	341
Nitrofurazone	337
Nitromethane	461
p-Nitrophenol	417
o-Nitrotoluene	504
p-Nitrotoluene	498
Ochratoxin A	358
Oleic Acid Diethanolamine Condensate	481
Oxazepam (Mice)	443
Oxazepam (Rats)	468
Oxymetholone	485
Oxytetracycline Hydrochloride	315
Ozone and Ozone/NNK	440
Penicillin VK	336
Pentachloroanisole	414
Pentachloroethane	232
Pentachloronitrobenzene	325
Pentachlorophenol, Purified	483
Pentachlorophenol, Technical Grade	349
Pentaerythritol Tetranitrate	365
Phenolphthalein	465
Phenylbutazone	367
Phenylephrine Hydrochloride	322
N-Phenyl-2-Naphthylamine	333
o-Phenylphenol	301
Polybrominated Biphenyl Mixture (Firemaster FF-1) (Gavage)	244
Polybrominated Biphenyl Mixture (Firemaster FF-1) (Feed)	398
Polysorbate 80 (Glycol)	415
Polyvinyl Alcohol	474
Primidone	476
Probenecid	395
Promethazine Hydrochloride	425
Propylene	272
Propylene Glycol Mono-*t*-butyl Ether	515
1,2-Propylene Oxide	267
Propyl Gallate	240
Pyridine	470
Quercetin	409
Riddelliine	508
Resorcinol	403
Rhodamine 6G	364
Rotenone	320
Roxarsone	345
Salicylazosulfapyridine	457
Scopolamine Hydrobromide Trihydrate	445
Sodium Azide	389
Sodium Fluoride	393
Sodium Nitrite	495
Sodium Xylenesulfonate	464
Stannous Chloride	231
Stoddard Solvent IIC	519
Succinic Anhydride	373
Talc	421
Tara Gum	224
2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (Dermal)	201
2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (Gavage)	209
1,1,1,2-Tetrachloroethane	237
Tetrachloroethylene	311
Tetracycline Hydrochloride	344
Tetrafluoroethylene	450
1-Trans-Delta^9-Tetrahydrocannabinol	446
Tetrahydrofuran	475
Tetrakis(Hydroxymethyl)Phosphonium Sulfate	296
Tetrakis(Hydroxymethyl)Phosphonium Chloride	296
Tetranitromethane	386
Theophylline	473
4,4-Thiobis(6-*t*-Butyl-*m*-Cresol)	435
Titanocene Dichloride	399
Toluene	371

Chemical	TR No.
2,4- & 2,6-Toluene Diisocyanate	251
Triamterene	420
Tribromomethane	350
Trichloroethylene	243
Trichloroethylene	273
1,2,3-Trichloropropane	384
Tricresyl Phosphate	433
Triethanolamine	449
Triethanolamine	518
Tris(2-Chloroethyl) Phosphate	391
Tris(2-Ethylhexyl) Phosphate	274
Turmeric Oleoresin (Curcumin)	427
Urethane, Ethanol, and Urethane/Ethanol	510
Vanadium Pentoxide	507
4-Vinylcyclohexene	303
4-Vinyl-1-Cyclohexene Diepoxide	362
Vinylidene Chloride	228
Vinyl Toluene	375
Xylenes (Mixed)	327
2,6-Xylidine	278
Zearalenone	235
Ziram	238