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TOXICOLOGY AND CARCINOGENESIS STUDIES OF C.I. ACID ORANGE 3

(CAS NO. 6373-74-6)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

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

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF C.I. ACID ORANGE 3

(CAS NO. 6373-74-6)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES

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NOTE TO THE READER

This study was performed under the direction of the National Institute of Environmental Health Sciences as a function of the National Toxicology Program. The studies described in this Technical Report have been conducted in compliance with NTP chemical 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 U.S. Public Health Service Policy on Humane Care and Use of Animals. All NTP toxicology and carcinogenesis studies are subjected to a data audit before being presented for public peer review.

Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to identify any mistakes so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in this report is encouraged to make this information known to the NTP. Comments and questions about the National Toxicology Program Technical Reports on Toxicology and Carcinogenesis Studies should be directed to Dr. J.E. Huff, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3780).

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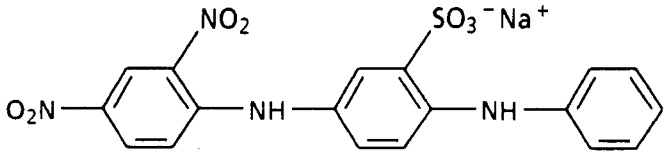

C.I. ACID ORANGE 3

CAS No. 6373-74-6

$C_{18}H_{13}N_4O_7SNa$ Molecular weight 452

Synonyms: 2-anilino-5-(2,4-dinitroanilino)-benzenesulfonic acid, monosodium salt; 5[(2,4-dinitrophenol)amine]-2-(phenylamine)-benzenesulfonic acid, monosodium salt; C.I. 10385; Tetracid Light Yellow 2R.

ABSTRACT

C.I. Acid Orange 3 is a dinitrodiphenylamine derivative used exclusively as a dye (up to 0.2%) in semipermanent hair coloring products. This study was one of a series on semipermanent hair dyes, which included HC Blue No. 1 (NTP TR 271), HC Blue No. 2 (NTP TR 293), HC Red No. 3 (NTP TR 281), and C.I. Disperse Blue 1 (NTP TR 299). Toxicology and carcinogenesis studies of C.I. Acid Orange 3 (90% pure, containing 10% water for short-term studies and containing 6%-8% water and 2%-4% acetone for 2-year studies) were conducted by administering the dye in corn oil by gavage to F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies (at 94-1,500 mg/kg in rats and 62-1,000 mg/kg in mice), no compound-related deaths or body weight changes were observed and no adverse effects were observed at necropsy.

In the 13-week studies (at 94-1,500 mg/kg in rats and 31-2,000 mg/kg in mice), compound-related kidney lesions were observed in rats and mice of each sex. These lesions included variable degrees of degeneration and necrosis of epithelial cells in the proximal convoluted tubules, regeneration of tubular epithelium, and granular casts in the tubules. In a few female rats of the highest dose group, necrosis of the renal papilla and suppurative inflammation were also observed. Mean body weights were generally comparable among groups of rats and mice. Mice receiving 2,000 mg/kg had body weights 11%-12% lower than those of vehicle controls. Five of 10 female rats that received the highest dose of 1,500 mg/kg died before the end of the study, but no compound-related deaths occurred in male rats or mice of either sex.

Based on these results, 2-year studies of C.I. Acid Orange 3 were conducted by administering the dye by gavage in corn oil at 0, 375, or 750 mg/kg to groups of 50 F344/N rats of each sex, 5 days per week for 103 weeks. Groups of 50 male B6C3F$_1$ mice were administered 0, 125, or 250 mg/kg C.I. Acid Orange 3 on the same schedule, and groups of 50 female B6C3F$_1$ mice were administered 0, 250, or 500 mg/kg. These doses were selected on the basis of the nature and severity of the renal lesions in both species.

Body Weights and Survival in the Two-Year Studies: Mean body weights of high dose rats were generally more than 10% lower than those of vehicle controls after week 52 for males and week 70 for females. Mean body weights for low dose groups were comparable to those of vehicle controls. The survival of high dose male (after week 33) and female (after week 14) rats was lower ($P<0.05$) than that of vehicle controls and was attributed to nephrotoxicity (final survival--male: vehicle control, 36/50;

low dose, 30/50; high dose, 0/50; female: 43/50; 34/50; 7/50). Mean body weights of dosed male and female mice were lower than those of vehicle controls (high dose, 5%-11% after week 74; low dose, 7%-17% after week 48). Survival of both the low dose (after week 102) and high dose (after week 100) groups of male mice was lower than that of the vehicle controls (final survival: 38/50; 25/50; 26/50). Although survival was lower than usual, no notable differences in survival were observed between groups of female mice (final survival: 23/50; 23/50; 24/50).

Nonneoplastic and Neoplastic Lesions in the Two-Year Studies: For both species, the kidney was the major target organ for C.I. Acid Orange 3. These findings are summarized in the accompanying table. The incidences of renal pelvic epithelial hyperplasia were increased in dosed rats of each sex. No renal neoplasms were observed in dosed male rats, but a tubular cell adenocarcinoma was observed in a vehicle control male rat. Six transitional cell carcinomas of the kidney were observed in high dose female rats; kidney transitional cell neoplasms have not been observed in 1,697 corn oil vehicle control female F344/N rats.

Nonneoplastic lesions characteristic of secondary renal hyperparathyroidism or secondary to uremia also occurred in dosed rats. These lesions included parathyroid hyperplasia, fibrous dysplasia of bone, erosion and ulcers of the glandular stomach, and mineralization of the aorta and glandular stomach.

Epithelial hyperplasia of the urinary bladder was observed in one low dose and three high dose female mice. A squamous cell carcinoma was seen in the urinary bladder of one low dose female mouse. Even though no squamous cell urinary bladder neoplasms have been observed in 1,665 corn oil vehicle control female B6C3F$_1$ mice, this single neoplasm in a low dose animal was not considered to be related to the administration of C.I. Acid Orange 3.

SUMMARY OF KIDNEY LESIONS IN MALE AND FEMALE F344/N RATS AND B6C3F$_1$ MICE IN THE TWO-YEAR GAVAGE STUDIES OF C.I. ACID ORANGE 3

Lesion	Male			Female		
	Vehicle Control	Low Dose	High Dose	Vehicle Control	Low Dose	High Dose
RATS		**375 mg/kg**	**750 mg/kg**		**375 mg/kg**	**750 mg/kg**
No. of animals examined	50	50	50	50	50	50
Nephropathy	50	50	49	23	(a) 45	(a) 48
Papillary necrosis	0	1	1	0	0	(a) 10
Suppurative inflammation	7	(a) 37	(a) 44	0	(a) 10	(a) 45
Pigmentation	4	4	(a) 39	0	0	(b) 5
Pelvic epithelial hyperplasia	0	(b) 6	(a) 13	0	2	(a) 13
Transitional cell carcinoma	0	0	0	0	0	(a) 6
MICE		**125 mg/kg**	**250 mg/kg**		**250 mg/kg**	**500 mg/kg**
No. of animals examined	50	50	50	50	50	50
Nephrosis	47	47	45	13	(a) 42	(a) 50
Fibrosis	0	(b) 5	(a) 19	4	9	(a) 31
Inflammation	1	4	(a) 12	7	7	(a) 22
Papillary degeneration	0	4	(a) 18	0	3	(a) 19
Medullary (papillary) necrosis	0	0	(b) 6	2	5	(b) 8
Tubular dilatation	2	(a) 39	(a) 33	2	(a) 35	(a) 42
Tubular mineralization	31	20	25	3	(a) 15	(a) 22
Lymphoid hyperplasia	18	(a) 35	(a) 33	20	24	29

(a) $P<0.01$ vs. vehicle control
(b) $P<0.05$ vs. vehicle control

Genetic Toxicology: C.I. Acid Orange 3 was mutagenic with and without exogenous metabolic activation in *Salmonella typhimurium* strains TA97, TA98, and TA100; no mutagenicity was observed for strain TA1535.

Audit: The data, documents, and pathology materials from the 2-year studies of C.I. Acid Orange 3 have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of C.I. Acid Orange 3 for male F344/N rats administered 375 mg/kg; because of a marked reduction in survival and no indication of carcinogenicity, the 750 mg/kg group was considered to be inadequate for assessment of carcinogenic activity. There was *clear evidence of carcinogenic activity* of C.I. Acid Orange 3 for female F344/N rats as shown by the occurrence of transitional cell carcinomas of the kidney in the 750 mg/kg group; this group had reduced survival and chemically related nonneoplastic lesions of the kidney. There was *no evidence of carcinogenic activity* of C.I. Acid Orange 3 for male B6C3F_1 mice administered 125 or 250 mg/kg or for female B6C3F_1 mice administered 250 or 500 mg/kg. Nonneoplastic lesions of the kidney were observed in both dose groups of both sexes of rats and mice.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 9.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 12.

SUMMARY OF THE TWO-YEAR GAVAGE AND GENETIC TOXICOLOGY STUDIES OF C.I. ACID ORANGE 3

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses			
0, 375, or 750 mg/kg C.I. Acid Orange 3 in corn oil, 5 d/wk	0, 375, or 750 mg/kg C.I. Acid Orange 3 in corn oil, 5 d/wk	0, 125, or 250 mg/kg C.I. Acid Orange 3 in corn oil, 5 d/wk	0, 250, or 500 mg/kg C.I. Acid Orange 3 in corn oil, 5 d/wk
Body weights in the 2-year study			
High dose lower than controls	High dose lower than controls	Dosed lower than controls	Dosed lower than controls
Survival rates in the 2-year study			
36/50; 30/50; 0/50	43/50; 34/50; 7/50	38/50; 25/50; 26/50	23/50; 23/50; 24/50
Nonneoplastic effects			
Suppurative inflammation and pigmentation of the kidney; epithelial hyperplasia of the renal pelvis	Suppurative inflammation and pigmentation of the kidney; epithelial hyperplasia of the renal pelvis; nephropathy and necrosis of the renal papilla	Renal inflammation, fibrosis, and necrosis; degeneration of the renal papilla; renal tubule dilatation	Renal inflammation, fibrosis, and necrosis; degeneration of the renal papilla; renal tubule dilatation; nephrosis and renal tubule mineralization
Neoplastic effects			
None	Transitional cell carcinomas of the renal pelvis	None	None
Level of evidence of carcinogenic activity			
No evidence	Clear evidence	No evidence	No evidence
Other considerations			
The study in the 750 mg/kg group was inadequate for assessment of carcinogenic activity because of reduced survival			
Genetic toxicology			
Mutagenic with and without S9 in *S. typhimurium* strains TA97, TA98, and TA100 but not TA1535			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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. Selection per se is not an indicator of a chemical's carcinogenic potential.

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.

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. 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 because of major flaws cannot be evaluated ("Inadequate Study"). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Reports 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 quintet is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either 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 chemically 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 chemically related.
- **No Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing no chemically 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.

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. This 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:

- The 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 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 incidences 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);
- The presence or absence of dose relationships;
- The statistical significance of the observed tumor increase;
- The 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.

These considerations together with the definitions as written should be used as composite guidelines for selecting one of the five categories. Additionally, the following concepts (as patterned from the International Agency for Research on Cancer Monographs) have been adopted by the NTP to give further clarification of these issues:

> The term *chemical carcinogenesis* generally means the induction by chemicals of neoplasms not usually observed, the induction by chemicals of more neoplasms than are generally found, or the earlier induction by chemicals of neoplasms that are commonly observed. Different mechanisms may be involved in these situations. Etymologically, the term *carcinogenesis* means induction of cancer, that is, of malignant neoplasms; however, the commonly accepted meaning is the induction of various types of neoplasms or of a combination of malignant and benign neoplasms. In the Technical Reports, the words *tumor* and *neoplasm* are used interchangeably.

CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of C.I. Acid Orange 3 is based on the 13-week studies that began in July 1979 or March 1980 and ended in October 1979 or June 1980 and on 2-year studies that began in October 1980 for rats or December 1980 for mice and ended in October 1982 for rats or December 1982 for mice at Southern Research Institute (Birmingham, Alabama).

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The members of the Peer Review Panel who evaluated the draft Technical Report on C.I. Acid Orange 3 on July 14, 1987, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenicity and other observed toxic responses.

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SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF C.I. ACID ORANGE 3

On July 14, 1987, the draft Technical Report on the toxicology and carcinogenesis studies of C.I. Acid Orange 3 received peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Dr. J.H. Mennear, NIEHS, introduced the toxicology and carcinogenesis studies by reviewing the experimental design, results, and proposed conclusions (no evidence of carcinogenic activity for male rats; clear evidence of carcinogenic activity for female rats; no evidence of carcinogenic activity for male or female mice).

Dr. Gallo, a principal reviewer, agreed with the conclusions. He thought that the increased incidences of rare renal tumors in female rats allowed an opportunity to study mechanisms without having to evoke the caveats for such tumors in male rats. He questioned use of the oral route for studying a chemical used almost exclusively as a hair dye.

As a second principal reviewer, Dr. Popp agreed with the conclusions for male rats and male and female mice. He stated that the high dose for rats of both sexes was clearly excessive. He felt that the renal toxicity and poor survival in female rats tended to confound interpretation of the renal tumors in females.

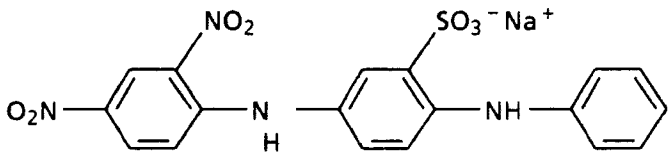
As a third principal reviewer, Dr. Mirer agreed with the conclusions and agreed that the high mortality in male rats reduced the sensitivity of the study to detect a typically late-appearing carcinogenic response. He suggested that the presence of kidney toxicity in all four experiments, contrasted with the occurrence of tumors in only one experiment (female rats), argued against a coupling of toxicity and tumorigenesis. Dr. Mirer asked for clarification of the impurities present in the technical-grade chemical used in the studies. Dr. Mennear replied that the commercial product received contained 33% impurities, including water, wetting agents, and surfactants. These impurities were not analyzed but were removed by solvent extraction, leaving 10% impurities, these being the acetone and water used in the extraction procedure.

Dr. Gallo moved that the Technical Report on C.I. Acid Orange 3 be accepted with the revisions discussed and with the conclusions as written for male rats and male and female mice, no evidence of carcinogenic activity, and for female rats, clear evidence of carcinogenic activity. Dr. Mirer seconded the motion, which was approved by five votes to two (Dr. Hooper and Dr. Popp) with one abstention (Dr. Ashby).

I. INTRODUCTION

Use and Production
Metabolism
Toxicity, Teratogenicity, and Effects on Reproduction
Epidemiology
Mutagenicity
Study Rationale

I. INTRODUCTION



C.I. ACID ORANGE 3

CAS No. 6373-74-6

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Synonyms: 2-anilino-5-(2,4-dinitroanilino)-benzenesulfonic acid, monosodium salt; 5[(2,4-dinitrophenol)amine]-2-(phenylamine)-benzenesulfonic acid, monosodium salt; C.I. 10385; Tetracid Light Yellow 2R.

Use and Production

C.I. Acid Orange 3 is a dinitrodiphenylamine derivative used exclusively as a dye in semipermanent hair color products that are generally shampooed into the hair, lathered, and then allowed to remain in contact with the hair and scalp for 30-45 minutes (Frenkel and Brody, 1973). At the concentrations (up to 0.2%) used in these preparations, C.I. Acid Orange 3 is in solution. In the United States, approximately 1,500 kg of C.I. Acid Orange 3 was used in 1984 (personal communication from Clairol, Inc., to J. Mennear, NTP).

Metabolism

No studies have been published on the dermal absorption, distribution, metabolism, or excretion of C.I. Acid Orange 3.

Toxicity, Teratogenicity, and Effects on Reproduction

C.I. Acid Orange 3 was administered to laboratory animals in studies of complex mixtures of dyes, dye intermediates, and product base chemicals (solvents and detergents). Wernick et al. (1975) administered a composite of 15 semipermanent hair dyes formulated in product base materials to dogs, rats, and rabbits. The composite, which was 6.95% dye chemicals including 0.24% C.I. Acid Orange 3, was tested for systemic effects in beagle dogs (administration in feed for 2 years), for teratologic effects in Sprague Dawley rats (administration in feed on days 6-15 of gestation) and New Zealand rabbits (administration by gavage on days 6-18 of gestation), and for reproductive effects in Sprague Dawley rats (administration in feed). The largest doses of C.I. Acid Orange 3 delivered by the mixture were 0.24 mg/kg per day to dogs and rabbits and 1.92 mg/kg per day (estimated) to rats. No compound-related effects were observed.

Burnett et al. (1976) studied a formulation of 13 dyes and dye intermediates and 8 base chemicals. This mixture, 0.2% of which was C.I. Acid Orange 3, was applied to the shaved skin of New Zealand white rabbits (1.0 ml/kg twice weekly for 13 weeks) and to pregnant Charles River rats (2.9 ml/kg on days 1, 4, 7, 13, 16, and 19 of gestation). Systemic or teratologic effects were not observed.

Epidemiology

Epidemiologic information relating the incidence of various human cancers to either employment as a hairdresser or personal use of hair dyes was evaluated as inconclusive in a monograph on aromatic amines, including hair dye preparations, published by the International Agency for Research on Cancer (IARC, 1982).

Mutagenicity

C.I. Acid Orange 3 was mutagenic in *Salmonella typhimurium* strains TA97, TA98, and TA100 when tested in a preincubation protocol in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster

liver S9; these three strains, which are deficient in DNA excision repair capabilities, demonstrate enhanced error-prone repair of damaged DNA (Appendix E, Table E1). No increase in revertant colonies was observed in strain TA1535, which lacks both error-prone DNA repair and excision repair capabilities. Additional in vitro assays for induction of gene mutations, sister chromatid exchanges, and chromosomal aberrations in mammalian cells are in progress.

Study Rationale

C.I. Acid Orange 3 is one of five semipermanent hair dyes selected for toxicology and carcinogenesis assessment. HC Blue No. 1 (NTP, 1985a), HC Blue No. 2 (NTP, 1985b), C.I. Disperse Blue 1 (NTP, 1986a), and HC Red No. 3 (NTP, 1986b) have already been evaluated in oral administration studies. C.I. Acid Orange 3 does not bear a close structural relationship to the other four dyes, but all five have the potential of being metabolized to aromatic amines. HC Blue No. 1 caused an increase in hepatocellular neoplastic nodules and carcinomas in male rats, alveolar/bronchiolar adenomas or carcinomas in female rats, hepatocellular carcinomas in male and female mice, and thyroid gland follicular cell adenomas in male mice. HC Blue No. 2 did not cause increased incidences of any neoplasms in either rats or mice. C.I. Disperse Blue 1 caused increased incidences of transitional cell papillomas and carcinomas, leiomyomas and leiomyosarcomas, and squamous cell papillomas and carcinomas of the urinary bladder in male and female rats and a marginal increase in the incidence of hepatocellular adenomas or carcinomas in male mice; there was no evidence of carcinogenicity for female mice. HC Red No. 3 produced a marginal increase in the incidence of hepatocellular adenomas or carcinomas (combined) in male mice, but the study in female mice was considered to be inadequate for the assessment of carcinogenicity because of poor survival. HC Red No. 3 did not cause an increase in the incidence of neoplasms in rats of either sex.

All dyes in this series of studies were nominated by the National Cancer Institute and were administered by the oral route to maximize systemic exposure.

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF C.I. ACID ORANGE 3

PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES

FOURTEEN-DAY STUDIES

FIRST THIRTEEN-WEEK STUDIES

SECOND THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

- Study Design
- Source and Specifications of Animals
- Animal Maintenance
- Clinical Examinations and Pathology
- Statistical Methods

PROCUREMENT AND CHARACTERIZATION OF C.I. ACID ORANGE 3

The C.I. Acid Orange 3 used for these studies was obtained in two lots from Clairol Research Laboratories (Table 1). Purity and identity analyses were conducted at Midwest Research Institute (MRI) (Kansas City, Missouri). MRI reports on the analyses performed in support of the C.I. Acid Orange 3 studies are on file at NIEHS.

Initial analysis of lot no. C122881 by ultraviolet spectroscopy indicated that it was a formulated product and that it contained only 67% C.I. Acid Orange 3 compared with a reference standard. This lot was subsequently purified to technical-grade dye specifications by Soxhlet extraction of the bulk chemical with acetone. The extract was dried in a vacuum oven at 40° C for 24 hours. The solid was then ground to a fine powder in a mortar with a pestle. All subsequent analyses were performed on the purified material. This purification was performed so that the second batch of study material would be similar in purity to the initial batch.

Both lots of study material were identified as C.I. Acid Orange 3 by spectral analysis. The infrared spectra were consistent with that expected for the structure and with that in the literature (Sadtler Standard Spectra) (see Figure 1 for a representative spectrum). The nuclear magnetic resonance spectra were consistent with a spectrum provided by the supplier and were also consistent with the structure of C.I. Acid Orange 3 (see Figure 2 for a representative spectrum). The ultraviolet spectra were consistent with the structure and were comparable to the molar absorptivity values at 355 nm provided by the supplier for a purified and a typical commercial batch of the dye.

TABLE 1. IDENTITY AND SOURCE OF C.I. ACID ORANGE 3 USED IN THE GAVAGE STUDIES

Fourteen-Day Studies	First Thirteen-Week Studies	Second Thirteen-Week Studies	Two-Year Studies (a)
Lot Number			
0095-130-3	0095-130-3	0095-130-3	0095-130-3; C122881
Date of Initial Use			
4/25/79	7/6/79	3/25/80	Lot no. 0095-130-3: rats--10/16/80; mice--12/9/80; lot no. C122881: 2/4/82
Supplier			
Clairol Research Laboratories (New York, NY)	Clairol Research Laboratories (New York, NY)	Clairol Research Laboratories (New York, NY)	Clairol Research Laboratories (New York, NY)

(a) Lot no. C122881 was purified at MRI by Soxhlet extraction with acetone.

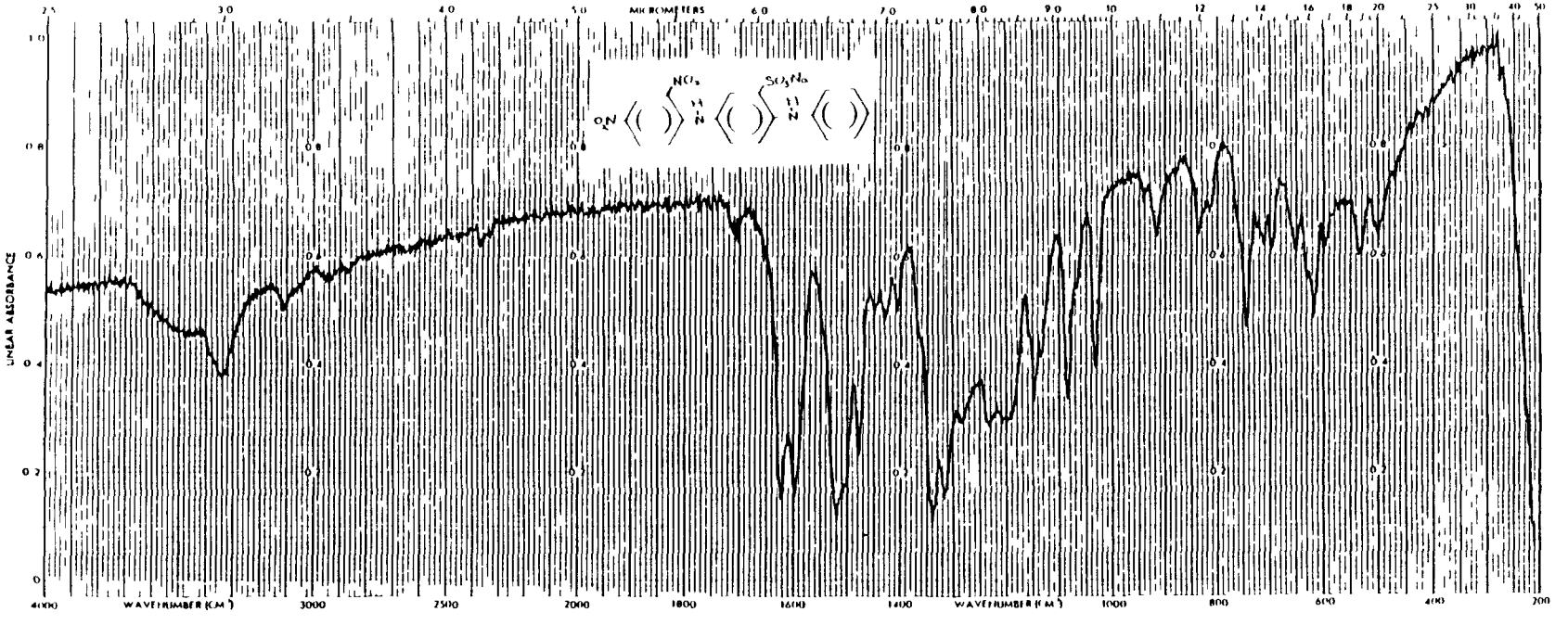
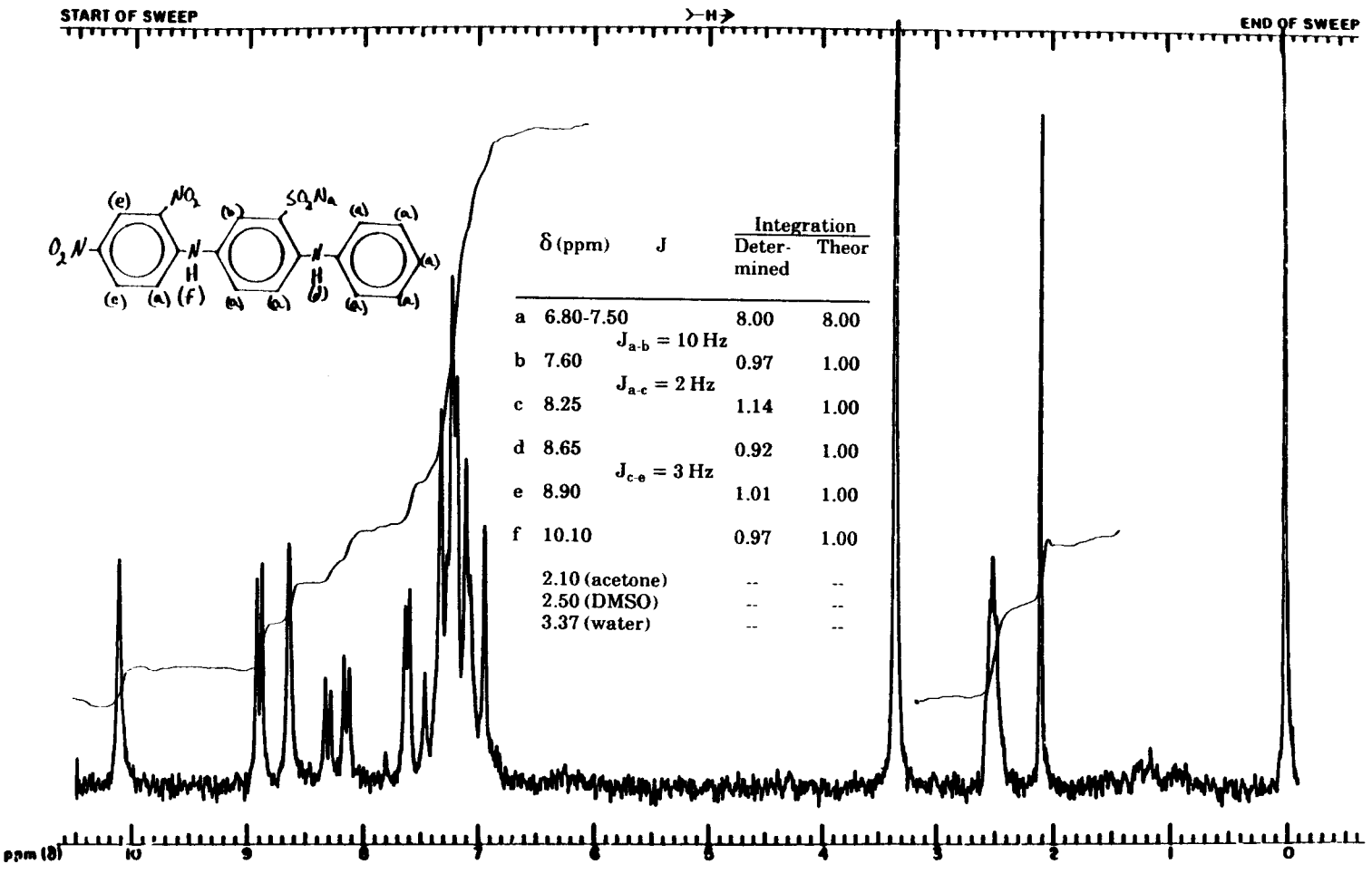


FIGURE 1. INFRARED ABSORPTION SPECTRUM OF C.I. ACID ORANGE 3 (PURIFIED LOT NO. C122881)



	δ (ppm)	J	Integration Determined	Integration Theor
a	6.80-7.50		8.00	8.00
		J_{a-b} = 10 Hz		
b	7.60		0.97	1.00
		J_{a-c} = 2 Hz		
c	8.25		1.14	1.00
d	8.65		0.92	1.00
		J_{c-e} = 3 Hz		
e	8.90		1.01	1.00
f	10.10		0.97	1.00
	2.10 (acetone)		--	--
	2.50 (DMSO)		--	--
	3.37 (water)		--	--

FIGURE 2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF C.I. ACID ORANGE 3 (PURIFIED LOT NO. C122881)

Both lots of study material were dark orange-brown microcrystals. Results of purity analyses confirm that the study materials were of technical-grade purity. Elemental analysis of the study material for carbon, nitrogen, sulfur, and hydrogen were consistent with the molecular formula of C.I. Acid Orange 3. The two lots were analyzed for water content by Karl Fischer analysis and for purity by high-performance liquid chromatography (HPLC) with a μBondapak C_{18} column, a mobile phase of 5mM tetrabutylammonium hydroxide in water (pH 7.4):5mM tetrabutylammonium hydroxide in methanol (30:70) at a flow rate of 1 ml/minute and detection at either 280 nm or 365 nm. Purity also was determined by ultraviolet spectroscopy by comparison with data obtained from the supplier on a purified sample. Acetone content of purified lot no. C122881 also was determined by gas chromatographic analysis of a tetrahydrofuran solution of the purified dye with an 80/100 Carbopack/0.1% SP1000 column and a flame ionization detector. The results of the purity analyses are summarized in Table 2.

The high-performance liquid chromatography impurity profile analysis of lot no. 0095-130-3 did not detect any impurities other than water greater than 1%. One impurity at the 1% level was detected at 280 nm in lot no. C122881; however, this impurity was not identified.

Stability studies performed with the same high-performance liquid chromatographic system with a 25:75 solvent ratio and detection at 356 nm indicated that the bulk chemical was stable when stored for 2 weeks at temperatures up to 65° C. Further confirmation of the stability of the bulk chemical (stored at 5° C) during the toxicity studies was obtained by spectroscopic analysis (350 nm) vs. a reference standard. No degradation was seen over the course of the studies. Identity of the chemical at the study laboratory was confirmed by infrared spectroscopy.

PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES

C.I. Acid Orange 3 was suspended in corn oil to give the desired concentrations (Table 3). Studies performed at the analytical chemistry laboratory by extraction with methanol and high-performance liquid chromatography with a μBondapak C_{18} column, a mobile phase of 1% acetic acid in methanol:1% acetic acid in water (77:23) at a flow rate of 1 ml/minute, and detection at 280 nm indicated that C.I. Acid Orange 3 (50 mg/ml) suspensions in corn oil were stable when stored for 7 days in the dark at room temperature. At the study laboratory, measurements of absorption at 355 nm of methanol extracts indicated that the chemical suspended in corn oil (25 and 200 mg/ml) was stable when stored for 14 days in the dark at room temperature. Suspensions of C.I. Acid Orange 3 in corn oil were stored at room temperature for no longer than 2 weeks.

TABLE 2. RESULTS OF PURITY ANALYSIS OF C.I. ACID ORANGE 3

Lot No.	Ultraviolet Analysis (percent)	HPLC (percent)	Water (percent)	Acetone (percent)
0095-130-3	89.1	89.0	11.2	--
C122881 (average of three purifications)	94.3	88.7	6.0	2.7

TABLE 3. PREPARATION AND STORAGE OF DOSE MIXTURES IN THE GAVAGE STUDIES OF C.I. ACID ORANGE 3

Fourteen-Day Studies	First Thirteen-Week Studies	Second Thirteen-Week Studies	Two-Year Studies
Preparation			
Weighed amount of chemical placed in bottle and appropriate amount of corn oil added. Mixture shaken vigorously by hand for 1 min, stirred magnetically for 5 min, and poured into serum bottles. Daily doses shaken vigorously and then stirred magnetically 5 min before dosing.	Same as 14-d studies	Same as 14-d studies	Same as 14-d studies except that stirring done with a magnetic stirrer or a Brinkman Polytron®
Maximum Storage Time			
6 d	Not available	Not available	2 wk
Storage Conditions			
5° C	Not available	Not available	Room temperature in the dark

Periodic analyses for C.I. Acid Orange 3 in dose mixtures were performed by the study and analytical chemistry laboratories by extracting samples with methanol and determining the absorption at 355 nm (study laboratory) or at 290-293 nm (analytical chemistry laboratory). Dose preparations were analyzed five times during the 13-week studies. The results ranged from 61% to 109% of the target concentrations (Table 4). During the 2-year studies, dose mixtures were analyzed approximately once every 8 weeks; concentrations varied from 84% to 113% of the target concentrations (Table 5). Because 59/65 dose mixtures analyzed were within 10% of the target concentrations, the dose mixtures were estimated to have been within specifications 91% of the time throughout the entire studies. Referee analyses were performed periodically by the analytical chemistry laboratory. Good agreement was generally found between the study and analytical chemistry laboratories (Table 6).

FOURTEEN-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Harlan Industries and held for 14 days before the studies began. Groups of five rats of each sex were administered 0, 94, 187, 375, 750, or 1,500 mg/kg C.I. Acid Orange 3 in corn oil by gavage for 14 consecutive days. Groups of five mice of each sex were administered 0, 62, 125, 250, 500, or 1,000 mg/kg C.I. Acid Orange 3 on the same schedule.

TABLE 4. RESULTS OF ANALYSIS OF DOSE MIXTURES IN THE THIRTEEN-WEEK GAVAGE STUDIES OF C.I. ACID ORANGE 3

Date Mixed	Concentration of C.I. Acid Orange 3 in Corn Oil (mg/ml) (a) Target	Determined	Determined as a Percent of Target
07/13/79	3.1	3.3	106
	6.2	5.8	94
	12.5	11.8	94
	18.8	19.2	102
	25.0	(b) 20.3	81
	37.4	35.6	95
	50.0	47.2	94
	75.0	75.0	100
	150.0	144.5	96
	300.0	(b) 241.5	81
07/27/79	3.1	(b) 1.9	61
	6.2	5.9	95
	12.5	12.2	98
	18.8	17.7	94
	25.0	23.8	95
	37.4	(b) 32.7	87
	50.0	(b) 44.6	89
	75.0	69.3	92
	150.0	140.1	93
	300.0	(b) 253.4	84
08/03/79	3.1	3.2	103
	6.2	6.0	97
	12.5	(b) 11.2	90
	18.8	20.4	109
	25.0	23.2	93
	37.4	34.3	92
	50.0	52.9	106
	75.0	69.6	93
	150.0	142.0	95
	300.0	(b) 260.5	87
09/21/79	3.1	(b) 2.0	65
	6.2	5.7	92
	12.5	12.0	96
	18.8	20.0	106
	25.0	27.3	109
	37.4	39.6	106
	50.0	51.5	103
	75.0	78.1	104
	150.0	141.5	94
	300.0	(b) 262.0	87
04/02/80 (second 13-week studies in mice)			
	25.0	24.2	97
	50.0	44.9	90
	100.0	98.8	99
	200.0	211.0	106

(a) Results of duplicate analysis
(b) Out of specifications; not remixed.

TABLE 5. RESULTS OF ANALYSIS OF DOSE MIXTURES IN THE TWO-YEAR GAVAGE STUDIES OF C.I. ACID ORANGE 3

Date Mixed	Concentration of C.I. Acid Orange 3 in Corn Oil for Target Concentration (mg/ml) (a)				
	12.5	25	50	75	150
10/09/80	--	--	--	(b) 75.8	155
11/06/80	--	--	--	73.2	(c) 169
11/26/80	13.7	26.9	48.5	--	--
12/04/80	13.8	--	55.0	81.4	--
01/01/81	--	24.9	--	--	154
01/29/81	12.0	--	45.7	73.5	--
02/26/81	--	(d) 21.4	--	--	151
03/03/81	--	(e) 27.2	--	--	--
03/26/81	(d) 11.1	--	45.0	(d) 66.5	--
03/31/81	(e) 11.3	--	--	(e) 80.3	--
04/23/81	--	24.8	--	--	161
05/21/81	(d) 10.7	--	49.7	73.6	--
05/27/81	(e) 12.0	--	--	--	--
06/18/81	--	(d) 21.0	--	--	146
06/22/81	--	(e) 25.9	--	--	--
07/16/81	12.8	--	48.7	75.5	--
08/20/81	--	26.0	--	--	151
09/10/81	12.7	--	51.9	74.6	--
10/08/81	--	26.5	--	--	151
11/05/81	12.9	--	50.1	73.5	--
12/03/81	--	25.3	--	--	151
03/11/82	12.4	23.0	45.3	70.4	144
05/06/82	12.6	24.5	48.0	72.1	145
07/01/82	11.9	23.9	49.1	72.6	145
08/26/82	12.2	24.0	47.2	72.2	137
10/21/82	11.6	23.4	46.0	--	--
Mean (mg/ml)	12.3	24.3	48.5	73.5	150.8
Standard deviation	0.90	1.78	2.84	3.37	8.12
Coefficient of variation (percent)	7.3	7.3	5.9	4.6	5.4
Range (mg/ml)	10.7-13.8	21.0-26.9	45.0-55.0	66.5-81.4	137-169
Number of samples	13	13	13	13	13

(a) Results of duplicate analysis unless otherwise specified
(b) Result of a single analysis
(c) Out of specifications; used in the study.
(d) Out of specifications; not used in the study.
(e) Remix; not included in the mean.

TABLE 6. RESULTS OF REFEREE ANALYSIS OF DOSE MIXTURES IN THE TWO-YEAR GAVAGE STUDIES OF C.I. ACID ORANGE 3

Date Mixed	Target Concentration (mg/ml)	Determined Concentration (mg/ml)	
		Study Laboratory (a)	Referee Laboratory (b)
11/06/80	150	169	154
04/23/81	25	24.8	25.7
07/16/81	50	48.7	50.3
08/26/82	150	137	142

(a) Results of duplicate analysis
(b) Results of triplicate analysis

Animals were housed five per cage and received water and feed ad libitum. Details of animal maintenance are presented in Table 7. Rats and mice were observed twice per day and were weighed on days 1, 7, and 15. A necropsy was performed on all animals.

FIRST THIRTEEN-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxic effects of repeated administration of C.I. Acid Orange 3 and to determine the doses to be used in the 2-year studies.

Four-week-old male and female F344/N rats and 4- to 6-week-old male and female $B6C3F_1$ mice were obtained from Charles River Breeding Laboratories and observed for 16 days before the studies began. Rats and mice were housed five per cage in polycarbonate cages. Feed and water were available ad libitum.

Groups of 10 rats of each sex were administered 0, 94, 187, 375, 750, or 1,500 mg/kg C.I. Acid Orange 3 in corn oil by gavage, 5 days per week for 13 weeks. Groups of 10 mice of each sex received 0, 31, 62, 125, 250, or 500 mg/kg C.I. Acid Orange 3 on the same schedule.

Animals were checked two times per day; moribund animals were killed. Individual animal weights were recorded once per week. At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Tissues and groups examined are listed in Table 7.

SECOND THIRTEEN-WEEK STUDIES

Thirteen-week studies were repeated in $B6C3F_1$ mice to evaluate the cumulative toxic effects of repeated administration of C.I. Acid Orange 3 at higher doses than those used in the first 13-week studies and to determine the doses to be used for mice in the 2-year studies.

Four- to six-week-old male and female $B6C3F_1$ mice were obtained from Charles River Breeding Laboratories and observed for 13 days before the studies began. Mice were housed five per cage in polycarbonate cages. Feed and water were available ad libitum.

Groups of 10 mice of each sex were administered 0, 250, 500, 1,000, or 2,000 mg/kg C.I. Acid Orange 3 in corn oil by gavage, 5 days per week for 13 weeks.

Animals were checked two times per day; moribund animals were killed. Animal weights were recorded once per week. At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized, and microscopic examination was performed on tissues from vehicle control and high dose animals. Tissues and groups examined are listed in Table 7.

TWO-YEAR STUDIES

Study Design

Groups of 50 F344/N rats of each sex were administered 0, 375, or 750 mg/kg C.I. Acid Orange 3 in corn oil by gavage, 5 days per week for 103 weeks. Groups of 50 male $B6C3F_1$ mice were administered 0, 125, or 250 mg/kg C.I. Acid Orange 3, and groups of 50 female $B6C3F_1$ mice were administered 0, 250, or 500 mg/kg on the same schedule.

Source and Specifications of Animals

The male and female F344/N rats and $B6C3F_1$ (C57BL/6N, female × C3H/HeN MTV^-, male) mice used in these studies were produced under strict barrier conditions at Charles River Breeding Laboratories under a contract to the Carcinogenesis Program. Breeding stock for the foundation colonies at the production facility originated at the National Institutes of Health Repository. Animals shipped for study were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Rats were shipped to the study laboratory at 4-5 weeks of age and mice, at 5-6 weeks of age. The rats were quarantined at the study facility for 2 weeks and the mice, for 3 weeks. Thereafter, a complete necropsy was performed on five animals of each sex and species to assess their health status. The rats were placed on study at 6-7 weeks of age and the mice, at 8-9 weeks of age. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix F).

TABLE 7. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF C.I. ACID ORANGE 3

Fourteen-Day Studies	First Thirteen-Week Studies	Second Thirteen-Week Studies	Two-Year Studies
EXPERIMENTAL DESIGN			
Size of Study Groups			
5 males and 5 females of each species	10 males and 10 females of each species	10 male and 10 female mice	50 males and 50 females of each species
Doses			
Rats--0, 94, 187, 375, 750, or 1,500 mg/kg C.I. Acid Orange 3 in corn oil by gavage; dose vol--5 ml/kg; mice--0, 62, 125, 250, 500, or 1,000 mg/kg; dose vol--10 ml/kg	Rats--0, 94, 187, 375, 750, or 1,500 mg/kg C.I. Acid Orange 3 in corn oil by gavage; dose vol--5 ml/kg; mice--0, 31, 62, 125, 250, or 500 mg/kg; dose vol--10 ml/kg	Mice--0, 250, 500, 1,000, or 2,000 mg/kg C.I. Acid Orange 3 in corn oil by gavage; dose vol--10 ml/kg; rats--not applicable	Rats--0, 375, or 750 mg/kg C.I. Acid Orange 3 in corn oil by gavage; dose vol--5 ml/kg; mice--male: 0, 125, or 250 mg/kg; female: 0, 250, or 500 mg/kg; dose vol--10 ml/kg
Date of First Dose			
4/25/79	7/6/79	3/25/80	Rats--10/16/80; mice--12/9/80
Date of Last Dose			
5/8/79	10/4/79	6/23/80	Rats--8/20/82 for high dose males, 10/6/82 for other groups; mice--11/29/82
Duration of Dosing			
14 consecutive d	5 d/wk for 13 wk	5 d/wk for 13 wk	5 d/wk for 103 wk
Type and Frequency of Observation			
Observed 2 × d; weighed initially and 1 × wk thereafter	Same as 14-d studies	Same as 14-d studies	Observed 2 × d; weighed initially, 1 × wk for 13 wk, (rats) or 12 wk (mice), and then 1 × mo; palpated at weighing starting at week 43 (rats) or 39 (mice)
Necropsy and Histologic Examination			
Necropsy performed on all animals	Necropsy performed on all animals except one rat; histologic exam performed on all vehicle control and high dose animals. Tissues examined include: adrenal glands, brain, colon, esophagus, femur including marrow, heart, kidneys, liver, lungs and bronchi, mandibular and mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes/seminal vesicles or ovaries/uterus, salivary glands, skin, small intestine, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder. Tissues examined in the 750 mg/kg rat groups include kidneys and urinary bladder	Necropsy performed on all animals; histologic exam performed on all vehicle control and high dose animals and on animals dying before the end of the studies. Tissues examined: same as first 13-wk studies; kidneys examined from all mice in the 500 and 1,000 mg/kg groups	Necropsy and histologic examination performed on all animals; the following tissues were examined: adrenal glands, aorta, brain, cecum, colon, costochondral junction, duodenum, esophagus, eyes, femur including marrow, gallbladder (mice), gross lesions, heart, ileum, jejunum, kidneys, larynx including oral cavity, liver, lungs and bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroids, pituitary gland, preputial or clitoral gland (after 6/1/82), prostate/testes/seminal vesicles/epididymis/tunica vaginalis/scrotal sac or ovaries/uterus, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, tissue masses, trachea, urinary bladder, and Zymbal gland (after 6/1/82)

TABLE 7. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF C.I. ACID ORANGE 3 (Continued)

Fourteen-Day Studies	First Thirteen-Week Studies	Second Thirteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE			
Strain and Species			
F344/N rats; $B6C3F_1$ mice	F344/N rats; $B6C3F_1$ mice	$B6C3F_1$ mice	F344/N rats; $B6C3F_1$ mice
Animal Source			
Harlan Industries (Indianapolis, IN)	Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Kingston, NY)
Study Laboratory			
Southern Research Institute	Southern Research Institute	Southern Research Institute	Southern Research Institute
Method of Animal Identification			
Ear marked with poultry punch	Ear marked with poultry punch	Ear marked with poultry punch	Ear marked with poultry punch
Time Held Before Study			
14 d	16 d	13 d	Rats--14 d; mice--19 d
Age When Placed on Study			
7-8 wk	Rats: 6 wk; mice 6-8 wk	6-8 wk	Rats--6-7 wk; mice--8-9 wk
Age When Killed			
9-10 wk	20-22 wk	19-22 wk	Rats--110-112 wk; mice--112-113 wk
Necropsy Dates			
5/10/79-5/11/79	10/6/79-10/11/79	6/24/80-6/27/80	Rats--8/20/82 for high dose males, 10/14/82-10/20/82 for other groups; mice--12/7/82-12/13/82
Method of Animal Distribution			
Assigned to cages by one table of random numbers and then to groups according to another table of random numbers	Same as 14-d studies	Same as 14-d studies	Animals distributed to weight classes and assigned to cages by one table of random numbers and to groups by another table of random numbers
Feed			
Wayne Lab Blox® pellets (Allied Mills, Chicago, IL); available ad libitum	Same as 14-d studies	NIH 07 Rat and Mouse Ration (Zeigler Bros., Gardners, PA); available ad libitum	Same as second 13-wk studies
Bedding			
Beta Chips--heat-treated hardwood chips (Northeastern Products Corp., Warrensburg, NY)	Same as 14-d studies	Same as 14-d studies	Same as 14-d studies
Water			
Automatic watering system (Edstrom Industries, Waterford, WI); available ad libitum	Same as 14-d studies	Same as 14-d studies	Same as 14-d studies
Cages			
Polycarbonate (Lab Products, Garfield, NJ)	Same as 14-d studies	Same as 14-d studies	Same as 14-d studies

TABLE 7. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF C.I. ACID ORANGE 3 (Continued)

Fourteen-Day Studies	First Thirteen-Week Studies	Second Thirteen-Week Studies	Two-Year Studies
Cage Filters			
Reemay spun-bonded polyester filters (Snow Filtration, Cincinnati, OH)	Same as 14-d studies	Same as 14-d studies	Same as 14-d studies
Animals per Cage			
5	5	5	5
Other Chemicals on Study in the Same Room			
None	None	None	None
Animal Room Environment			
Temp--21°-23° C; hum--30%-50%; fluorescent light 12 h/d; 15 room air changes/h	Same as 14-d studies	Temp--22°-24° C; hum--34%-67%; fluorescent light 12 h/d; 15 room air changes/h	Temp--22.2°-24.4° C (83% of the time); hum--50% ± 10% (84% of the time); fluorescent light 12 h/d; 15 room air changes/h

A quality control skin grafting program has been in effect since early 1978 to monitor the genetic integrity of the inbred mice used to produce the hybrid $B6C3F_1$ study animal. In mid-1981, data were obtained that showed incompatibility between the NIH C3H reference colony and the C3H colony from a Program supplier. In August 1981, inbred parental lines of mice were further tested for genetic integrity via isozyme and protein electrophoresis profiles that demonstrate phenotype expressions of known genetic loci.

The C57BL/6N mice were homogeneous at all loci tested. Eighty-five percent of the C3H mice monitored were variant at one to three loci, indicating some heterogeneity in the C3H line from this supplier. Nevertheless, the genome of this line is more homogeneous than that of randomly bred stocks.

Male mice from the C3H colony and female mice from the C57BL/6N colony were used as parents for the hybrid $B6C3F_1$ mice used in these studies. The influence of the potential genetic nonuniformity in the hybrid mice on these results is not known, but results of the studies are not affected because concurrent controls were included in each study.

Animal Maintenance

Animals were housed five per cage. Feed and water were available ad libitum. Further details of animal maintenance are given in Table 7.

Clinical Examinations and Pathology

All animals were observed two times per day, and clinical signs were recorded at least once per month. Body weights were recorded once per week for the first 13 (rats) or 12 (mice) weeks of the studies and once per month thereafter. Mean body weights were calculated for each group. Animals found moribund and those surviving to the end of the studies were humanely killed. A necropsy was performed on all animals including those found dead, unless they were excessively autolyzed or cannibalized, missexed, or found missing. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study.

During necropsy, all organs and tissues were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin,

embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Tissues examined are listed in Table 7.

When the pathology evaluation was completed, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tumor diagnoses, all target tissues, and all tissues from a randomly selected 10% of the animals were evaluated by a quality assessment pathologist. The quality assessment report and slides were submitted to the Pathology Working Group (PWG) Chairperson, who reviewed all target tissues and those about which there was a disagreement between the laboratory and quality assessment pathologists.

Representative slides selected by the Chairperson were reviewed by the PWG, which includes the laboratory pathologist, without knowledge of previously rendered diagnoses. When the consensus diagnosis of the PWG differed from that of the laboratory pathologist, the laboratory pathologist was asked to reconsider the original diagnosis. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). The final diagnoses represent a consensus of contractor pathologists and the NTP Pathology Working Group. For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are combined according to the guidelines of McConnell et al. (1986).

Slides/tissues are generally not evaluated in a blind fashion (i.e., without knowledge of dose group) unless the lesions in question are subtle or unless there is an inconsistent diagnosis of lesions by the laboratory pathologist. Nonneoplastic lesions are not examined routinely by the quality assessment pathologist or PWG unless they are considered part of the toxic effect of the chemical.

Statistical Methods

Data Recording: Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Survival Analyses: The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses at the time they were found to be missing or dead from other than natural causes; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend. When significant survival differences were detected, additional analyses using these procedures were carried out to determine the time point at which significant differences in the survival curves were first detected. All reported P values for the survival analysis are two-sided.

Calculation of Incidence: The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators include only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence: Three statistical methods are used to analyze tumor incidence data: life table tests, incidental tumor analysis, and Fisher exact/Cochran-Armitage trend analyses. Tests of significance include pairwise comparisons of high dose and low dose groups with vehicle controls and tests for overall dose-response trends. For studies in which administration of the study compound has little effect on survival, the results of the three alternative

analyses will generally be similar. When differing results are obtained by the three methods, the final interpretation of the data will depend on the extent to which the tumor under consideration is regarded as being the cause of death. Continuity-corrected tests are used in the analysis of tumor incidence, and reported P values are one-sided. The procedures described below also were used to evaluate selected nonneoplastic lesions.

Life Table Analyses--The first method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "fatal"; i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and vehicle control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results, including the data from animals killed at the end of the study, were then combined by the Mantel-Haenszel method (1959) to obtain an overall P value. This method of adjusting for intercurrent mortality is the life table method of Cox (1972) and of Tarone (1975). The underlying variable considered by this analysis is time to death due to tumor. If the tumor is rapidly lethal, then time to death due to tumor closely approximates time to tumor onset. In this case, the life table test also provides a comparison of the time-specific tumor incidences.

Incidental Tumor Analyses--The second method of analysis assumed that all tumors of a given type observed in animals that died before the end of the study were "incidental"; i.e., they were merely observed at necropsy in animals dying of an unrelated cause. According to this approach, the proportions of tumor-bearing animals in dosed and vehicle control groups were compared in each of five time intervals: weeks 0-52, weeks 53-78, weeks 79-92, week 93 to the week before the terminal-kill period, and the terminal-kill period. The denominators of these proportions were the number of animals actually examined for tumors during the time interval. The individual time interval comparisons were then combined by the previously described method to obtain a single overall result. (See Haseman, 1984, for the computational details of both methods.)

Fisher Exact/Cochran-Armitage Trend Analyses--In addition to survival-adjusted methods, the results of the Fisher exact test for pairwise comparisons and the Cochran-Armitage linear trend test (Armitage, 1971; Gart et al., 1979) are given in the appendixes containing the analyses of tumor incidence. These two tests are based on the overall proportion of tumor-bearing animals and do not adjust for survival differences.

Historical Control Data: Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Consequently, control tumor incidences from the NTP historical control data base (Haseman et al., 1984, 1985) are included for those tumors appearing to show compound-related effects.

III. RESULTS

RATS

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MICE

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III. RESULTS: RATS

FOURTEEN-DAY STUDIES

One female rat that received 1,500 mg/kg died on day 16 just before the terminal kill (Table 8). All other rats survived to the end of the studies. Final mean body weights of males and females were not adversely affected by C.I. Acid Orange 3. Orange urine or extremities were observed for rats that received 750 or 1,500 mg/kg, 3/5 males and 4/5 females that received 375 mg/kg, 2/5 females that received 187 mg/kg, and 1/5 females that received 94 mg/kg. No compound-related effects were observed at necropsy.

THIRTEEN-WEEK STUDIES

Although C.I. Acid Orange 3 at 1,500 mg/kg had no effect in the 14-day studies, this was the highest dose administered in the 13-week studies in rats (Table 9). The highest dose in rats was limited by the viscosity of the corn oil suspension and by the diameter of the gavaging needle rather than by toxicity. The most concentrated suspension that could be administered with precision was 300 mg/ml. The largest dose volume of corn oil used in NTP 2-year studies in rats is 5 ml/kg.

TABLE 8. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE FOURTEEN-DAY GAVAGE STUDIES OF C.I. ACID ORANGE 3

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	5/5	97 ± 3	140 ± 6	+43 ± 4	--
94	5/5	103 ± 4	145 ± 6	+42 ± 2	104
187	5/5	95 ± 1	145 ± 3	+50 ± 2	104
375	5/5	100 ± 4	142 ± 6	+42 ± 2	101
750	5/5	103 ± 4	150 ± 6	+47 ± 4	107
1,500	5/5	102 ± 3	136 ± 4	+34 ± 2	97
FEMALE					
0	5/5	81 ± 2	109 ± 4	+28 ± 2	--
94	5/5	87 ± 3	112 ± 4	+25 ± 2	103
187	5/5	86 ± 3	113 ± 5	+27 ± 2	104
375	5/5	84 ± 2	107 ± 3	+23 ± 2	98
750	5/5	86 ± 3	113 ± 4	+27 ± 3	104
1,500	(d) 4/5	86 ± 4	114 ± 1	+27 ± 4	105

(a) Number surviving/number initially in the group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on those animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Day of death: 16

TABLE 9. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF C.I. ACID ORANGE 3

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	10/10	127 ± 1	336 ± 4	+209 ± 4	--
94	10/10	126 ± 2	329 ± 4	+203 ± 3	98
187	10/10	126 ± 1	327 ± 4	+201 ± 3	97
375	10/10	128 ± 2	330 ± 6	+202 ± 5	98
750	10/10	128 ± 1	330 ± 5	+202 ± 5	98
1,500	10/10	128 ± 1	310 ± 3	+182 ± 2	92
FEMALE					
0	10/10	103 ± 1	193 ± 2	+90 ± 2	--
94	10/10	103 ± 1	191 ± 2	+88 ± 1	99
187	10/10	103 ± 1	193 ± 2	+90 ± 2	100
375	10/10	102 ± 1	190 ± 3	+88 ± 2	98
750	10/10	104 ± 1	186 ± 2	+82 ± 1	96
1,500	(d) 5/10	102 ± 1	183 ± 4	+80 ± 4	95

(a) Number surviving/number initially in the group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on those animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Week of death: 1,7,7,8,8

Final mean body weights of rats that received 1,500 mg/kg were from 5% to 8% lower than those of vehicle controls (Table 9). Yellow fur was observed for all dosed groups of females and for males that received 750 or 1,500 mg/kg.

Five of 10 female rats that received 1,500 mg/kg C.I. Acid Orange 3 died before the end of the study (Table 9). Nephrosis was observed in 9/10 males and 2/9 females that received 1,500 mg/kg. In the 1,500 mg/kg group, suppurative inflammation of the kidney occurred in 3/9 females, and necrosis of the renal papilla was observed in 2/9 females. Acidophilic cytoplasmic inclusion bodies or granules were observed in the transitional epithelium of the urinary bladder of all five females that received 1,500 mg/kg and survived to the end of the study. Hyperplasia of the transitional epithelium of the urinary bladder was observed in 2/5 females that received 1,500 mg/kg and survived to the end of the study. These lesions were not observed in the vehicle controls.

Dose Selection Rationale: Because of the incidence of nephrosis and deaths, doses of C.I. Acid Orange 3 selected for rats for the 2-year studies were 375 and 750 mg/kg, administered in corn oil by gavage, 5 days per week.

TWO-YEAR STUDIES

Body Weights

Mean body weights of high dose male rats were 5%-10% lower than those of vehicle controls after week 25 and 11%-16% lower after week 52 (Table 10 and Figure 3). Mean body weights of high dose female rats were 5%-10% lower than those of vehicle controls after week 47 and 11%-19% lower after week 70.

TABLE 10. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR GAVAGE STUDIES OF C.I. ACID ORANGE 3

Weeks on Study	Vehicle Control Av. Wt. (grams)	Vehicle Control No. of Survivors	375 mg/kg Av. Wt. (grams)	375 mg/kg Wt. (percent of veh. controls)	375 mg/kg No. of Survivors	750 mg/kg Av. Wt. (grams)	750 mg/kg Wt. (percent of veh. controls)	750 mg/kg No. of Survivors
MALE								
0	139	50	138	99	50	139	100	50
1	177	50	176	99	50	176	99	50
2	206	50	210	102	50	211	102	50
3	231	50	233	101	50	234	101	50
4	255	50	254	100	50	255	100	50
5	273	50	270	99	50	270	99	50
6	288	50	284	99	50	284	99	50
7	301	50	297	99	50	297	99	49
8	314	50	311	99	50	310	99	49
9	324	50	323	100	50	320	99	49
10	334	50	332	99	50	328	98	49
11	338	50	337	100	50	331	98	48
12	350	50	348	99	50	342	98	48
13	345	50	353	102	50	347	101	48
17	379	50	373	98	50	365	96	48
21	400	50	396	99	50	387	97	48
25	415	50	411	99	50	395	95	47
30	438	50	429	98	49	403	92	46
34	451	50	438	97	49	414	92	39
38	465	50	453	97	49	425	91	39
43	477	50	464	97	49	432	91	35
47	480	50	464	97	49	437	91	35
52	493	50	479	97	49	445	90	35
56	500	50	483	97	48	445	89	34
60	508	50	489	96	47	444	87	33
66	502	50	493	98	47	445	89	31
70	506	50	491	97	46	433	86	27
73	506	49	484	96	46	436	86	24
78	503	48	481	96	43	431	86	18
82	497	45	480	97	39	429	86	13
87	497	44	475	96	33	427	86	9
92	489	43	469	96	32	413	84	8
97	472	41	460	97	32	--	--	0
101	457	40	449	98	30	--	--	0
104	445	36	446	100	30	--	--	0
FEMALE								
0	108	50	107	99	50	109	101	50
1	129	50	129	100	50	130	101	50
2	145	50	145	100	50	145	100	50
3	153	50	154	101	50	155	101	49
4	164	50	164	100	50	163	99	49
5	172	50	171	99	50	170	99	49
6	178	50	179	101	50	177	99	49
7	185	50	184	99	50	182	98	49
8	187	50	189	101	50	186	99	49
9	191	50	193	101	50	189	99	49
10	194	50	196	101	50	192	99	49
11	198	50	200	101	50	191	96	48
12	205	50	205	100	50	196	96	46
13	203	50	194	96	50	199	98	44
17	217	50	217	100	50	205	94	39
21	221	50	226	102	50	218	99	37
25	227	50	231	102	50	224	99	37
30	239	50	239	100	50	232	97	37
34	241	50	243	101	50	235	98	37
38	248	50	249	100	50	239	96	36
43	255	50	258	101	50	244	96	35
47	255	50	260	102	50	243	95	35
52	265	50	268	101	50	249	94	33
56	273	50	277	101	50	255	93	32
60	283	50	284	100	50	261	92	31
66	290	49	292	101	49	265	91	31
70	299	47	299	100	48	270	90	26
73	303	47	301	99	48	270	89	23
78	308	47	300	97	48	271	88	22
82	316	47	305	97	48	273	86	21
87	319	47	306	96	46	274	86	18
92	323	46	305	94	43	264	82	14
97	317	44	303	96	41	261	82	11
101	320	43	299	93	38	258	81	8
104	317	43	300	95	34	256	81	7

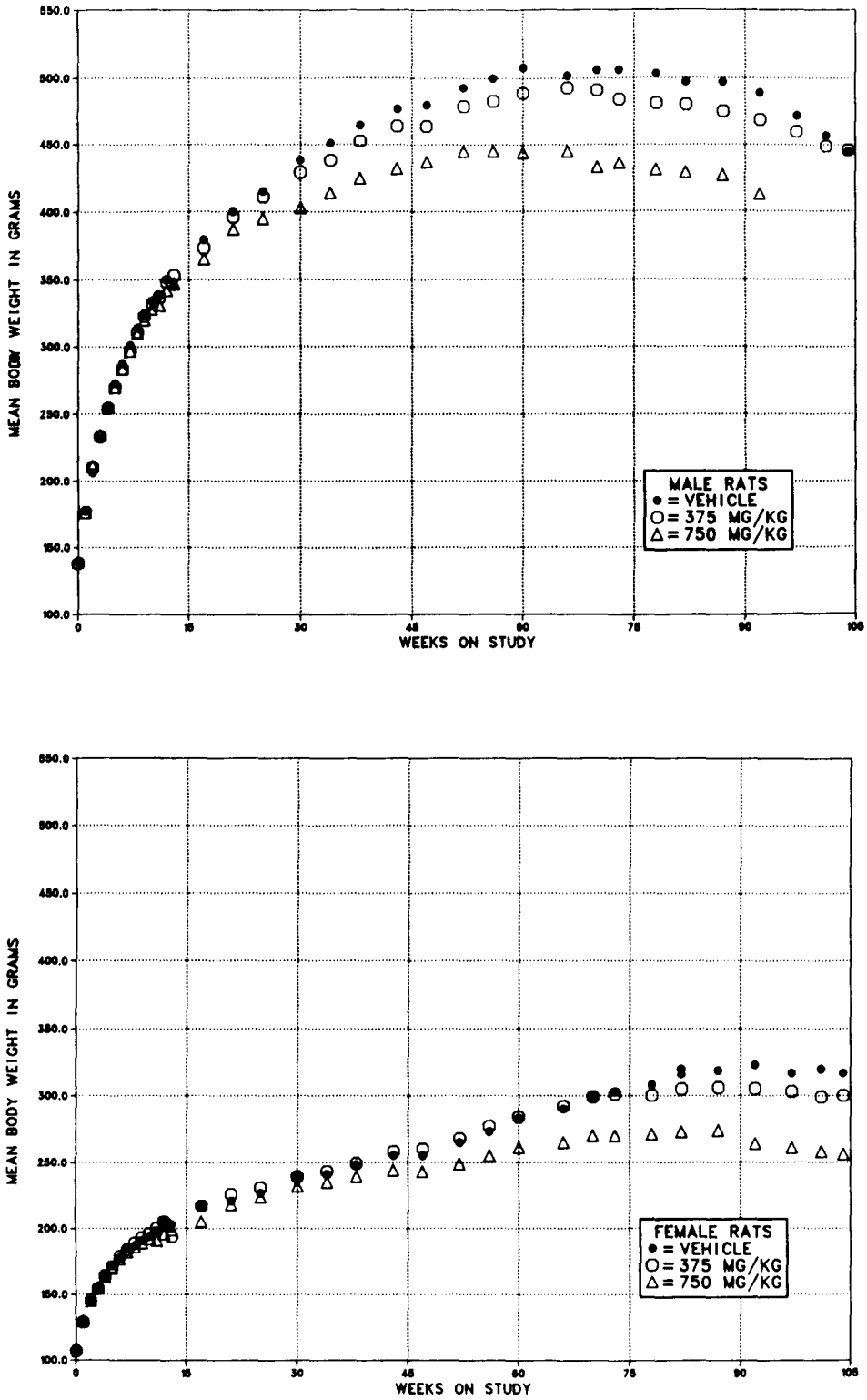


FIGURE 3. GROWTH CURVES FOR RATS ADMINISTERED C.I. ACID ORANGE 3 IN CORN OIL BY GAVAGE FOR TWO YEARS

Survival

Estimates of the probabilities of survival for male and female rats administered C.I. Acid Orange 3 at the doses used in these studies and for vehicle controls are shown in Table 11 and in the Kaplan and Meier curves in Figure 4. The survival of the high dose group of both males (after week 33) and females (after week 14) was significantly lower than that of the vehicle controls. By week 97, all of the males receiving 750 mg/kg had died.

Pathology and Statistical Analyses of Results

This section describes the significant or noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions of the kidney, glandular stomach, bone, circulatory system, parathyroids, colon, cecum, and testis.

Lesions in male rats are summarized in Appendix A. Histopathologic findings on neoplasms are summarized in Table A1. Table A2 gives the survival and tumor status for individual male rats. Table A3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table A3 (footnotes). Findings on nonneoplastic lesions are summarized in Table A4. Analysis of trends and pairwise comparisons of the high dose group with the vehicle controls are not presented because the reduced survival in the high dose group markedly lowered both the sensitivity of the tests for the detection of tumors and the opportunity for compound-related tumors to develop. There were no tumors showing increased incidences in the high dose group relative to those in the vehicle controls.

Lesions in female rats are summarized in Appendix B. Histopathologic findings on neoplasms are summarized in Table B1. Table B2 gives the survival and tumor status for individual female rats. Table B3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table B3 (footnotes). Historical incidences of tumors in corn oil vehicle control female rats are listed in Table B4. Findings on nonneoplastic lesions are summarized in Table B5.

TABLE 11. SURVIVAL OF RATS IN THE TWO-YEAR GAVAGE STUDIES OF C.I. ACID ORANGE 3

	Vehicle Control	375 mg/kg	750 mg/kg
MALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	10	15	44
Accidentally killed (c)	4	5	6
Killed at termination	36	30	0
Survival P values (d)	<0.001	0.208	<0.001
FEMALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	7	16	42
Accidentally killed (c)	0	0	1
Killed at termination	43	33	7
Died during termination period	0	1	0
Survival P values (d)	<0.001	0.070	<0.001

(a) Terminal-kill period: weeks 104-105
(b) Includes animals killed in a moribund condition
(c) All deaths were related to errors in gavage technique.
(d) The result of the life table trend test is in the vehicle control column, and the results of the life table pairwise comparisons with the vehicle controls are in the dosed columns.

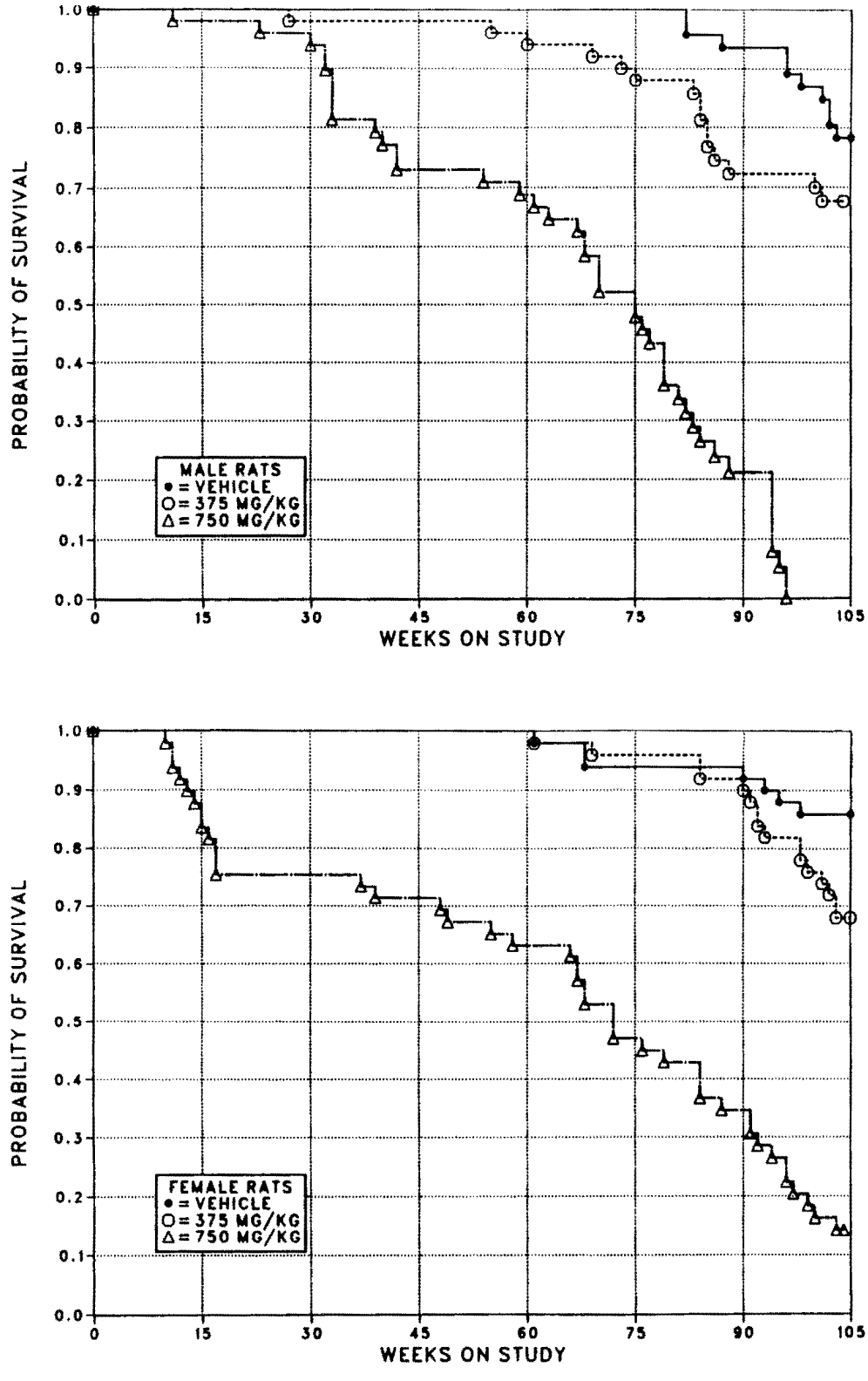


FIGURE 4. KAPLAN-MEIER SURVIVAL CURVES FOR RATS ADMINISTERED C.I. ACID ORANGE 3 IN CORN OIL BY GAVAGE FOR TWO YEARS

Kidney: The administration of C.I. Acid Orange 3 was associated with a spectrum of nonneoplastic lesions in male and female rats (Table 12). These included an increased incidence and/or severity of nephropathy, hyperplasia of the pelvic epithelium, papillary necrosis, inflammation, and pigmentation.

Six transitional cell carcinomas were observed in female rats given 750 mg/kg C.I. Acid Orange 3. These neoplasms originated from the transitional epithelium of the renal pelvis and exhibited cellular atypia and local invasion of the submucosa. The incidence of this rare tumor in the high dose group was significantly greater than that in the vehicle control group. Renal transitional cell carcinomas have not been observed in 1,697 historical corn oil vehicle control female F344/N rats. No renal neoplasms were observed in dosed male rats; however, one vehicle control male was found to have a tubular cell adenocarcinoma.

TABLE 12. ANALYSIS OF SELECTED RENAL LESIONS IN RATS IN THE TWO-YEAR GAVAGE STUDIES OF C.I. ACID ORANGE 3 (a)

Lesion	Vehicle Control	375 mg/kg	750 mg/kg
MALE			
No. of animals examined	50	50	50
Nephropathy	50	50	49
Mild	33	35	6
Moderate	16	15	14
Severe	1	0	29
Papillary necrosis	0	1	1
Suppurative inflammation	7	(b) 37	(b) 44
Pigmentation	4	4	(b) 39
Pelvic epithelial hyperplasia	0	(c) 6	(b) 13
FEMALE			
No. of animals examined	50	50	50
Nephropathy	23	(b) 45	(b) 48
Mild	22	39	12
Moderate	1	5	14
Severe	0	1	22
Papillary necrosis	0	0	(b) 10
Suppurative inflammation	0	(b) 10	(b) 45
Pigmentation	0	0	(c) 5
Pelvic epithelial hyperplasia	0	2	(b) 13
Transitional Cell Carcinoma (d)			
Overall Rates	0/50 (0%)	0/50 (0%)	6/50 (12%)
Adjusted Rates	0.0%	0.0%	50.1%
Terminal Rates	0/43 (0%)	0/34 (0%)	2/7 (29%)
Week of First Observation			87
Life Table Tests	P<0.001	(e)	P<0.001
Incidental Tumor Tests	P<0.001	(e)	P=0.007

(a) The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix A, Table A3 (footnotes).
(b) P<0.01 vs. vehicle controls
(c) P<0.05 vs. vehicle controls
(d) Historical incidence of transitional cell neoplasms at study laboratory: 0/400; historical incidence in NTP studies: 0/1,697
(e) No P value is reported because no tumors were observed in the 375 mg/kg and vehicle control groups.

Glandular Stomach, Bone, Circulatory System, and Parathyroids: Mineralization, erosion of the epithelium, and ulcers occurred in the glandular stomach of some dosed rats (Table 13). Mineralization of the aorta also occurred in some dosed rats. These lesions are probably related to uremia caused by kidney failure. Parathyroid hyperplasia (to a degree evident by light microscopic examination) was increased in high dose male rats. Fibrous dysplasia of bones was also increased in high dose male and female rats and is considered to be secondary to the renal disease and parathyroid hyperplasia (renal secondary hyperparathyroidism).

Colon and Cecum: Chronic and suppurative inflammation of the colon and cecum were observed in a number of dosed male and female rats. Whether these changes are directly related to the administration of C.I. Acid Orange 3 or are related to uremia from kidney failure is uncertain.

Testis: Interstitial cell hyperplasia was observed at increased incidences in dosed male rats (vehicle control, 1/50; low dose, 8/50; high dose, 10/48; $P<0.05$); the incidences of interstitial cell tumors in the dosed groups were significantly lower than that in the vehicle controls (47/50; 34/50; 22/48; $P<0.02$). Interstitial cell hyperplasia and neoplasia represent a morphologic continuum, and at the end of 2 years, nearly all male F344/N rats are expected to have interstitial cell tumors. Male rats dying early would be expected to have a greater incidence of hyperplasia and a lower incidence of tumors than those surviving for 2 years.

TABLE 13. INCIDENCES OF LESIONS CONSIDERED SECONDARY TO KIDNEY TOXICITY IN RATS IN THE TWO-YEAR GAVAGE STUDIES OF C.I. ACID ORANGE 3

Site/Lesion	Male			Female		
	0	375 mg/kg	750 mg/kg	0	375 mg/kg	750 mg/kg
Glandular Stomach	(a) 50	50	50	50	50	50
Ulcer	0	(b) 5	(c) 8	0	2	2
Erosion	1	5	0	0	1	1
Mineralization	6	1	(c) 12	0	1	(b) 5
Parathyroids	48	46	45	48	45	40
Hyperplasia	0	0	(c) 8	0	0	1
Femur	50	50	50	50	50	50
Fibrous dysplasia	0	0	(c) 26	0	0	(c) 12
Aorta	50	50	50	50	50	50
Mineralization	0	1	3	0	0	(c) 9

(a) Number of tissues examined
(b) $P<0.05$ vs. vehicle controls
(c) $P<0.01$ vs. vehicle controls

FOURTEEN-DAY STUDIES

None of the mice died before the end of the studies (Table 14). A malfunction of the watering system during the first week resulted in decreased water availability to male mice that received 500 or 1,000 mg/kg C.I. Acid Orange 3. This probably accounted for initial weight losses and overall decreased weight gains in these groups. Vehicle control female mice lost weight during the first week of the studies. All dosed groups had orange urine. All but two mice that received 1,000 mg/kg were inactive. No compound-related effects were observed at necropsy.

TABLE 14. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE FOURTEEN-DAY GAVAGE STUDIES OF C.I. ACID ORANGE 3

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	5/5	21.6 ± 0.8	26.4 ± 0.6	+4.8 ± 0.6	--
62	5/5	21.4 ± 0.9	25.4 ± 0.7	+4.0 ± 0.7	96.2
125	5/5	20.6 ± 1.4	24.8 ± 1.0	+4.2 ± 1.0	93.9
250	5/5	22.8 ± 0.8	26.0 ± 0.7	+3.2 ± 0.7	98.5
500	5/5	22.6 ± 0.5	(d) 24.8 ± 0.2	+2.2 ± 0.4	93.9
1,000	5/5	24.0 ± 0.4	(d) 25.4 ± 0.5	+1.4 ± 0.4	96.2
FEMALE					
0	5/5	16.8 ± 0.4	19.4 ± 0.5	+2.6 ± 0.4	--
62	5/5	17.8 ± 0.5	20.6 ± 0.4	+2.8 ± 0.4	106.2
125	5/5	18.6 ± 0.4	21.8 ± 0.4	+3.2 ± 0.6	112.4
250	5/5	17.4 ± 0.5	19.8 ± 0.5	+2.4 ± 0.2	102.1
500	5/5	16.6 ± 0.4	20.2 ± 0.7	+3.6 ± 0.5	104.1
1,000	5/5	18.2 ± 0.7	20.8 ± 0.4	+2.6 ± 0.7	107.2

(a) Number surviving/number initially in the group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Automatic watering system malfunction during the first week of the study may have contributed to the reduced body weights in these groups.

FIRST THIRTEEN-WEEK STUDIES

No compound-related deaths occurred (Table 15). Final mean body weights were not adversely affected by C.I. Acid Orange 3. Because no toxicologic effects were produced, the 13-week studies in mice were repeated at doses of 250, 500, 1,000, or 2,000 mg/kg.

SECOND THIRTEEN-WEEK STUDIES

No compound-related deaths occurred in mice (Table 16). Orange urine was observed in the 1,000 and 2,000 mg/kg groups. Final mean body weights of males and females that received 2,000 mg/kg were 12% and 11% lower, respectively, than those of vehicle controls. Mild to severe nephropathy consisting of increased basophilia of the tubular epithelial cells, tubular dilatation, and cast formation was observed in 10/10 males and 9/10 females that received 2,000 mg/kg and in 5/10 males and 2/10 females that received 1,000 mg/kg. These kidney changes were not observed in vehicle controls.

Dose Selection Rationale: Because of the severity of the kidney lesions, doses of C.I. Acid Orange 3 selected for mice in the 2-year studies were 125 and 250 mg/kg for males and 250 and 500 mg/kg for females, administered in corn oil by gavage, 5 days per week.

TABLE 15. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE FIRST THIRTEEN-WEEK GAVAGE STUDIES OF C.I. ACID ORANGE 3

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	10/10	25.6 ± 0.5	39.6 ± 0.6	+14.0 ± 0.4	--
31	10/10	25.7 ± 0.5	39.6 ± 0.7	+13.9 ± 0.5	100.0
62	10/10	25.3 ± 0.3	37.1 ± 0.5	+11.8 ± 0.4	93.7
125	10/10	25.9 ± 0.4	38.1 ± 0.7	+12.2 ± 0.7	96.2
250	9/10	25.9 ± 0.4	38.9 ± 1.6	+13.0 ± 1.3	98.2
500	10/10	26.3 ± 0.3	38.4 ± 0.8	+12.1 ± 0.7	97.0
FEMALE					
0	9/10	19.5 ± 0.2	27.8 ± 0.7	+8.3 ± 0.7	--
31	9/10	19.1 ± 0.3	27.9 ± 1.0	+8.7 ± 0.7	100.4
62	9/10	19.2 ± 0.3	26.2 ± 0.4	+7.2 ± 0.4	94.2
125	10/10	19.2 ± 0.3	27.2 ± 0.3	+8.0 ± 0.4	97.8
250	10/10	18.9 ± 0.2	27.1 ± 0.8	+8.2 ± 0.6	97.5
500	9/10	18.7 ± 0.3	27.3 ± 0.6	+8.6 ± 0.6	98.2

(a) Number surviving/number initially in the group; all deaths judged to be related to gavage error.
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on those animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean

TABLE 16. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE SECOND THIRTEEN-WEEK GAVAGE STUDIES OF C.I. ACID ORANGE 3

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	10/10	24.6 ± 0.5	34.6 ± 1.6	+10.0 ± 1.4	--
250	9/10	24.9 ± 0.5	36.8 ± 1.2	+12.0 ± 0.9	106.4
500	10/10	24.8 ± 0.7	35.1 ± 1.6	+10.3 ± 1.3	101.4
1,000	10/10	25.1 ± 0.5	35.3 ± 0.8	+10.2 ± 0.6	102.0
2,000	10/10	25.1 ± 0.5	30.5 ± 0.6	+5.4 ± 0.6	88.2
FEMALE					
0	10/10	20.3 ± 0.2	28.2 ± 0.5	+7.9 ± 0.5	--
250	9/10	20.1 ± 0.2	28.6 ± 0.7	+8.4 ± 0.6	101.4
500	10/10	20.6 ± 0.4	28.7 ± 0.6	+8.1 ± 0.5	101.8
1,000	8/10	19.9 ± 0.2	(d) 25.6 ± 1.1	+5.8 ± 1.0	90.8
2,000	9/10	19.9 ± 0.2	25.2 ± 0.7	+5.3 ± 0.7	89.4

(a) Number surviving/number initially in the group; all deaths occurred during week 1 and were attributable to gavage error.
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on those animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) The final body weight of four of these animals was reduced during the last week of the study due to malfunctioning of the automatic watering apparatus. No difference in mean body weight relative to that of the vehicle controls was apparent before 12 weeks.

TWO-YEAR STUDIES

Body Weights

Mean body weights of high dose male mice were 6%-10% lower than those of vehicle controls from week 74 to the end of the studies (Table 17 and Figure 5). Mean body weights of low dose male mice were 5%-8% lower than those of vehicle controls from week 44 to week 70 and then were 9%-14% lower. Mean body weights of high dose female mice were 5%-11% lower than those of vehicle controls from week 74 to the end of the studies. Mean body weights of low dose female mice were 5%-8% lower than those of vehicle controls from week 30 to week 48 and then were 9%-17% lower.

TABLE 17. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR GAVAGE STUDIES OF C.I. ACID ORANGE 3

Weeks on Study	Vehicle Control Av. Wt. (grams)	Vehicle Control No. of Survivors	Low Dose Av. Wt. (grams)	Low Dose Wt. (percent of veh. controls)	Low Dose No. of Survivors	High Dose Av. Wt. (grams)	High Dose Wt. (percent of veh. controls)	High Dose No. of Survivors
MALE				**125 mg/kg**			**250 mg/kg**	
0	25.9	50	26.3	102	50	25.6	99	50
1	27.5	50	28.4	103	50	27.8	101	50
2	28.6	50	29.1	102	50	29.1	102	50
3	29.3	50	30.4	104	50	30.2	103	50
4	31.0	50	31.5	102	50	31.4	101	50
5	31.5	50	31.8	101	50	31.7	101	50
6	31.9	50	33.1	104	50	32.7	103	50
7	32.9	50	34.1	104	50	34.4	105	50
8	34.3	50	34.2	100	50	34.0	99	50
9	35.4	50	32.8	93	50	33.3	94	50
10	36.0	50	35.2	98	50	36.1	100	50
11	36.7	50	36.1	98	50	37.1	101	50
12	37.6	50	36.6	97	50	37.9	101	50
17	38.2	50	37.6	98	50	39.8	104	50
22	41.5	50	40.1	97	50	41.1	99	50
26	43.1	50	42.2	98	50	43.6	101	49
30	46.6	50	44.7	96	48	45.5	98	49
35	45.9	50	44.4	97	48	46.1	100	49
39	46.8	50	45.3	97	48	46.0	98	49
44	48.4	49	45.6	94	48	44.8	93	48
48	48.3	49	45.7	95	48	46.9	97	47
52	49.0	49	45.7	93	48	46.1	94	47
58	48.3	49	44.2	92	46	47.0	97	47
62	49.1	49	45.3	92	45	47.2	96	47
65	49.3	49	45.9	93	44	47.1	96	47
70	49.0	49	45.3	92	44	47.2	96	47
74	50.1	47	44.0	88	44	45.9	92	46
79	49.7	45	45.1	91	42	45.6	92	43
84	50.3	45	44.7	89	39	45.7	91	41
89	48.8	41	43.2	89	37	44.7	92	35
93	48.6	40	43.4	89	35	43.7	90	32
97	48.9	39	42.0	86	31	44.6	91	29
101	48.8	38	42.0	86	27	44.3	91	27
104	47.0	38	42.1	90	25	44.3	94	26
FEMALE				**250 mg/kg**			**500 mg/kg**	
0	18.8	50	20.3	108	50	19.7	105	50
1	20.5	50	21.6	105	50	21.4	104	50
2	21.3	50	22.1	104	50	21.5	101	50
3	22.0	50	22.8	104	50	22.4	102	50
4	23.4	50	23.7	101	50	23.9	102	50
5	23.2	50	23.9	103	50	24.0	103	50
6	23.8	50	22.9	96	50	24.3	102	50
7	24.2	50	24.7	102	50	25.4	105	50
8	24.7	50	25.4	103	50	25.2	102	50
9	25.4	50	26.0	102	50	24.0	94	50
10	26.0	50	25.9	100	50	26.0	100	50
11	26.4	50	27.1	103	50	26.9	102	50
12	27.5	50	26.9	98	50	27.5	100	50
17	27.8	50	28.0	101	50	28.8	104	50
22	29.9	50	30.1	101	50	30.2	101	50
26	32.0	50	31.5	98	50	32.2	101	50
30	33.8	50	32.2	95	50	33.2	98	50
35	35.3	50	33.5	95	50	34.5	98	50
39	35.9	50	33.8	94	50	35.4	99	50
44	37.3	50	35.3	95	50	35.6	95	50
48	38.1	50	35.0	92	50	37.0	97	49
52	39.3	50	35.7	91	50	37.8	96	49
58	41.0	50	35.9	88	49	39.4	96	49
62	42.2	50	37.3	88	48	39.9	95	49
65	42.6	50	38.2	90	46	40.1	94	49
70	43.2	50	37.9	88	45	41.8	97	49
74	43.9	48	39.0	89	43	41.6	95	46
79	44.2	43	38.8	88	40	42.1	95	44
84	44.2	39	39.7	90	39	41.5	94	44
89	43.1	32	39.2	91	38	39.7	92	40
93	44.4	27	37.8	85	36	40.1	90	38
97	45.3	25	37.5	83	31	40.3	89	33
101	44.9	25	37.9	84	24	40.3	90	26
104	45.0	23	38.4	85	23	41.1	91	24

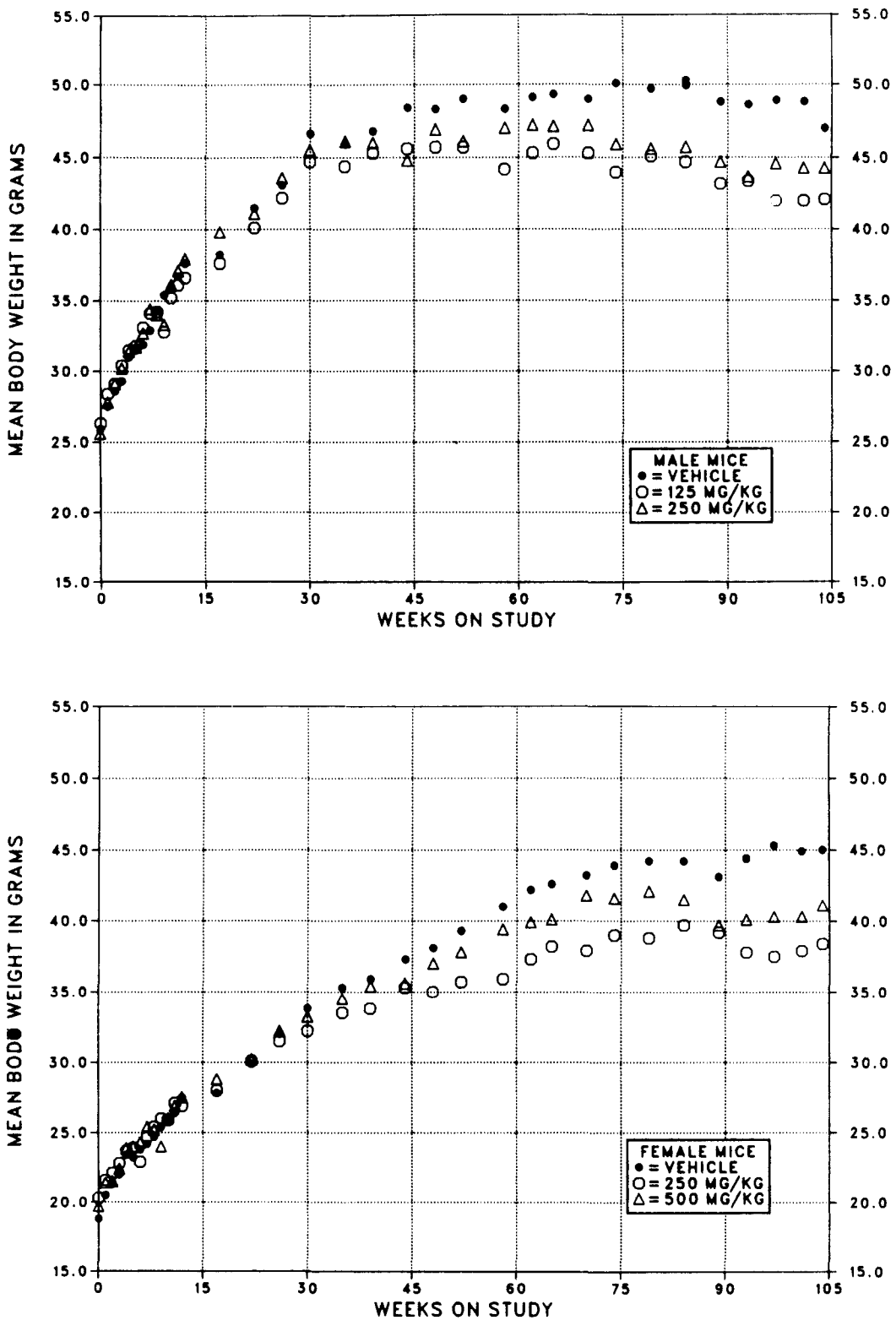


FIGURE 5. GROWTH CURVES FOR MICE ADMINISTERED C.I. ACID ORANGE 3 IN CORN OIL BY GAVAGE FOR TWO YEARS

Survival

Estimates of the probabilities of survival for male and female mice administered C.I. Acid Orange 3 at the doses used in these studies and for vehicle controls are shown in Table 18 and in the Kaplan and Meier curves in Figure 6. Survival of both the low dose (after week 102) and high dose (after week 100) groups of male mice was significantly lower than that of the vehicle controls. No significant differences in survival were observed between any groups of female mice.

Pathology and Statistical Analyses of Results

This section describes the significant or noteworthy changes in the incidences of mice with neoplastic or nonneoplastic lesions of the kidney, urinary bladder, skin, circulatory system, and forestomach.

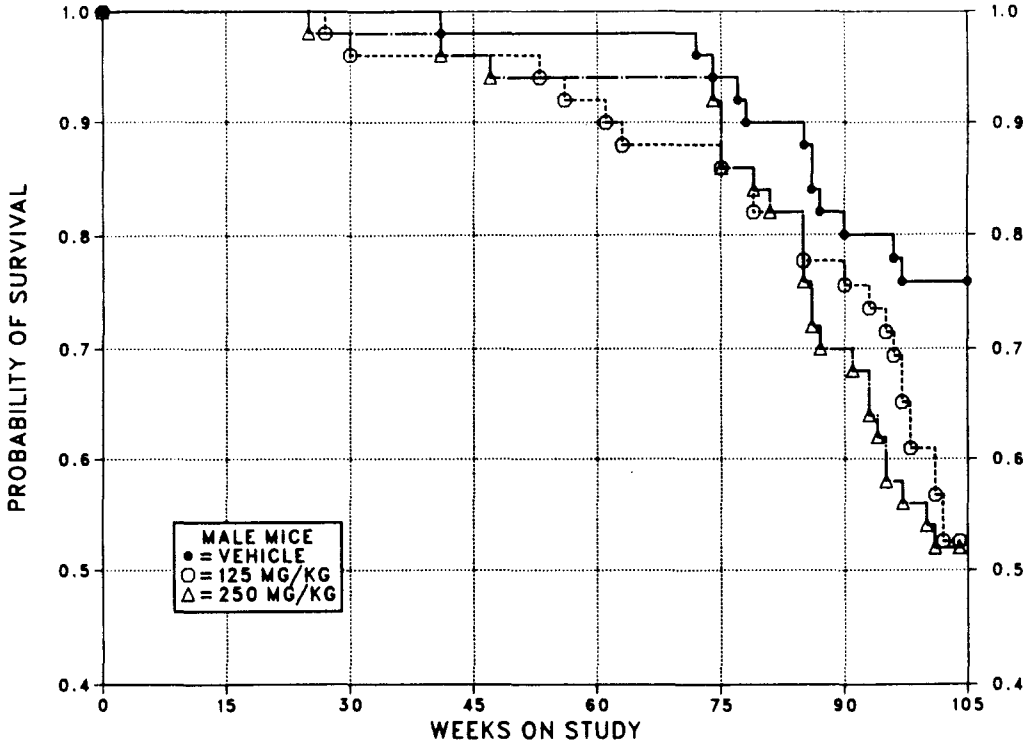
Lesions in male mice are summarized in Appendix C. Histopathologic findings on neoplasms are summarized in Table C1. Table C2 gives the survival and tumor status for individual male mice. Table C3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table C3 (footnotes). Historical incidences of tumors in corn oil vehicle control male mice are listed in Table C4. Findings on nonneoplastic lesions are summarized in Table C5.

Lesions in female mice are summarized in Appendix D. Histopathologic findings on neoplasms are summarized in Table D1. Table D2 gives the survival and tumor status for individual female mice. Table D3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table D3 (footnotes). Historical incidences of tumors in corn oil vehicle control female mice are listed in Table D4. Findings on nonneoplastic lesions are summarized in Table D5.

TABLE 18. SURVIVAL OF MICE IN THE TWO-YEAR GAVAGE STUDIES OF C.I. ACID ORANGE 3

	Vehicle Control	Low Dose	High Dose
MALE (a)		**125 mg/kg**	**250 mg/kg**
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	12	23	24
Accidentally killed	0	2	0
Killed at termination	38	25	26
Survival P values (c)	0.021	0.036	0.025
FEMALE (a)		**250 mg/kg**	**500 mg/kg**
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	27	27	26
Killed at termination	23	23	23
Died during termination period	0	0	1
Survival P values (c)	0.580	0.948	0.615

(a) Terminal-kill period: weeks 104-105
(b) Includes animals killed in a moribund condition
(c) The result of the life table trend test is in the vehicle control column, and the results of the life table pairwise comparisons with the vehicle controls are in the dosed columns



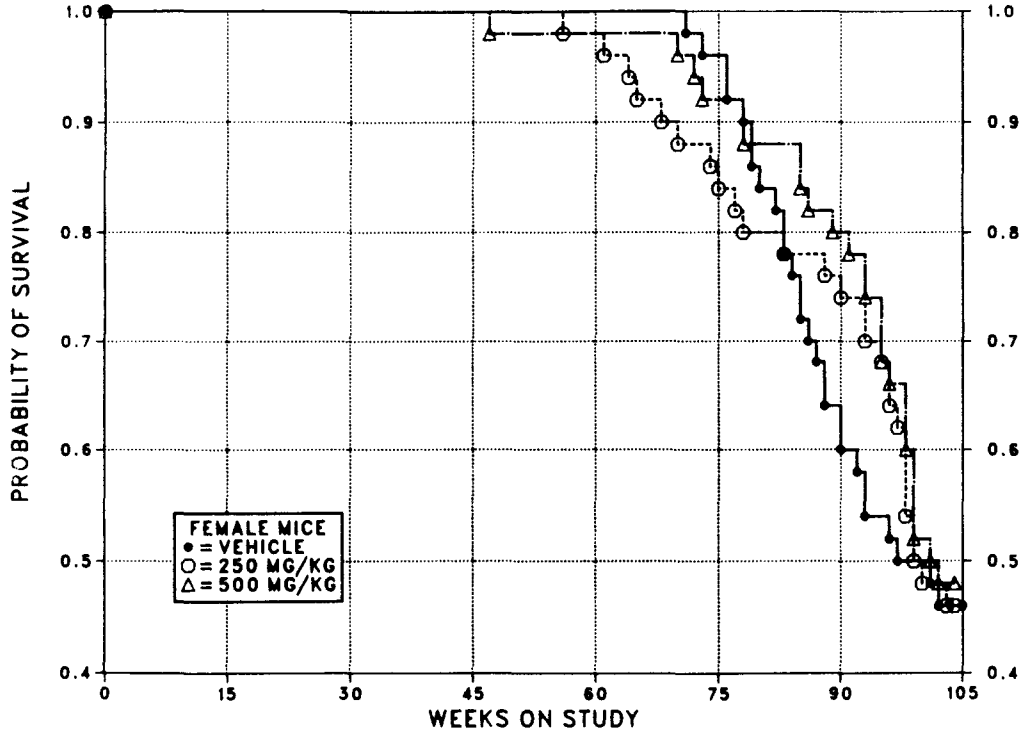


FIGURE 6. KAPLAN-MEIER SURVIVAL CURVES FOR MICE ADMINISTERED C.I. ACID ORANGE 3 IN CORN OIL BY GAVAGE FOR TWO YEARS

Kidney: A spectrum of chemical-related nonneoplastic lesions occurred in the kidney of male and female mice (Table 19). These lesions included an increased incidence and/or severity of inflammation, fibrosis, nephrosis, papillary degeneration, medullary (papillary) necrosis, tubular dilatation, tubular mineralization, and lymphoid hyperplasia. All mice with medullary necrosis died before the termination of the studies. Despite this chemically induced nephrotoxicity, the only kidney neoplasm observed was a tubular cell adenoma in a vehicle control male mouse.

Urinary Bladder: Epithelial hyperplasia was observed in 0/50 vehicle control, 1/49 low dose, and 3/50 high dose female mice. A squamous cell carcinoma was seen in one low dose female mouse. No squamous cell urinary bladder neoplasms were observed in 1,665 historical corn oil vehicle control female $B6C3F_1$ mice.

Skin: Chronic inflammation was observed at increased incidences in dosed male mice (vehicle control, 3/50; low dose, 15/50; high dose, 15/50; $P<0.01$). These lesions are believed to be secondary to fighting. The animals were group housed, and the position of the lesions correlated with typical fighting wounds in group housed male mice.

Negative Trends: Negative trends occurred for the incidences of hemangiosarcomas in male mice (vehicle control, 6/50; low dose, 1/50; high dose, 2/50) and squamous cell papillomas of the forestomach in female mice (4/50; 0/50; 0/50). The vehicle control values for both lesions were notably greater than the mean historical incidences (Appendix C, Table C4; Appendix D, Table D4a).

TABLE 19. NUMBERS OF MICE WITH RENAL LESIONS IN THE TWO-YEAR GAVAGE STUDIES OF C.I. ACID ORANGE 3

Lesion	Male			Female		
	0	125 mg/kg	250 mg/kg	0	250 mg/kg	500 mg/kg
No. of animals examined	50	50	50	50	50	50
Inflammation	1	4	(a) 12	7	7	(a) 22
Fibrosis	0	(b) 5	(a) 19	4	9	(a) 31
Nephrosis	47	47	45	13	(a) 42	(a) 50
Papillary degeneration	0	4	(a) 18	0	3	(a) 19
Medullary (papillary) necrosis	0	0	(b) 6	2	5	(b) 8
Tubular dilatation	2	(a) 39	(a) 33	2	(a) 35	(a) 42
Tubular mineralization	31	20	25	3	(a) 15	(a) 22
Lymphoid hyperplasia	18	(a) 35	(a) 33	20	24	29

(a) $P<0.01$ vs. vehicle controls
(b) $P<0.05$ vs. vehicle controls

IV. DISCUSSION AND CONCLUSIONS

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In rats, the administration of C.I. Acid Orange 3 at 1,500 mg/kg per day for 13 weeks produced renal toxicity. The nephropathy was characterized by nephrosis in males and females and suppurative inflammation and papillary necrosis in females. In the 1,500 mg/kg dose group, 5/10 females died between weeks 1 and 8. The final mean body weight of surviving animals was 8% lower than that of the vehicle control group for males and 5% lower for females. Because of the extent and severity of the renal lesions, doses of 375 and 750 mg/kg per day were selected for the 2-year studies in rats.

In mice, no animals died after the administration of C.I. Acid Orange 3 at doses as high as 2,000 mg/kg for 13 weeks; however, this dose did reduce final body weights by 11%-12% in each sex. C.I. Acid Orange 3 was nephrotoxic to mice of either sex. Nephropathy in males and females dosed at 1,000 and 2,000 mg/kg consisted of increased basophilia of the tubular epithelium, tubular dilatation, and cast formation.

Because the renal lesions observed were considered to be potentially life threatening, doses of 125 and 250 mg/kg were selected for the 2-year study in male mice and 250 and 500 mg/kg were selected for the 2-year study in females. The lower dose for males was selected because the renal lesions were judged to be more severe in male mice dosed with 1,000 mg/kg for 13 weeks than in females. This suggested that males might be somewhat more sensitive than females to the chronic renal effects of C.I. Acid Orange 3.

In the 2-year studies, the administration of 750 mg/kg C.I. Acid Orange 3 reduced the survival of male and female rats, and the administration of the dye at 125 or 250 mg/kg significantly reduced the survival of dosed male mice. C.I. Acid Orange 3 at 250 or 500 mg/kg did not reduce survival of female mice. The primary cause of death in both species was the spectrum of nonneoplastic lesions in the kidney. These included nephropathy, hyperplasia of the pelvic epithelium, papillary necrosis, inflammation, and pigmentation (see Tables 12 and 19). Nephropathy is an age-related disease process characterized by varied degrees of degeneration, regeneration, and atrophy of the tubular epithelium; hyaline tubular casts; glomerulosclerosis; and interstitial fibrosis. Nephropathy was present in nearly all male rats of each group, but the severity of this lesion was judged to be greater in dosed animals. The incidence and severity of nephropathy were also increased in dosed female rats. Hyperplasia of the transitional epithelium overlying the renal papilla frequently accompanies severe nephropathy, and the increased incidences in dosed rats may reflect the enhanced nephropathy.

Suppurative inflammation consisting of focal aggregates of neutrophils within the lumens of tubules in the papilla, medulla, and cortex also occurred at increased incidences in dosed rats. Necrosis of the renal papilla occurred primarily in eight high dose females that died between weeks 11 and 17 of the study. A single high dose female died with papillary necrosis during week 97. Two dosed males also had papillary necrosis.

Renal papillary necrosis is a hallmark lesion of chronic abuse of nonsteroid anti-inflammatory drugs in humans (Stygles and Iuliucci, 1981). This lesion is produced in laboratory animals by several analgesics, such as aspirin, phenacetin, and sodium salicylate, given at large doses for a long period of time. The mechanism of production of papillary necrosis by nonsteroid anti-inflammatory agents is unknown, but microscopic evidence of impaired blood flow to the renal papilla in rats administered aspirin has been reported (Kincaid-Smith, 1967; Nanra and Kincaid-Smith, 1970; Nanra, 1974).

No evidence of renal vascular change was observed in animals dosed with C.I. Acid Orange 3, but such changes could have been masked by the extensive pathologic renal effects produced by the dye.

An increased incidence of orange-brown pigment located within the epithelium of cortical tubules and interstitial macrophages was observed in high dose male rats and, to a much lesser extent, in female rats. The amount of accumulated pigment was minimal and may represent hemosiderin and/or C.I. Acid Orange 3 or a metabolite.

A spectrum of nonneoplastic lesions characteristic of uremia and renal secondary hyperparathyroidism occurred in male and female rats.

These lesions in dosed rats reflect the increased severity of nephropathy associated with the administration of C.I. Acid Orange 3. Mineralization of the glandular stomach and aorta and erosion and ulcers of the glandular stomach are frequently associated with uremia. Fibrous dysplasia (osteodystrophy and osteitis fibrosa cystica) reflects profound disturbances in divalent ion metabolism. The pathophysiology of this metabolic bone disease is complex. The severe renal disease results in phosphate retention and abnormal vitamin D metabolism wherein formation of the active 1,25-dihydroxy metabolite of vitamin D is diminished. These factors reduce plasma calcium and cause increased secretion of parathyroid hormone and eventually parathyroid hyperplasia. The parathyroid hormone mobilizes calcium from the bone and increases urinary phosphate excretion to return the plasma concentrations of calcium and phosphate to normal. As the kidney loses the ability to compensate and respond to parathyroid hormone, bone becomes more resistant to the effects of parathyroid hormone and the absorption of calcium from the intestine is reduced by the impaired synthesis of 1,25-dihydroxycholecalciferol. This leads to reduced calcification of bone and excessive production of fibrous connective tissue in bone. Mineralization of soft tissues occurs because the high levels of plasma phosphate and the calcium mobilized from bone upset the normal plasma calcium/phosphate ratio.

Parathyroid hyperplasia was diagnosed in eight high dose male rats and one high dose female rat. Although there was no strong correlation between parathyroid hyperplasia and fibrous dysplasia of the femur and mineralization of the glandular stomach and aorta in rats, these latter lesions are considered to be due to hyperparathyroidism secondary to renal disease.

Despite the reduced survival of high dose male rats, the low dose male rat group is considered to be adequate for a long-term study of carcinogenicity because survival in the 375 mg/kg dose group (30/50) was similar to that in vehicle controls (36/50) Deaths in the 750 mg/kg group were chemically related and were probably caused by adverse effects on the kidney. The 375 mg/kg dose, at which notable kidney toxicity was produced, is considered to be the largest dose that could be administered under the conditions of these studies.

Although survival of dosed male mice was reduced, the study is considered to be adequate for the assessment of carcinogenicity because a sufficient number of animals were at risk of a carcinogenic effect for over 90 weeks. As late as week 93, 70% of the low dose and 64% of the high dose males were still alive, and the survival of vehicle controls was somewhat higher than normal.

The administration of C.I. Acid Orange 3 at the high dose produced an increase in the incidence of transitional cell carcinomas of the renal pelvis in female rats (see Table 12). The carcinomas originated from the transitional epithelium of the renal pelvis and exhibited cellular atypia and local invasion of the submucosa. Renal transitional cell carcinomas have not been previously observed in approximately 1,700 historical corn oil vehicle control F344/N female rats. In addition, there was a dose-related increase in the incidence of epithelial hyperplasia of the renal pelvis. Although epithelial hyperplasia of the renal pelvis was increased in dosed male rats, no kidney neoplasms were found. Perhaps this is a reflection of the reduced survival in high dose males. The first pelvic transitional cell carcinoma was detected in a female rat that died during week 87. Only nine males in the high dose group were alive during week 87, and all were dead by week 97.

Increased incidences of testicular interstitial cell hyperplasia and concomitant decreases in the incidences of interstitial cell tumors were seen in dosed male rats. These trends are consistent with the reduced survival pattern of the dosed male rats.

The administration of C.I. Acid Orange 3 to mice did not produce any significant increases in neoplasia. There was a marginally increased incidence of epithelial hyperplasia of the urinary bladder in female mice, and one low dose female had a squamous cell carcinoma of the urinary bladder. Squamous cell urinary bladder neoplasms have not been previously observed in 1,665 corn oil vehicle control female B6C3F$_1$ mice in NTP studies. Whether this neoplasm

was due to the administration of C.I. Acid Orange 3 cannot be determined.

The experimental and tabulated data for the NTP Technical Report on C.I. Acid Orange 3 were examined for accuracy, consistency, completeness, and compliance with Good Laboratory Practice regulations. As summarized in Appendix H, the audit revealed no major problems with the conduct of the studies or with collection and documentation of the experimental data. No discrepancies were found that influenced the final interpretation of the results of these studies.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of C.I. Acid Orange 3 for male F344/N rats administered 375 mg/kg; because of a marked reduction in survival and no indication of carcinogenicity, the 750 mg/kg group was considered to be inadequate for assessment of carcinogenic activity. There was *clear evidence of carcinogenic activity* of C.I. Acid Orange 3 for female F344/N rats as shown by the occurrence of transitional cell carcinomas of the kidney in the 750 mg/kg group; this group had reduced survival and chemically related nonneoplastic lesions of the kidney. There was *no evidence of carcinogenic activity* of C.I. Acid Orange 3 for male $B6C3F_1$ mice administered 125 or 250 mg/kg or for female $B6C3F_1$ mice administered 250 or 500 mg/kg. Nonneoplastic lesions of the kidney were observed in both dose groups of both sexes of rats and mice.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 9.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 12.

V. REFERENCES

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APPENDIX A

SUMMARY OF LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Papilloma, NOS	4 (8%)		
Basal cell carcinoma		1 (2%)	
Sebaceous adenoma	1 (2%)		
Keratoacanthoma		1 (2%)	
Fibroma	5 (10%)	2 (4%)	
Fibrosarcoma	1 (2%)		
Fibrous histiocytoma, malignant	1 (2%)		
Myxosarcoma		1 (2%)	
RESPIRATORY SYSTEM			
#Lung	(48)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)		
Alveolar/bronchiolar carcinoma	1 (2%)	1 (2%)	
C-cell carcinoma, metastatic	1 (2%)		
Interstitial cell tumor, metastatic	1 (2%)		
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Leukemia, mononuclear cell	10 (20%)	10 (20%)	2 (4%)
#Spleen	(50)	(50)	(49)
Leukemia, mononuclear cell		1 (2%)	
#Thymus	(36)	(39)	(35)
Thymoma, benign	1 (3%)		1 (3%)
CIRCULATORY SYSTEM			
None			
DIGESTIVE SYSTEM			
#Liver	(50)	(50)	(50)
Neoplastic nodule	2 (4%)		
#Pancreas	(50)	(50)	(50)
Acinar cell adenoma	5 (10%)	6 (12%)	1 (2%)
Acinar cell carcinoma	1 (2%)		1 (2%)
#Forestomach	(50)	(50)	(50)
Squamous cell papilloma			1 (2%)
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Tubular cell adenocarcinoma	1 (2%)		
#Urinary bladder	(50)	(50)	(49)
Transitional cell papilloma	1 (2%)		
ENDOCRINE SYSTEM			
#Pituitary	(49)	(46)	(48)
Carcinoma, NOS	3 (6%)	1 (2%)	
Adenoma, NOS	11 (22%)	9 (20%)	1 (2%)
#Adrenal	(50)	(50)	(50)
Pheochromocytoma	15 (30%)	13 (26%)	8 (16%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Thyroid	(50)	(50)	(49)
Follicular cell adenoma	1 (2%)		1 (2%)
C-cell adenoma	7 (14%)	5 (10%)	
C-cell carcinoma	1 (2%)		
#Pancreatic islets	(50)	(50)	(50)
Islet cell adenoma	3 (6%)	1 (2%)	1 (2%)
Islet cell carcinoma	1 (2%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Fibroadenoma	2 (4%)	1 (2%)	
*Preputial gland	(50)	(50)	(50)
Carcinoma, NOS	1 (2%)	1 (2%)	
Adenoma, NOS		1 (2%)	
#Testis	(50)	(50)	(48)
Interstitial cell tumor	46 (92%)	34 (68%)	22 (46%)
Interstitial cell tumor, malignant	1 (2%)		
NERVOUS SYSTEM			
#Cerebrum	(50)	(50)	(50)
Astrocytoma		1 (2%)	
SPECIAL SENSE ORGANS			
*Zymbal gland	(50)	(50)	(50)
Carcinoma, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
*Mesentery	(50)	(50)	(50)
Mesothelioma, NOS	1 (2%)		1 (2%)
*Tunica vaginalis	(50)	(50)	(50)
Mesothelioma, NOS	1 (2%)		
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Mesothelioma, NOS	1 (2%)		
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	3	4	13
Moribund sacrifice	7	11	31
Terminal sacrifice	36	30	
Dosing accident	4	4	4
Accidentally killed, NOS		1	2

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	49	41	25
Total primary tumors	131	91	40
Total animals with benign tumors	49	39	24
Total benign tumors	104	73	36
Total animals with malignant tumors	19	16	3
Total malignant tumors	22	18	3
Total animals with secondary tumors##	2		
Total secondary tumors	2		
Total animals with tumors uncertain--benign or malignant	5		1
Total uncertain tumors	5		1

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3: VEHICLE CONTROL

ANIMAL NUMBER	026	013	035	040	033	045	019	006	015	029	022	025	047	050	010	028	001	002	003	004	005	007	008	009	011
WEEKS ON STUDY	070	073	081	081	082	082	087	096	096	098	101	102	102	103	104	104	105	105	105	105	105	105	105	105	105
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma, NOS										X					X										X
Sebaceous adenoma																									
Fibroma										X															
Fibrosarcoma																								X	
Fibrous histiocytoma, malignant																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																								X	
Alveolar/bronchiolar carcinoma																							X		
C-cell carcinoma, metastatic																									
Interstitial cell tumor, metastatic																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	–	+	+	+	–	+	+	–	–	+	+	–	–	+	+	+	+	–	+	–	–	+	–	+	+
Thymoma, benign																									
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
Neoplastic nodule															X										
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell adenoma																							X		
Acinar cell carcinoma																				X					
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenocarcinoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional cell papilloma							X																		
ENDOCRINE SYSTEM																									
Pituitary	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS									X			X													
Adenoma, NOS								X		X			X	X		X			X				X		
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma			X							X	X	X	X							X	X	X	X		
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																									
C-cell adenoma																X				X					
C-cell carcinoma																									
Parathyroid	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+	
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									X
Islet cell carcinoma											X														
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	N	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																						X			
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X
Interstitial cell tumor, malignant																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BODY CAVITIES																									
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma, NOS																									
Mesentery	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, NOS					X																				
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, NOS						X																			
Leukemia, mononuclear cell								X	X				X		X		X		X					X	

+: Tissue examined microscopically
–: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
.: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: VEHICLE CONTROL (Continued)

ANIMAL NUMBER	0 1 2	0 1 4	0 1 6	0 1 7	0 1 8	0 2 0	0 2 1	0 2 3	0 2 4	0 2 7	0 3 0	0 3 1	0 3 2	0 3 4	0 3 6	0 3 7	0 3 8	0 3 9	0 4 1	0 4 2	0 4 3	0 4 4	0 4 6	0 4 8	0 4 9	TOTAL:
WEEKS ON STUDY	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Papilloma, NOS																						X				4
Sebaceous adenoma																						X				1
Fibroma		X																	X		X			X		5
Fibrosarcoma																										1
Fibrous histiocytoma, malignant																				X						1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma																									X	2
Alveolar/bronchiolar carcinoma																										1
C-cell carcinoma, metastatic			X																							1
Interstitial cell tumor, metastatic							X																			1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	36
Thymoma, benign																									X	1
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule																								X		2
Bile duct	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinar cell adenoma		X									X							X		X						5
Acinar cell carcinoma																										1
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenocarcinoma				X																						1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional cell papilloma																										1
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, NOS				X																						3
Adenoma, NOS		X					X									X	X									11
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma					X		X			X				X	X				X							15
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma												X														1
C-cell adenoma				X	X	X						X								X						7
C-cell carcinoma			X																							1
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma						X															X					3
Islet cell carcinoma																										1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroadenoma				X																						2
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell tumor	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	46
Interstitial cell tumor, malignant							X																			1
Prostate	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS												X														1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
BODY CAVITIES																										
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Mesothelioma, NOS													X													1
Mesentery	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mesothelioma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mesothelioma, NOS																										1
Leukemia, mononuclear cell							X							X		X										10

* Animals necropsied

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3: LOW DOSE

ANIMAL NUMBER	0013	0002	0024	0021	0028	0007	0025	0011	0017	0008	0046	0031	0012	0018	0009	0027	0026	0003	0048	0006	0001	0004	0005	0010	0014
WEEKS ON STUDY	0027	0055	0060	0069	0073	0075	0077	0079	0079	0080	0080	0083	0084	0084	0085	0085	0086	0088	1000	1001	1004	1004	1004	1004	1004
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																									
Keratoacanthoma																									
Fibroma																		X							
Myxosarcoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia, mononuclear cell													X												
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	-	-	+	-	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell adenoma																					X				X
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Carcinoma, NOS	X																								
Adenoma, NOS					X							X						X							
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma													X		X			X	X			X			
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma													X												
Parathyroid	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	N
Fibroadenoma																									
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor									X	X	X		X				X		X	X	X	X	X	X	X
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
Adenoma, NOS																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma																	X								
SPECIAL SENSE ORGANS																									
Zymbal gland	+	N	+	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	+	N	+	N	+
Carcinoma, NOS			X																						
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell															X				X					X	

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: LOW DOSE (Continued)

ANIMAL NUMBER	0 1 5	0 1 6	0 1 9	0 2 0	0 2 2	0 2 3	0 2 9	0 3 0	0 3 2	0 3 3	0 3 4	0 3 5	0 3 6	0 3 7	0 3 8	0 3 9	0 4 0	0 4 1	0 4 2	0 4 3	0 4 4	0 4 5	0 4 7	0 4 9	0 5 0	TOTAL:
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Basal cell carcinoma											X															1
Keratoacanthoma				X																						1
Fibroma												X														2
Myxosarcoma									X																	1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																		X								1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia, mononuclear cell																										1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	–	–	+	+	+	+	+	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	39
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	50
Acinar cell adenoma							X		X		X		X													6
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	46
Carcinoma, NOS																										1
Adenoma, NOS			X			X									X	X		X			X					9
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma	X		X		X						X			X	X			X			X					13
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell adenoma								X				X					X			X						5
Parathyroid	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma														X												1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	N	+	+	N	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	*50
Fibroadenoma								X																		1
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell tumor	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X		X	X	X	X	34
Prostate	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																							X			1
Adenoma, NOS													X													1
NERVOUS SYSTEM																										
Brain	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma																										1
SPECIAL SENSE ORGANS																										
Zymbal gland	+	N	+	N	N	+	N	N	+	N	+	N	N	N	N	N	N	+	N	N	N	N	N	N	+	*50
Carcinoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell						X			X					X		X		X		X					X	10

* Animals necropsied

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3: HIGH DOSE

ANIMAL NUMBER	0015	0004	0003	0288	0409	0045	0166	0333	0411	0488	0337	0244	0022	0122	0255	0446	0338	0227	0221	0144	0335	0066	0311	0420
WEEKS ON STUDY	0006	0111	0235	0255	0330	0332	0332	0333	0333	0333	0333	0339	0400	0422	0422	0545	0559	0561	0563	0567	0568	0568	0570	0700	0700
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	–	+	+	–	–	+	–	+	–	+	–	+	+	+	–	+	+	+	+	+	+
Thymoma, benign																									
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell adenoma																									
Acinar cell carcinoma																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																							X		
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																									
Parathyroid	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	+	+	N	+	+	N	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor																			X		X	X			
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BODY CAVITIES																									
Mesentery	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell																									

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: HIGH DOSE (Continued)

ANIMAL NUMBER	0262	0113	0334	0001	0111	0222	0449	0118	0119	0443	0550	0229	0330	0220	0444	0447	0110	0055	0007	0117	0223	0339	0336	0008	0332	TOTAL:
WEEKS ON STUDY	0772	0755	0756	0776	0766	0766	0777	0799	0799	0799	0811	0822	0833	0844	0844	0866	0888	0944	0994	0994	0994	0994	0995	0996	0996	TISSUES TUMORS
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	–	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	–	–	+	–	+	–	+	–	35
Thymoma, benign																						X				1
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinar cell adenoma																						X				1
Acinar cell carcinoma																					X					1
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma							X																			1
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	48
Adenoma, NOS																										1
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma							X						X			.	X	X	X		X			X	X	8
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	49
Follicular cell adenoma																		X								1
Parathyroid	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+	–	–	+	+	+	45
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma																				X						1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	N	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	*50
Testis	+	+	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	48
Interstitial cell tumor		X	X	X	X		X			X	X		X		X	X	X	X	X	X	X	X	X	X	X	22
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
BODY CAVITIES																										
Mesentery	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mesothelioma, NOS					X																					1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell												X								X						2

* Animals necropsied

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

	Vehicle Control	375 mg/kg	750 mg/kg
Skin: Papilloma			
Overall Rates (a)	4/50 (8%)	0/50 (0%)	0/50 (0%)
Adjusted Rates (b)	10.6%	0.0%	0.0%
Terminal Rates (c)	3/36 (8%)	0/30 (0%)	0/0
Week of First Observation	98		
Life Table Test (d)		P=0.093N	
Incidental Tumor Test (d)		P=0.125N	
Fisher Exact Test (d)		P=0.059N	
Skin: Fibroma			
Overall Rates (a)	5/50 (10%)	2/50 (4%)	0/50 (0%)
Adjusted Rates (b)	13.3%	6.3%	0.0%
Terminal Rates (c)	4/36 (11%)	1/30 (3%)	0/0
Week of First Observation	98	88	
Life Table Test (d)		P=0.308N	
Incidental Tumor Test (d)		P=0.292N	
Fisher Exact Test (d)		P=0.218N	
Skin: Fibroma or Fibrosarcoma			
Overall Rates (a)	6/50 (12%)	2/50 (4%)	0/50 (0%)
Adjusted Rates (b)	16.0%	6.3%	0.0%
Terminal Rates (c)	5/36 (14%)	1/30 (3%)	0/0
Week of First Observation	98	88	
Life Table Test (d)		P=0.210N	
Incidental Tumor Test (d)		P=0.196N	
Fisher Exact Test (d)		P=0.134N	
Skin: Fibroma, Fibrosarcoma, or Myxosarcoma			
Overall Rates (a)	6/50 (12%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	16.0%	9.5%	0.0%
Terminal Rates (c)	5/36 (14%)	2/30 (7%)	0/0
Week of First Observation	98	88	
Life Table Test (d)		P=0.350N	
Incidental Tumor Test (d)		P=0.336N	
Fisher Exact Test (d)		P=0.243N	
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	3/48 (6%)	1/50 (2%)	0/50 (0%)
Adjusted Rates (b)	8.6%	3.3%	0.0%
Terminal Rates (c)	3/35 (9%)	1/30 (3%)	0/0
Week of First Observation	105	104	
Life Table Test (d)		P=0.361N	
Incidental Tumor Test (d)		P=0.361N	
Fisher Exact Test (d)		P=0.293N	
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	10/50 (20%)	11/50 (22%)	2/50 (4%)
Adjusted Rates (b)	25.2%	32.7%	18.7%
Terminal Rates (c)	7/36 (19%)	8/30 (27%)	0/0
Week of First Observation	96	84	82
Life Table Test (d)		P=0.311	
Incidental Tumor Test (d)		P=0.232	
Fisher Exact Test (d)		P=0.500	
Pancreas: Acinar Cell Adenoma			
Overall Rates (a)	5/50 (10%)	6/50 (12%)	1/50 (2%)
Adjusted Rates (b)	13.9%	20.0%	12.5%
Terminal Rates (c)	5/36 (14%)	6/30 (20%)	0/0
Week of First Observation	105	104	94
Life Table Test (d)		P=0.371	
Incidental Tumor Test (d)		P=0.371	
Fisher Exact Test (d)		P=0.500	

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
Pancreas: Acinar Cell Adenoma or Carcinoma			
Overall Rates (a)	6/50 (12%)	6/50 (12%)	2/50 (4%)
Adjusted Rates (b)	16.7%	20.0%	25.0%
Terminal Rates (c)	6/36 (17%)	6/30 (20%)	0/0
Week of First Observation	105	104	94
Life Table Test (d)		P=0.488	
Incidental Tumor Test (d)		P=0.488	
Fisher Exact Test (d)		P=0.620	
Pituitary Gland: Adenoma			
Overall Rates (a)	11/49 (22%)	9/46 (20%)	1/48 (2%)
Adjusted Rates (b)	27.2%	26.7%	3.6%
Terminal Rates (c)	7/36 (19%)	6/29 (21%)	0/0
Week of First Observation	96	73	70
Life Table Test (d)		P=0.583	
Incidental Tumor Test (d)		P=0.486	
Fisher Exact Test (d)		P=0.464N	
Pituitary Gland: Carcinoma			
Overall Rates (a)	3/49 (6%)	1/46 (2%)	0/48 (0%)
Adjusted Rates (b)	7.5%	2.0%	0.0%
Terminal Rates (c)	1/36 (3%)	0/29 (0%)	0/0
Week of First Observation	96	27	
Life Table Test (d)		P=0.378N	
Incidental Tumor Test (d)		P=0.383N	
Fisher Exact Test (d)		P=0.333N	
Pituitary Gland: Adenoma or Carcinoma			
Overall Rates (a)	14/49 (29%)	10/46 (22%)	1/48 (2%)
Adjusted Rates (b)	33.2%	28.2%	3.6%
Terminal Rates (c)	8/36 (22%)	6/29 (21%)	0/0
Week of First Observation	96	27	70
Life Table Test (d)		P=0.458N	
Incidental Tumor Test (d)		P=0.578N	
Fisher Exact Test (d)		P=0.299N	
Adrenal Gland: Pheochromocytoma			
Overall Rates (a)	15/50 (30%)	13/50 (26%)	8/50 (16%)
Adjusted Rates (b)	36.2%	37.8%	100.0%
Terminal Rates (c)	10/36 (28%)	9/30 (30%)	0/0
Week of First Observation	81	84	79
Life Table Test (d)		P=0.520	
Incidental Tumor Test (d)		P=0.500	
Fisher Exact Test (d)		P=0.412N	
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	7/50 (14%)	5/50 (10%)	0/49 (0%)
Adjusted Rates (b)	19.4%	15.6%	0.0%
Terminal Rates (c)	7/36 (19%)	4/30 (13%)	0/0
Week of First Observation	105	84	
Life Table Test (d)		P=0.509N	
Incidental Tumor Test (d)		P=0.453N	
Fisher Exact Test (d)		P=0.380N	
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	8/50 (16%)	5/50 (10%)	0/49 (0%)
Adjusted Rates (b)	22.2%	15.6%	0.0%
Terminal Rates (c)	8/36 (22%)	4/30 (13%)	0/0
Week of First Observation	105	84	
Life Table Test (d)		P=0.400N	
Incidental Tumor Test (d)		P=0.348N	
Fisher Exact Test (d)		P=0.277N	

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
Pancreatic Islets: Islet Cell Adenoma			
Overall Rates (a)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted Rates (b)	8.3%	3.3%	12.5%
Terminal Rates (c)	3/36 (8%)	1/30 (3%)	0/0
Week of First Observation	105	104	94
Life Table Test (d)		P=0.372N	
Incidental Tumor Test (d)		P=0.372N	
Fisher Exact Test (d)		P=0.309N	
Pancreatic Islets: Islet Cell Adenoma or Carcinoma			
Overall Rates (a)	4/50 (8%)	1/50 (2%)	1/50 (2%)
Adjusted Rates (b)	10.6%	3.3%	12.5%
Terminal Rates (c)	3/36 (8%)	1/30 (3%)	0/0
Week of First Observation	101	104	94
Life Table Test (d)		P=0.245N	
Incidental Tumor Test (d)		P=0.305N	
Fisher Exact Test (d)		P=0.181N	
Testis: Interstitial Cell Tumor			
Overall Rates (a)	46/50 (92%)	34/50 (68%)	22/48 (46%)
Adjusted Rates (b)	95.8%	91.8%	100.0%
Terminal Rates (c)	34/36 (94%)	27/30 (90%)	0/0
Week of First Observation	73	79	63
Life Table Test (d)		P=0.237N	
Incidental Tumor Test (d)		P=0.044N	
Fisher Exact Test (d)		P=0.003N	
Testis: Interstitial Cell Tumor or Interstitial Cell Tumor, Malignant			
Overall Rates (a)	47/50 (94%)	34/50 (68%)	22/48 (46%)
Adjusted Rates (b)	97.9%	91.8%	100.0%
Terminal Rates (c)	35/36 (97%)	27/30 (90%)	0/0
Week of First Observation	73	79	63
Life Table Test (d)		P=0.178N	
Incidental Tumor Test (d)		P=0.018N	
Fisher Exact Test (d)		P=0.001N	
All Sites: Mesothelioma			
Overall Rates (a)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted Rates (b)	7.0%	0.0%	4.5%
Terminal Rates (c)	1/36 (3%)	0/30 (0%)	0/0
Week of First Observation	82		76
Life Table Test (d)		P=0.155N	
Incidental Tumor Test (d)		P=0.049N	
Fisher Exact Test (d)		P=0.122N	
All Sites: Benign Tumors			
Overall Rates (a)	49/50 (98%)	39/50 (78%)	24/50 (48%)
Adjusted Rates (b)	100.0%	95.1%	100.0%
Terminal Rates (c)	36/36 (100%)	28/30 (93%)	0/0
Week of First Observation	73	73	63
Life Table Test (d)		P=0.441N	
Incidental Tumor Test (d)		P=0.041N	
Fisher Exact Test (d)		P=0.002N	
All Sites: Malignant Tumors			
Overall Rates (a)	19/50 (38%)	16/50 (32%)	3/50 (6%)
Adjusted Rates (b)	46.1%	43.1%	30.4%
Terminal Rates (c)	14/36 (39%)	10/30 (33%)	0/0
Week of First Observation	96	27	82
Life Table Test (d)		P=0.555	
Incidental Tumor Test (d)		P=0.579N	
Fisher Exact Test (d)		P=0.338N	

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
All Sites: All Tumors			
Overall Rates (a)	49/50 (98%)	41/50 (82%)	25/50 (50%)
Adjusted Rates (b)	100.0%	95.3%	100.0%
Terminal Rates (c)	36/36 (100%)	28/30 (93%)	0/0
Week of First Observation	73	27	63
Life Table Test (d)		P=0.555	
Incidental Tumor Test (d)		P=0.070N	
Fisher Exact Test (d)		P=0.008N	

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Trend and high dose pairwise comparison with the vehicle control statistics are not presented because the reduced survival in the high dose group markedly lowered both the sensitivity of the tests for the detection of tumors and the opportunity for compound-related tumors to develop. Beneath the low dose group incidence are the P values corresponding to pairwise comparisons between that dosed group and the vehicle controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Fisher exact test compares directly the overall incidence rates. A lower incidence in a dosed group is indicated by (N).

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Epidermal inclusion cyst	4 (8%)	3 (6%)	
Hemorrhage		1 (2%)	
Ulcer, chronic			1 (2%)
Reaction, foreign body	1 (2%)		
Inflammation, pyogranulomatous	2 (4%)		
Hyperkeratosis		1 (2%)	
RESPIRATORY SYSTEM			
*Nasal cavity	(50)	(50)	(50)
Inflammation, suppurative	11 (22%)	13 (26%)	4 (8%)
Inflammation, chronic	1 (2%)	1 (2%)	
Infection, fungal	6 (12%)	8 (16%)	2 (4%)
Hyperplasia, epithelial		1 (2%)	
Metaplasia, squamous		1 (2%)	
#Lung	(48)	(50)	(50)
Aspiration, NOS	1 (2%)	4 (8%)	5 (10%)
Atelectasis	1 (2%)	1 (2%)	
Congestion, NOS		6 (12%)	1 (2%)
Edema, NOS	4 (8%)	3 (6%)	2 (4%)
Edema, interstitial			1 (2%)
Hemorrhage	4 (8%)	2 (4%)	2 (4%)
Inflammation, interstitial	1 (2%)		1 (2%)
Inflammation, suppurative	1 (2%)		
Inflammation, acute	1 (2%)		1 (2%)
Inflammation, chronic	2 (4%)	2 (4%)	
Inflammation, granulomatous	3 (6%)		
Alveolar macrophages	11 (23%)	11 (22%)	5 (10%)
Hyperplasia, alveolar epithelium	2 (4%)	3 (6%)	
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Depletion, lymphoid			2 (4%)
#Bone marrow	(50)	(50)	(50)
Atrophy, NOS		1 (2%)	
Metaplasia, osseous	1 (2%)		
Myelofibrosis	2 (4%)		
#Spleen	(50)	(50)	(49)
Congestion, NOS		1 (2%)	
Hemorrhage		1 (2%)	
Necrosis, NOS		1 (2%)	
Hemosiderosis	1 (2%)		
Atrophy, focal	1 (2%)		
Hematopoiesis	2 (4%)		
#Splenic red pulp	(50)	(50)	(49)
Fibrosis	1 (2%)		
Atrophy, NOS	2 (4%)		1 (2%)
#Lymph node	(50)	(50)	(50)
Hyperplasia, NOS		1 (2%)	

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Mandibular lymph node	(50)	(50)	(50)
Plasmacytosis	8 (16%)		
Erythrophagocytosis	1 (2%)		
Hyperplasia, lymphoid		1 (2%)	
#Mediastinal lymph node	(50)	(50)	(50)
Hemorrhage	2 (4%)	1 (2%)	
Pigmentation, NOS	2 (4%)	2 (4%)	
Histiocytosis	1 (2%)		
Erythrophagocytosis	3 (6%)	2 (4%)	
#Mesenteric lymph node	(50)	(50)	(50)
Hemorrhage	2 (4%)		1 (2%)
Pigmentation, NOS	11 (22%)	2 (4%)	
Atrophy, NOS	8 (16%)	12 (24%)	17 (34%)
Mastocytosis	1 (2%)		
#Renal lymph node	(50)	(50)	(50)
Hemorrhage			2 (4%)
Atrophy, NOS			3 (6%)
#Iliac lymph node	(50)	(50)	(50)
Pigmentation, NOS	1 (2%)		
Histiocytosis	1 (2%)		
#Inguinal lymph node	(50)	(50)	(50)
Plasmacytosis	1 (2%)		
*Nasal cavity	(50)	(50)	(50)
Hyperplasia, lymphoid		1 (2%)	
#Liver	(50)	(50)	(50)
Hematopoiesis	2 (4%)		
#Kidney	(50)	(50)	(50)
Hyperplasia, lymphoid			1 (2%)
#Thymus	(36)	(39)	(35)
Hemorrhage		1 (3%)	1 (3%)
Inflammation, suppurative			1 (3%)
Atrophy, NOS		1 (3%)	2 (6%)
CIRCULATORY SYSTEM			
#Mandibular lymph node	(50)	(50)	(50)
Lymphangiectasis	5 (10%)	2 (4%)	
#Mediastinal lymph node	(50)	(50)	(50)
Lymphangiectasis	2 (4%)		1 (2%)
#Mesenteric lymph node	(50)	(50)	(50)
Lymphangiectasis			1 (2%)
#Renal lymph node	(50)	(50)	(50)
Lymphangiectasis			3 (6%)
#Inguinal lymph node	(50)	(50)	(50)
Lymphangiectasis	1 (2%)		
#Heart	(50)	(50)	(50)
Mineralization			2 (4%)
Inflammation, chronic	5 (10%)	4 (8%)	9 (18%)
Fibrosis	41 (82%)	33 (66%)	32 (64%)
Fibrosis, focal	1 (2%)		
Pigmentation, NOS	1 (2%)		2 (4%)
Atrophy, focal			1 (2%)
#Heart/atrium	(50)	(50)	(50)
Thrombosis, NOS	3 (6%)		
*Artery	(50)	(50)	(50)
Thrombosis, NOS			1 (2%)
Periarteritis	5 (10%)	3 (6%)	4 (8%)
Degeneration, hyaline			3 (6%)
*Aorta	(50)	(50)	(50)
Mineralization		1 (2%)	3 (6%)
Inflammation, chronic		1 (2%)	

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
CIRCULATORY SYSTEM (Continued)			
*Coronary artery	(50)	(50)	(50)
Mineralization	1 (2%)		1 (2%)
Inflammation, necrotizing	1 (2%)		
*Pulmonary artery	(50)	(50)	(50)
Mineralization	3 (6%)	4 (8%)	
*Cerebral artery	(50)	(50)	(50)
Mineralization		1 (2%)	
*Mesenteric artery	(50)	(50)	(50)
Mineralization			1 (2%)
*Hepatic vein	(50)	(50)	(50)
Thrombosis, NOS			1 (2%)
DIGESTIVE SYSTEM			
#Salivary gland	(49)	(49)	(49)
Ectopia			2 (4%)
Retention of content		1 (2%)	
Cyst, NOS	1 (2%)		
#Liver	(50)	(50)	(50)
Cyst, NOS	2 (4%)		
Inflammation, acute	1 (2%)		1 (2%)
Inflammation, chronic	11 (22%)	6 (12%)	1 (2%)
Inflammation, granulomatous	1 (2%)		
Adhesion, NOS		1 (2%)	
Basophilic cyto change	1 (2%)		
Hyperplasia, nodular	1 (2%)		
Angiectasis	2 (4%)	2 (4%)	
Nodular regeneration	1 (2%)		
#Liver/hepatocytes	(50)	(50)	(50)
Necrosis, NOS	2 (4%)	1 (2%)	1 (2%)
Cytoplasmic vacuolization	17 (34%)	22 (44%)	5 (10%)
Basophilic cyto change	7 (14%)	11 (22%)	1 (2%)
Atrophy, focal	9 (18%)	6 (12%)	3 (6%)
#Bile duct	(50)	(50)	(50)
Hyperplasia, NOS	46 (92%)	36 (72%)	10 (20%)
#Pancreas	(50)	(50)	(50)
Cyst, NOS		1 (2%)	
Fibrosis, focal		1 (2%)	
#Pancreatic acinus	(50)	(50)	(50)
Inflammation, granulomatous		1 (2%)	
Atrophy, NOS	10 (20%)	9 (18%)	4 (8%)
Hyperplasia, NOS	1 (2%)	2 (4%)	
#Glandular stomach	(50)	(50)	(50)
Mineralization	6 (12%)	1 (2%)	12 (24%)
Cyst, NOS	2 (4%)		
Ulcer, NOS		5 (10%)	8 (16%)
Inflammation, chronic			1 (2%)
Erosion	1 (2%)	5 (10%)	
#Gastric muscularis	(50)	(50)	(50)
Mineralization	1 (2%)		
Inflammation, necrotizing		1 (2%)	1 (2%)
Degeneration, NOS			3 (6%)
#Gastric serosa	(50)	(50)	(50)
Inflammation, granulomatous	1 (2%)		
Fibrosis	1 (2%)		
#Forestomach	(50)	(50)	(50)
Ulcer, NOS	1 (2%)		2 (4%)
Hyperplasia, epithelial	1 (2%)	2 (4%)	2 (4%)
#Duodenum	(49)	(50)	(50)
Ulcer, NOS			1 (2%)
Inflammation, fibrinous		1 (2%)	
Inflammation, chronic			1 (2%)

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Colon	(48)	(49)	(50)
Inflammation, suppurative		1 (2%)	4 (8%)
Inflammation, chronic	1 (2%)	4 (8%)	14 (28%)
Parasitism	7 (15%)	3 (6%)	
#Cecum	(48)	(49)	(50)
Ulcer, NOS			1 (2%)
Inflammation, suppurative			8 (16%)
Inflammation, chronic		3 (6%)	14 (28%)
Fibrosis, diffuse			1 (2%)
*Rectum	(50)	(50)	(50)
Parasitism	4 (8%)	4 (8%)	
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Mineralization		3 (6%)	2 (4%)
Hydronephrosis	5 (10%)	2 (4%)	2 (4%)
Cyst, NOS	2 (4%)	1 (2%)	3 (6%)
Hemorrhage			1 (2%)
Inflammation, suppurative	7 (14%)	37 (74%)	44 (88%)
Inflammation, chronic	39 (78%)	34 (68%)	38 (76%)
Nephropathy	50 (100%)	50 (100%)	49 (98%)
Infarct, NOS		1 (2%)	
Pigmentation, NOS	4 (8%)	4 (8%)	39 (78%)
#Kidney/medulla	(50)	(50)	(50)
Necrosis, focal			1 (2%)
#Renal papilla	(50)	(50)	(50)
Mineralization		2 (4%)	
Fibrosis		1 (2%)	
Necrosis, NOS		1 (2%)	1 (2%)
#Kidney/tubule	(50)	(50)	(50)
Necrosis, NOS	1 (2%)		1 (2%)
#Kidney/pelvis	(50)	(50)	(50)
Mineralization		2 (4%)	
Hemorrhage		1 (2%)	
Necrosis, NOS		1 (2%)	1 (2%)
Hyperplasia, epithelial		6 (12%)	13 (26%)
#Urinary bladder	(50)	(50)	(49)
Hemorrhage			1 (2%)
Inflammation, suppurative		1 (2%)	1 (2%)
Hyperplasia, epithelial		1 (2%)	
ENDOCRINE SYSTEM			
#Pituitary	(49)	(46)	(48)
Cyst, NOS	4 (8%)	3 (7%)	3 (6%)
Congestion, NOS		1 (2%)	
Hemorrhage	1 (2%)		
Hematoma, NOS		1 (2%)	
Hyperplasia, NOS	5 (10%)	3 (7%)	
Angiectasis	2 (4%)	2 (4%)	
Dysplasia, NOS	1 (2%)		
#Adrenal	(50)	(50)	(50)
Mineralization	1 (2%)		
#Adrenal cortex	(50)	(50)	(50)
Hemorrhage	2 (4%)		
Necrosis, NOS		1 (2%)	
Pigmentation, NOS		1 (2%)	
Cytoplasmic vacuolization	11 (22%)	9 (18%)	5 (10%)
Hyperplasia, NOS	1 (2%)	2 (4%)	1 (2%)

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Adrenal medulla	(50)	(50)	(50)
Cytoplasmic vacuolization		1 (2%)	
Hyperplasia, NOS	2 (4%)		2 (4%)
Hyperplasia, focal	2 (4%)	1 (2%)	1 (2%)
#Thyroid	(50)	(50)	(49)
Embryonal duct cyst	4 (8%)	1 (2%)	
Hemosiderosis	1 (2%)		
Hyperplasia, C-cell	19 (38%)	10 (20%)	2 (4%)
Hyperplasia, follicular cell	2 (4%)		
#Thyroid follicle	(50)	(50)	(49)
Dilatation, NOS	1 (2%)	1 (2%)	
Degeneration, NOS		1 (2%)	
Pigmentation, NOS		2 (4%)	
#Parathyroid	(48)	(46)	(45)
Hyperplasia, NOS			8 (18%)
#Pancreatic islets	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Cyst, NOS		3 (6%)	1 (2%)
Hemorrhagic cyst			1 (2%)
Hyperplasia, cystic	25 (50%)	11 (22%)	5 (10%)
*Bulbourethral gland	(50)	(50)	(50)
Retention of content	1 (2%)		
*Preputial gland	(50)	(50)	(50)
Retention of content	1 (2%)	1 (2%)	
Steatitis	25 (50%)	16 (32%)	4 (8%)
Inflammation, suppurative	9 (18%)	4 (8%)	1 (2%)
Inflammation, chronic	11 (22%)	5 (10%)	
Inflammation, granulomatous	1 (2%)		
Fibrosis	1 (2%)		
Atrophy, NOS	1 (2%)		
Hyperplasia, NOS	1 (2%)		1 (2%)
#Prostate	(48)	(50)	(50)
Dilatation, NOS		1 (2%)	
Inflammation, suppurative	22 (46%)	27 (54%)	20 (40%)
Inflammation, chronic	3 (6%)	3 (6%)	1 (2%)
Fibrosis		1 (2%)	
Corpora amylacea	2 (4%)	3 (6%)	
Hyperplasia, epithelial	2 (4%)	3 (6%)	
Metaplasia, squamous		2 (4%)	
#Testis	(50)	(50)	(48)
Mineralization	3 (6%)	5 (10%)	
Atrophy, NOS	15 (30%)	3 (6%)	4 (8%)
Hyperplasia, interstitial cell	1 (2%)	8 (16%)	10 (21%)
*Spermatic cord	(50)	(50)	(50)
Steatitis	1 (2%)		
NERVOUS SYSTEM			
#Cerebrum	(50)	(50)	(50)
Hemorrhage	2 (4%)		
Degeneration, NOS	3 (6%)	2 (4%)	
Malacia			1 (2%)

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
NERVOUS SYSTEM (Continued)			
#Brain/thalamus	(50)	(50)	(50)
Degeneration, NOS	1 (2%)		
#Pons	(50)	(50)	(50)
Hemorrhage	1 (2%)		
Degeneration, NOS	1 (2%)		
#Cerebellar peduncle	(50)	(50)	(50)
Hemorrhage		1 (2%)	
*Optic tract	(50)	(50)	(50)
Malacia			1 (2%)
SPECIAL SENSE ORGANS			
*Eye	(50)	(50)	(50)
Cataract		1 (2%)	
*Eye/anterior chamber	(50)	(50)	(50)
Hemorrhage		1 (2%)	
*Eye/retina	(50)	(50)	(50)
Atrophy, NOS		2 (4%)	4 (8%)
Dysplasia, NOS			1 (2%)
*Nasolacrimal duct	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)	
*Harderian gland	(50)	(50)	(50)
Inflammation, chronic	1 (2%)		
*Middle ear	(50)	(50)	(50)
Inflammation, chronic	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*Femur	(50)	(50)	(50)
Hyperostosis		1 (2%)	
Fibrous dysplasia			26 (52%)
*Skeletal muscle	(50)	(50)	(50)
Mineralization	1 (2%)		
BODY CAVITIES			
*Mediastinum	(50)	(50)	(50)
Hemorrhage			1 (2%)
Inflammation, acute			1 (2%)
Pigmentation, NOS		1 (2%)	
*Mesentery	(50)	(50)	(50)
Ectopia		1 (2%)	
Inflammation, granulomatous	1 (2%)		
Necrosis, fat	9 (18%)	8 (16%)	1 (2%)
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Mineralization			2 (4%)
Inflammation, suppurative		2 (4%)	
SPECIAL MORPHOLOGY SUMMARY			
None			

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX B

SUMMARY OF LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Papilloma, NOS		1 (2%)	1 (2%)
Basal cell tumor		3 (6%)	
Keratoacanthoma			1 (2%)
Fibroma	2 (4%)	3 (6%)	
Neurofibrosarcoma		1 (2%)	
RESPIRATORY SYSTEM			
None			
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Leukemia, mononuclear cell	9 (18%)	13 (26%)	1 (2%)
#Pelvic lymph node	(50)	(50)	(50)
Adenocarcinoma, NOS, invasive	1 (2%)		
#Liver	(50)	(50)	(49)
Leukemia, mononuclear cell			1 (2%)
#Thymus	(36)	(33)	(39)
Squamous cell carcinoma	1 (3%)		
CIRCULATORY SYSTEM			
None			
DIGESTIVE SYSTEM			
#Salivary gland	(50)	(50)	(48)
Adenoma, NOS	1 (2%)		
#Liver	(50)	(50)	(49)
Neoplastic nodule	1 (2%)	2 (4%)	
Hepatocellular carcinoma		1 (2%)	
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Transitional cell carcinoma			6 (12%)
#Urinary bladder	(48)	(49)	(48)
Endometrial stromal sarcoma, invasive	1 (2%)		
ENDOCRINE SYSTEM			
#Pituitary	(50)	(48)	(46)
Adenoma, NOS	25 (50%)	21 (44%)	4 (9%)
#Adrenal	(50)	(50)	(49)
Cortical adenoma	3 (6%)		
Pheochromocytoma	5 (10%)	2 (4%)	4 (8%)
Pheochromocytoma, malignant			1 (2%)
#Thyroid	(50)	(49)	(49)
C-cell adenoma	9 (18%)	8 (16%)	1 (2%)
C-cell carcinoma	1 (2%)		
#Pancreatic islets	(50)	(50)	(50)
Islet cell adenoma	1 (2%)	1 (2%)	1 (2%)

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Carcinoma, NOS	2 (4%)	1 (2%)	
Fibroadenoma	18 (36%)	10 (20%)	2 (4%)
*Clitoral gland	(50)	(50)	(50)
Carcinoma, NOS	1 (2%)		
Adenoma, NOS	1 (2%)	2 (4%)	
#Uterus	(50)	(49)	(50)
Adenoma, NOS	2 (4%)	2 (4%)	
Adenocarcinoma, NOS	1 (2%)		
Endometrial stromal polyp	8 (16%)	13 (27%)	2 (4%)
Endometrial stromal sarcoma	1 (2%)		
NERVOUS SYSTEM			
#Midbrain	(50)	(50)	(50)
Granular cell tumor, NOS			1 (2%)
SPECIAL SENSE ORGANS			
None			
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
*Abdominal cavity	(50)	(50)	(50)
Lipoma	1 (2%)		
*Mesentery	(50)	(50)	(50)
Lipoma		1 (2%)	
ALL OTHER SYSTEMS			
None			
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	3	6	17
Moribund sacrifice	4	11	25
Terminal sacrifice	43	33	7
Accidentally killed, NOS			1
TUMOR SUMMARY			
Total animals with primary tumors**	45	44	19
Total primary tumors	93	85	26
Total animals with benign tumors	42	39	14
Total benign tumors	76	67	16
Total animals with malignant tumors	16	15	8
Total malignant tumors	16	16	9
Total animals with secondary tumors##	2		
Total secondary tumors	2		
Total animals with tumors uncertain-- benign or malignant	1	2	1
Total uncertain tumors	1	2	1

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3: VEHICLE CONTROL

ANIMAL NUMBER	0046	0026	0032	0115	0330	0221	0449	0005	0448	0550	0001	0002	0003	0004	0006	0007	0008	0009	0110	0111	0112	0113	0114	0116	0117
WEEKS ON STUDY	0061	0668	0680	0990	0993	0995	0998	1004	1044	1044	1045	1055	1055	1055	1055	1055	1055	1055	1055	1055	1055	1055	1055	1055	1055
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																						X			
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS, invasive			X																						
Thymus	-	+	+	-	+	+	-	-	-	+	+	+	+	+	+	-	-	+	+	+	-	+	+	-	+
Squamous cell carcinoma																			X						
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS											X														
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal sarcoma, invasive		X																							
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS				X	X		X	X	X	X	X	X		X	X						X	X	X	X	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma																					X				
Pheochromocytoma								X						X											
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma													X		X										
C-cell carcinoma																									
Parathyroid	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma												X													
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS						X																			
Fibroadenoma									X			X						X	X	X		X	X	X	
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS												X													
Adenoma, NOS																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																		X							
Adenocarcinoma, NOS			X																						
Endometrial stromal polyp						X										X			X			X			
Endometrial stromal sarcoma		X																							
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Lipoma							X																		
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell				X				X	X	X										X					

+: Tissue examined microscopically
-: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: VEHICLE CONTROL (Continued)

ANIMAL NUMBER	0018	0019	0020	0022	0023	0024	0025	0027	0028	0029	0031	0033	0034	0035	0036	0037	0038	0039	0040	0041	0042	0043	0044	0045	0047	TOTAL:
WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma						X																				2
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, NOS, invasive																										1
Thymus	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	+	-	+	+	+	-	+	+	+	36
Squamous cell carcinoma																										1
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule								X																		1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endometrial stromal sarcoma, invasive																										1
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS			X	X		X	X		X		X	X	X	X									X		X	25
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cortical adenoma	X											X														3
Pheochromocytoma					X			X			X															5
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell adenoma		X			X			X	X							X	X							X		9
C-cell carcinoma																					X					1
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	48
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma																										1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Carcinoma, NOS							X																			2
Fibroadenoma								X	X	X			X		X		X			X	X	X			X	18
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
Adenoma, NOS				X																						1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS														X												2
Adenocarcinoma, NOS																										1
Endometrial stromal polyp	X		X	X			X																			8
Endometrial stromal sarcoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
BODY CAVITIES																										
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Lipoma																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell			X					X						X			X									9

* Animals necropsied

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3: LOW DOSE

ANIMAL NUMBER	0262	0264	0119	0330	0002	0005	0116	0331	0004	0445	0447	0112	0001	0222	0111	0339	0033	0006	0007	0008	0009	0110	0113	0114	0115
WEEKS ON STUDY	0661	0669	0884	0884	0990	0991	0992	0992	0993	0998	0999	0999	1011	1022	1033	1033	1044	1044	1044	1044	1044	1044	1044	1044	1044
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma, NOS																									X
Basal cell tumor													X								X	X			
Fibroma	X		X																						
Neurofibrosarcoma							X																		
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	-	-	+	-	+	-	+	+	-	+	-	+	+	-	+	+	+	+	-	+	-	+	+	-
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																						X			
Hepatocellular carcinoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
Adenoma, NOS	X	X			X				X				X		X	X			X	X	X				X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma															X										
Thyroid	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma	X									X									X						X
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																				X					
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+
Carcinoma, NOS																									
Fibroadenoma					X							X		X							X	X			
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Endometrial stromal polyp							X				X	X	X		X				X		X			X	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BODY CAVITIES																									
Mesentery	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Lipoma																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell				X		X					X				X	X	X				X				

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: LOW DOSE (Continued)

ANIMAL NUMBER	0117	0118	0120	0121	0123	0125	0127	0133	0141	0128	0129	0132	0134	0135	0136	0137	0138	0140	0142	0143	0144	0146	0148	0149	0150	TOTAL:
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Papilloma, NOS																										1
Basal cell tumor																										3
Fibroma					X																					3
Neurofibrosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	-	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-	-	-	+	33
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule													X													2
Hepatocellular carcinoma																							X			1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma, NOS		X	X			X		X		X	X								X			X	X	X		21
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma								X																		2
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell adenoma						X							X											X	X	8
Parathyroid	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma																										1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	N	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Carcinoma, NOS																								X		1
Fibroadenoma				X													X	X				X	X			10
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS																	X			X						2
Uterus	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS							X									X										2
Endometrial stromal polyp						X													X		X		X	X		13
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
BODY CAVITIES																										
Mesentery	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Lipoma																									X	1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell						X			X			X		X								X		X		13

* Animals necropsied

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3: HIGH DOSE

ANIMAL NUMBER	0 4 4	0 3 9	0 3 6	0 4 3	0 0 6	0 0 5	0 1 9	0 2 0	0 4 9	0 1 0	0 2 9	0 4 6	0 5 0	0 3 1	0 0 2	0 4 7	0 1 8	0 2 5	0 1 1	0 4 8	0 0 7	0 1 6	0 3 2	0 4 1	0 1 7
WEEKS ON STUDY	0 0 3	0 1 0	0 1 1	0 1 1	0 1 2	0 1 3	0 1 4	0 1 5	0 1 5	0 1 6	0 1 7	0 1 7	0 1 7	0 3 7	0 3 9	0 4 8	0 4 9	0 5 5	0 5 8	0 6 6	0 6 7	0 6 7	0 6 8	0 6 8	0 7 2
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma, NOS																				X					
Keratoacanthoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	-
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia, mononuclear cell																									
Bile duct	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional cell carcinoma																									
Urinary bladder	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																						X			
Adrenal	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma													X												
Pheochromocytoma, malignant																									
Thyroid	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma																									
Parathyroid	-	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp																		X						X	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granular cell tumor, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell																									

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: HIGH DOSE
(Continued)

ANIMAL NUMBER	033	037	015	003	001	004	024	012	013	014	008	042	021	026	045	035	038	034	009	022	023	027	028	030	040	TOTAL:
WEEKS ON STUDY	072	072	076	079	084	084	084	087	091	091	092	094	096	096	097	099	100	103	104	104	104	104	104	104	104	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Papilloma, NOS																										1
Keratoacanthoma																									X	1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	-	+	+	+	+	+	+	+	-	+	+	-	-	+	-	-	+	-	+	+	+	+	+	+	-	39
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia, mononuclear cell													X													1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional cell carcinoma								X					X	X				X				X			X	6
Urinary bladder	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																										
Pituitary	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	46
Adenoma, NOS				X			X											X								4
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma	X								X									X								4
Pheochromocytoma, malignant																				X						1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	49
C-cell adenoma																						X				1
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	40
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma																	X									1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroadenoma										X								X								2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrial stromal polyp																										2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	49
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granular cell tumor, NOS														X												1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell															X											1

* Animals necropsied

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

	Vehicle Control	375 mg/kg	750 mg/kg
Skin: Basal Cell Tumor			
Overall Rates (a)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	0.0%	8.4%	0.0%
Terminal Rates (c)	0/43 (0%)	2/34 (6%)	0/7 (0%)
Week of First Observation		101	
Life Table Tests (d)	P=0.249	P=0.090	(e)
Incidental Tumor Tests (d)	P=0.453	P=0.141	(e)
Cochran-Armitage Trend Test (d)	P=0.640		
Fisher Exact Test (d)		P=0.121	(e)
Skin: Fibroma			
Overall Rates (a)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	4.7%	6.9%	0.0%
Terminal Rates (c)	2/43 (5%)	1/34 (3%)	0/7 (0%)
Week of First Observation	105	61	
Life Table Tests (d)	P=0.540N	P=0.439	P=0.675N
Incidental Tumor Tests (d)	P=0.214N	P=0.531	P=0.675N
Cochran-Armitage Trend Test (d)	P=0.202N		
Fisher Exact Test (d)		P=0.500	P=0.247N
Skin: Fibroma or Neurofibrosarcoma			
Overall Rates (a)	2/50 (4%)	4/50 (8%)	0/50 (0%)
Adjusted Rates (b)	4.7%	9.0%	0.0%
Terminal Rates (c)	2/43 (5%)	1/34 (3%)	0/7 (0%)
Week of First Observation	105	61	
Life Table Tests (d)	P=0.605N	P=0.287	P=0.675N
Incidental Tumor Tests (d)	P=0.151N	P=0.477	P=0.675N
Cochran-Armitage Trend Test (d)	P=0.222N		
Fisher Exact Test (d)		P=0.339	P=0.247N
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	9/50 (18%)	13/50 (26%)	2/50 (4%)
Adjusted Rates (b)	20.3%	31.8%	16.1%
Terminal Rates (c)	8/43 (19%)	7/34 (21%)	0/7 (0%)
Week of First Observation	90	84	96
Life Table Tests (d)	P=0.265	P=0.123	P=0.606
Incidental Tumor Tests (d)	P=0.165N	P=0.420	P=0.212N
Cochran-Armitage Trend Test (d)	P=0.038N		
Fisher Exact Test (d)		P=0.235	P=0.026N
Liver: Neoplastic Nodule or Hepatocellular Carcinoma			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	0/49 (0%)
Adjusted Rates (b)	2.3%	8.8%	0.0%
Terminal Rates (c)	1/43 (2%)	3/34 (9%)	0/7 (0%)
Week of First Observation	105	104	
Life Table Tests (d)	P=0.432	P=0.225	P=0.850N
Incidental Tumor Tests (d)	P=0.432	P=0.225	P=0.850N
Cochran-Armitage Trend Test (d)	P=0.384N		
Fisher Exact Test (d)		P=0.309	P=0.505N
Kidney: Transitional Cell Carcinoma			
Overall Rates (a)	0/50 (0%)	0/50 (0%)	6/50 (12%)
Adjusted Rates (b)	0.0%	0.0%	50.1%
Terminal Rates (c)	0/43 (0%)	0/34 (0%)	2/7 (29%)
Week of First Observation			87
Life Table Tests (d)	P<0.001	(e)	P<0.001
Incidental Tumor Tests (d)	P<0.001	(e)	P=0.007
Cochran-Armitage Trend Test (d)	P=0.003		
Fisher Exact Test (d)		(e)	P=0.013

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
Adrenal Gland: Cortical Adenoma			
Overall Rates (a)	3/50 (6%)	0/50 (0%)	0/49 (0%)
Adjusted Rates (b)	7.0%	0.0%	0.0%
Terminal Rates (c)	3/43 (7%)	0/34 (0%)	0/7 (0%)
Week of First Observation	105		
Life Table Tests (d)	P=0.134N	P=0.166N	P=0.554N
Incidental Tumor Tests (d)	P=0.134N	P=0.166N	P=0.554N
Cochran-Armitage Trend Test (d)	P=0.038N		
Fisher Exact Test (d)		P=0.121N	P=0.125N
Adrenal Gland: Pheochromocytoma			
Overall Rates (a)	5/50 (10%)	2/50 (4%)	4/49 (8%)
Adjusted Rates (b)	11.6%	5.6%	22.8%
Terminal Rates (c)	5/43 (12%)	1/34 (3%)	0/7 (0%)
Week of First Observation	104	103	17
Life Table Tests (d)	P=0.140	P=0.318N	P=0.089
Incidental Tumor Tests (d)	P=0.435N	P=0.243N	P=0.662N
Cochran-Armitage Trend Test (d)	P=0.435N		
Fisher Exact Test (d)		P=0.218N	P=0.513N
Adrenal Gland: Pheochromocytoma or Malignant Pheochromocytoma			
Overall Rates (a)	5/50 (10%)	2/50 (4%)	5/49 (10%)
Adjusted Rates (b)	11.6%	5.6%	33.8%
Terminal Rates (c)	5/43 (12%)	1/34 (3%)	1/7 (14%)
Week of First Observation	104	103	17
Life Table Tests (d)	P=0.048	P=0.318N	P=0.022
Incidental Tumor Tests (d)	P=0.484	P=0.243N	P=0.394
Cochran-Armitage Trend Test (d)	P=0.562		
Fisher Exact Test (d)		P=0.218N	P=0.617
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	9/50 (18%)	8/49 (16%)	1/49 (2%)
Adjusted Rates (b)	20.9%	21.3%	14.3%
Terminal Rates (c)	9/43 (21%)	6/34 (18%)	1/7 (14%)
Week of First Observation	105	61	104
Life Table Tests (d)	P=0.478N	P=0.522	P=0.540N
Incidental Tumor Tests (d)	P=0.302N	P=0.548	P=0.540N
Cochran-Armitage Trend Test (d)	P=0.012N		
Fisher Exact Test (d)		P=0.518N	P=0.009N
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	10/50 (20%)	8/49 (16%)	1/49 (2%)
Adjusted Rates (b)	23.3%	21.3%	14.3%
Terminal Rates (c)	10/43 (23%)	6/34 (18%)	1/7 (14%)
Week of First Observation	105	61	104
Life Table Tests (d)	P=0.392N	P=0.593N	P=0.484N
Incidental Tumor Tests (d)	P=0.233N	P=0.570N	P=0.484N
Cochran-Armitage Trend Test (d)	P=0.006N		
Fisher Exact Test (d)		P=0.416N	P=0.004N
Mammary Gland: Fibroadenoma			
Overall Rates (a)	18/50 (36%)	10/50 (20%)	2/50 (4%)
Adjusted Rates (b)	41.9%	26.4%	17.6%
Terminal Rates (c)	18/43 (42%)	7/34 (21%)	0/7 (0%)
Week of First Observation	104	90	91
Life Table Tests (d)	P=0.160N	P=0.188N	P=0.368N
Incidental Tumor Tests (d)	P=0.018N	P=0.094N	P=0.110N
Cochran-Armitage Trend Test (d)	P<0.001N		
Fisher Exact Test (d)		P=0.059N	P<0.001N

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
Uterus: Adenoma or Adenocarcinoma			
Overall Rates (a)	3/50 (6%)	2/49 (4%)	0/50 (0%)
Adjusted Rates (b)	6.6%	6.1%	0.0%
Terminal Rates (c)	2/43 (5%)	2/33 (6%)	0/7 (0%)
Week of First Observation	68	104	
Life Table Tests (d)	P=0.343N	P=0.595N	P=0.417N
Incidental Tumor Tests (d)	P=0.198N	P=0.631N	P=0.187N
Cochran-Armitage Trend Test (d)	P=0.083N		
Fisher Exact Test (d)		P=0.510N	P=0.121N
Uterus: Endometrial Stromal Polyp			
Overall Rates (a)	8/50 (16%)	13/49 (27%)	2/50 (4%)
Adjusted Rates (b)	18.1%	33.4%	6.5%
Terminal Rates (c)	7/43 (16%)	8/33 (24%)	0/7 (0%)
Week of First Observation	95	92	55
Life Table Tests (d)	P=0.210	P=0.069	P=0.654
Incidental Tumor Tests (d)	P=0.296N	P=0.219	P=0.252N
Cochran-Armitage Trend Test (d)	P=0.064N		
Fisher Exact Test (d)		P=0.150	P=0.046N
All Sites: Benign Tumors			
Overall Rates (a)	42/50 (84%)	39/50 (78%)	14/50 (28%)
Adjusted Rates (b)	91.3%	82.9%	63.5%
Terminal Rates (c)	39/43 (91%)	26/34 (76%)	2/7 (29%)
Week of First Observation	93	61	17
Life Table Tests (d)	P=0.038	P=0.205	P=0.048
Incidental Tumor Tests (d)	P=0.003N	P=0.373N	P=0.017N
Cochran-Armitage Trend Test (d)	P<0.001N		
Fisher Exact Test (d)		P=0.306N	P<0.001N
All Sites: Malignant Tumors			
Overall Rates (a)	16/50 (32%)	15/50 (30%)	8/50 (16%)
Adjusted Rates (b)	33.8%	35.9%	63.7%
Terminal Rates (c)	12/43 (28%)	8/34 (24%)	3/7 (43%)
Week of First Observation	68	84	87
Life Table Tests (d)	P=0.051	P=0.445	P=0.036
Incidental Tumor Tests (d)	P=0.194N	P=0.294N	P=0.385N
Cochran-Armitage Trend Test (d)	P=0.044N		
Fisher Exact Test (d)		P=0.500N	P=0.050N
All Sites: All Tumors			
Overall Rates (a)	45/50 (90%)	44/50 (88%)	19/50 (38%)
Adjusted Rates (b)	91.8%	89.8%	78.8%
Terminal Rates (c)	39/43 (91%)	29/34 (85%)	3/7 (43%)
Week of First Observation	68	61	17
Life Table Tests (d)	P=0.002	P=0.110	P=0.003
Incidental Tumor Tests (d)	P=0.001N	P=0.430N	P=0.004N
Cochran-Armitage Trend Test (d)	P<0.001N		
Fisher Exact Test (d)		P=0.500N	P<0.001N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the vehicle controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) No P value is reported because no tumors were observed in the dosed and vehicle control groups.

TABLE B4. HISTORICAL INCIDENCE OF KIDNEY TRANSITIONAL CELL TUMORS IN FEMALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

	No. Examined	No. of Tumors
Historical Incidence at Southern Research Institute		
	400	0
Overall Historical Incidence		
	1,697	0

(a) Data as of August 7, 1986, for studies of at least 104 weeks

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)		
Inflammation, chronic	1 (2%)		
Hyperkeratosis		1 (2%)	
Acanthosis	1 (2%)	1 (2%)	
RESPIRATORY SYSTEM			
*Nasal cavity	(50)	(50)	(50)
Inflammation, suppurative	3 (6%)	6 (12%)	4 (8%)
Infection, fungal	1 (2%)	5 (10%)	2 (4%)
Foreign material, NOS		1 (2%)	
#Trachea	(50)	(50)	(50)
Inflammation, pyogranulomatous			1 (2%)
Necrosis, NOS			1 (2%)
Metaplasia, squamous		1 (2%)	
#Lung	(50)	(50)	(50)
Congestion, NOS	2 (4%)	2 (4%)	4 (8%)
Edema, NOS	1 (2%)	3 (6%)	3 (6%)
Hemorrhage	2 (4%)		
Inflammation, suppurative	1 (2%)	3 (6%)	
Inflammation, granulomatous	2 (4%)	2 (4%)	
Fibrosis	1 (2%)		
Cholesterol deposit		1 (2%)	
Alveolar macrophages	33 (66%)	17 (34%)	7 (14%)
Hyperplasia, alveolar epithelium	2 (4%)	4 (8%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Hematopoiesis	1 (2%)		
#Bone marrow	(50)	(50)	(50)
Congestion, NOS		1 (2%)	
Pigmentation, NOS		1 (2%)	
Atrophy, NOS			1 (2%)
Hyperplasia, NOS		1 (2%)	
Myelofibrosis			1 (2%)
Megakaryocytosis			1 (2%)
Hyperplasia, reticulum cell	6 (12%)	2 (4%)	1 (2%)
#Spleen	(50)	(50)	(50)
Fibrosis	3 (6%)	2 (4%)	
Necrosis, NOS	1 (2%)		
Hemosiderosis	3 (6%)		3 (6%)
Hematopoiesis	3 (6%)	4 (8%)	1 (2%)
#Mandibular lymph node	(50)	(50)	(50)
Hemorrhage		1 (2%)	
Atrophy, NOS			1 (2%)
Histiocytosis	1 (2%)		
Plasmacytosis	3 (6%)	2 (4%)	
Hyperplasia, lymphoid	1 (2%)		
#Mediastinal lymph node	(50)	(50)	(50)
Hemorrhage	1 (2%)		
Fibrosis	1 (2%)		
Pigmentation, NOS	4 (8%)	5 (10%)	1 (2%)
Erythrophagocytosis		3 (6%)	

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Celiac lymph node	(50)	(50)	(50)
Hemorrhage	1 (2%)		
Pigmentation, NOS	1 (2%)		
#Mesenteric lymph node	(50)	(50)	(50)
Hemorrhage	1 (2%)	1 (2%)	
Fibrosis	1 (2%)		
Pigmentation, NOS	13 (26%)		
Atrophy, NOS	10 (20%)	11 (22%)	21 (42%)
Hyperplasia, lymphoid	1 (2%)		1 (2%)
Mastocytosis		2 (4%)	
#Liver	(50)	(50)	(49)
Hematopoiesis		2 (4%)	
#Adrenal	(50)	(50)	(49)
Hematopoiesis		1 (2%)	
#Thymus	(36)	(33)	(39)
Cyst, NOS	1 (3%)		
CIRCULATORY SYSTEM			
#Lymph node	(50)	(50)	(50)
Lymphangiectasis	1 (2%)		
#Mandibular lymph node	(50)	(50)	(50)
Lymphangiectasis	2 (4%)	2 (4%)	
#Mediastinal lymph node	(50)	(50)	(50)
Lymphangiectasis	1 (2%)	1 (2%)	
#Mesenteric lymph node	(50)	(50)	(50)
Lymphangiectasis			1 (2%)
#Renal lymph node	(50)	(50)	(50)
Lymphangiectasis			2 (4%)
#Lung	(50)	(50)	(50)
Thrombus, fibrin			2 (4%)
Embolism, NOS			1 (2%)
#Heart	(50)	(50)	(50)
Mineralization			2 (4%)
Inflammation, suppurative			1 (2%)
Inflammation, chronic	5 (10%)	4 (8%)	7 (14%)
Fibrosis	14 (28%)	15 (30%)	15 (30%)
Degeneration, NOS	1 (2%)	1 (2%)	4 (8%)
*Artery	(50)	(50)	(50)
Mineralization		1 (2%)	2 (4%)
Periarteritis		1 (2%)	10 (20%)
Degeneration, hyaline			4 (8%)
Necrosis, fibrinoid		2 (4%)	
Hypertrophy, NOS		1 (2%)	1 (2%)
*Aorta	(50)	(50)	(50)
Mineralization			9 (18%)
Inflammation, acute/chronic			1 (2%)
Inflammation, chronic			1 (2%)
Degeneration, hyaline			1 (2%)
*Coronary artery	(50)	(50)	(50)
Necrosis, focal			1 (2%)
*Pulmonary artery	(50)	(50)	(50)
Mineralization	4 (8%)	6 (12%)	
#Renal papilla	(50)	(50)	(50)
Thrombosis, NOS			1 (2%)

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM			
#Salivary gland	(50)	(50)	(48)
Ectopia			1 (2%)
Retention of content		1 (2%)	2 (4%)
Atrophy, NOS		2 (4%)	
#Liver	(50)	(50)	(49)
Inflammation, suppurative	1 (2%)		
Inflammation, chronic	5 (10%)	4 (8%)	1 (2%)
Inflammation, granulomatous	20 (40%)		
Necrosis, focal			1 (2%)
Hyperplasia, nodular	1 (2%)	1 (2%)	
Angiectasis	2 (4%)	1 (2%)	
#Liver/Kupffer cell	(50)	(50)	(49)
Pigmentation, NOS	3 (6%)	2 (4%)	
#Liver/hepatocytes	(50)	(50)	(49)
Necrosis, focal	1 (2%)	1 (2%)	
Necrosis, central		1 (2%)	
Cytoplasmic change, NOS	1 (2%)		
Cytoplasmic vacuolization	4 (8%)	1 (2%)	6 (12%)
Basophilic cyto change	32 (64%)	31 (62%)	1 (2%)
Atrophy, focal	14 (28%)	10 (20%)	1 (2%)
#Bile duct	(50)	(50)	(49)
Hyperplasia, NOS	26 (52%)	29 (58%)	6 (12%)
#Pancreatic acinus	(50)	(50)	(50)
Inflammation, chronic	1 (2%)	3 (6%)	
Atrophy, NOS	14 (28%)	10 (20%)	3 (6%)
#Pancreatic interstitial tissue	(50)	(50)	(50)
Degeneration, hyaline			1 (2%)
*Pharynx	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)	
#Esophagus	(49)	(50)	(50)
Retention of content		1 (2%)	
#Glandular stomach	(50)	(50)	(50)
Mineralization		1 (2%)	5 (10%)
Cyst, NOS		2 (4%)	
Ulcer, NOS		2 (4%)	2 (4%)
Erosion		1 (2%)	1 (2%)
Dysplasia, epithelial	1 (2%)		1 (2%)
#Gastric muscularis	(50)	(50)	(50)
Mineralization	4 (8%)	1 (2%)	1 (2%)
Degeneration, NOS			7 (14%)
#Forestomach	(50)	(50)	(50)
Mineralization	1 (2%)		
Ulcer, NOS	1 (2%)		2 (4%)
Hyperplasia, focal			1 (2%)
#Colon	(49)	(49)	(50)
Inflammation, suppurative			7 (14%)
Inflammation, chronic	1 (2%)		12 (24%)
Parasitism	4 (8%)	3 (6%)	
#Colonic muscularis	(49)	(49)	(50)
Degeneration, NOS			1 (2%)
#Cecum	(49)	(49)	(50)
Edema, NOS			1 (2%)
Inflammation, suppurative		1 (2%)	6 (12%)
Inflammation, chronic		1 (2%)	12 (24%)
Parasitism	4 (8%)	1 (2%)	
*Rectum	(50)	(50)	(50)
Ulcer, NOS			1 (2%)
Inflammation, chronic			1 (2%)
Parasitism	4 (8%)	2 (4%)	1 (2%)

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Mineralization		2 (4%)	4 (8%)
Hydronephrosis	6 (12%)	8 (16%)	6 (12%)
Cyst, NOS	1 (2%)		5 (10%)
Inflammation, suppurative		10 (20%)	45 (90%)
Inflammation, chronic	4 (8%)	18 (36%)	37 (74%)
Fibrosis			5 (10%)
Nephropathy	23 (46%)	45 (90%)	48 (96%)
Pigmentation, NOS			5 (10%)
Atrophy, NOS			3 (6%)
Angiectasis		1 (2%)	
#Kidney/medulla	(50)	(50)	(50)
Hemorrhage			1 (2%)
Necrosis, NOS			2 (4%)
#Renal papilla	(50)	(50)	(50)
Mineralization	1 (2%)	5 (10%)	
Necrosis, NOS			10 (20%)
Dysplasia, epithelial			1 (2%)
#Kidney/tubule	(50)	(50)	(50)
Cytoplasmic vacuolization			1 (2%)
#Kidney/pelvis	(50)	(50)	(50)
Calculus, microscopic examination			1 (2%)
Mineralization	15 (30%)	23 (46%)	
Hemorrhage			1 (2%)
Necrosis, NOS			1 (2%)
Hyperplasia, epithelial		2 (4%)	13 (26%)
#Urinary bladder	(48)	(49)	(48)
Inflammation, suppurative			1 (2%)
Inflammation, chronic		4 (8%)	1 (2%)
Hyperplasia, epithelial	1 (2%)		2 (4%)
#Urinary bladder/serosa	(48)	(49)	(48)
Mineralization	1 (2%)	1 (2%)	
ENDOCRINE SYSTEM			
#Pituitary	(50)	(48)	(46)
Cyst, NOS	12 (24%)	15 (31%)	5 (11%)
Hyperplasia, NOS	6 (12%)	2 (4%)	6 (13%)
Angiectasis	4 (8%)	11 (23%)	
#Pituitary intermedia	(50)	(48)	(46)
Hyperplasia, NOS		1 (2%)	
#Adrenal	(50)	(50)	(49)
Congenital malformation, NOS		1 (2%)	
#Adrenal cortex	(50)	(50)	(49)
Hemorrhagic cyst	2 (4%)	2 (4%)	
Inflammation, granulomatous	1 (2%)		
Degeneration, lipoid		1 (2%)	
Cytoplasmic vacuolization	9 (18%)	10 (20%)	3 (6%)
Basophilic cyto change		1 (2%)	
Hypertrophy, NOS	1 (2%)		
Hyperplasia, focal	1 (2%)	2 (4%)	
Angiectasis		1 (2%)	
#Adrenal medulla	(50)	(50)	(49)
Cytoplasmic vacuolization		1 (2%)	
Hyperplasia, NOS	2 (4%)		
Hyperplasia, focal	3 (6%)		3 (6%)
Hyperplasia, diffuse			1 (2%)

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Thyroid	(50)	(49)	(49)
Embryonal duct cyst	1 (2%)	2 (4%)	6 (12%)
Cyst, NOS	1 (2%)		
Inflammation, pyogranulomatous			1 (2%)
Hyperplasia, C-cell	22 (44%)	8 (16%)	3 (6%)
Hyperplasia, follicular cell		1 (2%)	
#Thyroid follicle	(50)	(49)	(49)
Dilatation, NOS		1 (2%)	
#Parathyroid	(48)	(45)	(40)
Hyperplasia, NOS			1 (3%)
#Pancreatic islets	(50)	(50)	(50)
Hyperplasia, NOS	2 (4%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Fibrosis	1 (2%)	1 (2%)	
Hyperplasia, NOS		3 (6%)	1 (2%)
Hyperplasia, cystic	41 (82%)	31 (62%)	17 (34%)
*Clitoral gland	(50)	(50)	(50)
Retention of content		4 (8%)	
Inflammation, suppurative	3 (6%)	3 (6%)	
Inflammation, chronic	4 (8%)	1 (2%)	
Inflammation, granulomatous	1 (2%)	2 (4%)	
Hyperplasia, NOS	3 (6%)	2 (4%)	1 (2%)
*Vagina	(50)	(50)	(50)
Polyp, NOS	1 (2%)		1 (2%)
#Uterus	(50)	(49)	(50)
Hydrometra	8 (16%)		4 (8%)
Cyst, NOS		1 (2%)	
Hemorrhage			1 (2%)
Inflammation, suppurative	1 (2%)	1 (2%)	2 (4%)
Necrosis, NOS			1 (2%)
Polyp, inflammatory			1 (2%)
#Uterus/endometrium	(50)	(49)	(50)
Hyperplasia, epithelial		1 (2%)	1 (2%)
Hyperplasia, cystic		4 (8%)	
#Ovary	(50)	(50)	(49)
Cyst, NOS	4 (8%)	1 (2%)	5 (10%)
Parovarian cyst		1 (2%)	
Inflammation, granulomatous	1 (2%)		
Necrosis, NOS	1 (2%)		
NERVOUS SYSTEM			
#Cerebrum	(50)	(50)	(50)
Hemorrhage		1 (2%)	
Degeneration, NOS	1 (2%)	2 (4%)	2 (4%)
#Brain/thalamus	(50)	(50)	(50)
Degeneration, NOS	1 (2%)		
*Spinal cord	(50)	(50)	(50)
Degeneration, NOS	1 (2%)		
SPECIAL SENSE ORGANS			
*Eye	(50)	(50)	(50)
Cataract	2 (4%)	17 (34%)	
*Eye/sclera	(50)	(50)	(50)
Mineralization	1 (2%)		
*Eye/retina	(50)	(50)	(50)
Atrophy, NOS	3 (6%)	19 (38%)	2 (4%)
*Nasolacrimal duct	(50)	(50)	(50)
Inflammation, suppurative			1 (2%)

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
MUSCULOSKELETAL SYSTEM			
*Femur	(50)	(50)	(50)
Hyperostosis	1 (2%)		
Fibrous dysplasia			12 (24%)
BODY CAVITIES			
*Mediastinum	(50)	(50)	(50)
Foreign body, NOS			1 (2%)
*Mesentery	(50)	(50)	(50)
Mineralization		1 (2%)	
Necrosis, fat	6 (12%)	4 (8%)	4 (8%)
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Mineralization			1 (2%)
Inflammation, suppurative			2 (4%)
SPECIAL MORPHOLOGY SUMMARY			
None			

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX C

SUMMARY OF LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)		1 (2%)
Squamous cell carcinoma	1 (2%)		1 (2%)
Basal cell carcinoma		1 (2%)	
Keratoacanthoma	1 (2%)		
*Subcutaneous tissue	(50)	(50)	(50)
Sarcoma, NOS	1 (2%)	1 (2%)	2 (4%)
Fibroma		3 (6%)	
Fibrosarcoma	1 (2%)	3 (6%)	3 (6%)
Neurilemoma		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#Lung	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic		3 (6%)	2 (4%)
Alveolar/bronchiolar adenoma	8 (16%)	2 (4%)	5 (10%)
Alveolar/bronchiolar carcinoma	6 (12%)	7 (14%)	5 (10%)
Sarcoma, NOS, metastatic	1 (2%)		
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Malignant lymphoma, lymphocytic type		1 (2%)	2 (4%)
Malignant lymphoma, histiocytic type	1 (2%)	1 (2%)	2 (4%)
Malignant lymphoma, mixed type	5 (10%)	1 (2%)	2 (4%)
#Spleen	(50)	(50)	(50)
Malignant lymphoma, mixed type	1 (2%)		
#Mediastinal lymph node	(50)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic	1 (2%)		
#Mesenteric lymph node	(50)	(49)	(50)
Malignant lymphoma, mixed type	1 (2%)		
#Iliac lymph node	(50)	(49)	(50)
Fibrosarcoma, metastatic			1 (2%)
CIRCULATORY SYSTEM			
*Multiple organs	(50)	(50)	(50)
Hemangiosarcoma	3 (6%)		1 (2%)
#Bone marrow	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)
#Spleen	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)	
#Liver	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)		
#Urinary bladder	(49)	(49)	(50)
Hemangioma	1 (2%)		
DIGESTIVE SYSTEM			
#Liver	(50)	(50)	(50)
Hepatocellular adenoma	16 (32%)	4 (8%)	6 (12%)
Hepatocellular carcinoma	7 (14%)	16 (32%)	10 (20%)
Lipoma	1 (2%)		
#Forestomach	(49)	(50)	(50)
Squamous cell papilloma	3 (6%)	2 (4%)	1 (2%)
Squamous cell carcinoma	1 (2%)		

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Small intestine	(50)	(50)	(48)
Adenocarcinoma, NOS	1 (2%)		
#Duodenum	(50)	(50)	(48)
Adenocarcinoma, NOS	1 (2%)		
#Jejunum	(50)	(50)	(48)
Adenocarcinoma, NOS	1 (2%)	1 (2%)	1 (2%)
#Jejunal mucosa	(50)	(50)	(48)
Adenocarcinoma, NOS			1 (2%)
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Tubular cell adenoma	1 (2%)		
Fibrosarcoma, metastatic			1 (2%)
ENDOCRINE SYSTEM			
#Anterior pituitary	(44)	(42)	(44)
Adenoma, NOS			1 (2%)
#Adrenal/capsule	(49)	(49)	(50)
Adenoma, NOS	2 (4%)		1 (2%)
#Adrenal medulla	(49)	(49)	(50)
Pheochromocytoma	1 (2%)	2 (4%)	2 (4%)
#Thyroid	(50)	(50)	(49)
Follicular cell adenoma	1 (2%)		
Follicular cell carcinoma		1 (2%)	1 (2%)
#Pancreatic islets	(50)	(50)	(49)
Islet cell adenoma	1 (2%)		
REPRODUCTIVE SYSTEM			
*Scrotum	(50)	(50)	(50)
Sarcoma, NOS		1 (2%)	
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(50)	(50)	(50)
Carcinoma, NOS		1 (2%)	
Adenoma, NOS	3 (6%)	3 (6%)	6 (12%)
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Squamous cell carcinoma, metastatic	1 (2%)		

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	6	5	9
Moribund sacrifice	6	18	15
Terminal sacrifice	38	25	26
Accidentally killed, nda		2	
TUMOR SUMMARY			
Total animals with primary tumors**	42	36	39
Total primary tumors	74	53	56
Total animals with benign tumors	28	13	19
Total benign tumors	40	17	24
Total animals with malignant tumors	25	29	28
Total malignant tumors	34	36	32
Total animals with secondary tumors##	3	3	3
Total secondary tumors	3	3	4

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3: VEHICLE CONTROL

ANIMAL NUMBER	024	022	020	034	036	002	044	048	050	014	040	029	001	003	004	005	006	007	008	009	010	011	012	013	015
WEEKS ON STUDY	041	072	074	077	078	085	086	086	087	090	096	097	104	104	104	104	104	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Squamous cell carcinoma				X																					
Keratoacanthoma																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS										X															
Fibrosarcoma												X													
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X	X							X						X	X		X		
Alveolar/bronchiolar carcinoma			X						X		X									X					
Sarcoma, NOS, metastatic										X															
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Hemangiosarcoma																									X
Malignant lymphoma, mixed type																									
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic																									
Malignant lymphoma, mixed type																									
Thymus	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma					X											X	X	X		X		X	X		
Hepatocellular carcinoma						X	X										X	X							
Lipoma																					X				
Hemangiosarcoma	X																								
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	+	N	+	N	+	+	+	+	+	+	+	N	+	+	N	N	+	+	+	+	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma															X							X			
Squamous cell carcinoma																									
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																									
Large intestine	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma																				X					
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	−
Adrenal	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Pheochromocytoma																						X			
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma														X											
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																	X								X
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell carcinoma, metastatic				X																					
Hemangiosarcoma							X																	X	
Malignant lymphoma, histiocytic type																			X						
Malignant lymphoma, mixed type						X					X				X										

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: VEHICLE CONTROL (Continued)

ANIMAL NUMBER	0 1 6	0 1 7	0 1 8	0 1 9	0 2 1	0 2 3	0 2 5	0 2 6	0 2 7	0 2 8	0 3 0	0 3 1	0 3 2	0 3 3	0 3 5	0 3 7	0 3 8	0 3 9	0 4 1	0 4 2	0 4 3	0 4 5	0 4 6	0 4 7	0 4 9	TOTAL:
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 5	1 0 5	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell papilloma					X																					1
Squamous cell carcinoma																										1
Keratoacanthoma																			X							1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																										1
Fibrosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma									X		X															8
Alveolar/bronchiolar carcinoma																				X	X					6
Sarcoma, NOS, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Malignant lymphoma, mixed type													X													1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, meta																					X					1
Malignant lymphoma, mixed type																					X					1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma				X				X	X						X		X		X	X		X	X			16
Hepatocellular carcinoma	X	X																			X					7
Lipoma																										1
Hemangiosarcoma		X																								2
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	N	+	+	N	+	+	+	+	N	+	N	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell papilloma						X																				3
Squamous cell carcinoma																		X								1
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, NOS	X				X																X					3
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangioma															X											1
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	44
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS							X							X												2
Pheochromocytoma																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma																										1
Parathyroid	−	+	+	+	−	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma																		X								1
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	+	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS																		X								3
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell carcinoma, metastatic																										1
Hemangiosarcoma																				X						3
Malignant lymphoma, histiocytic type																										1
Malignant lymphoma, mixed type																X			X							5

* Animals necropsied

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3: LOW DOSE

ANIMAL NUMBER	0161	0281	0301	0481	0171	0110	0433	0191	0331	0477	0499	0451	0501	0381	0118	0299	0400	0083	0133	0093	0201	0404	0266	0235	0255
WEEKS ON STUDY	0277	0300	0535	0556	0661	0663	0755	0799	0799	0811	0811	0855	0855	0900	0933	0955	0966	0977	0997	0988	0988	1001	1001	1002	1002
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS															X										
Fibroma																									X
Fibrosarcoma																			X				X		X
Neurilemoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic										X		X										X			
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma														X			X				X				
Trachea	+	–	+	–	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																		X							
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	–	+	+	+	+	+	–	+	+	–	+	+	+	+	–	+	+	+	+	–	+	–	+	+	
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																							X		
Hepatocellular carcinoma			X	X	X	X	X		X	X	X	X	X						X			X		X	
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	N	+	N	+	+	+	N	+	N	+	+	N	+	+	+	N	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																						X			
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS												X													
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	–	–	+	+	+	–	+	+	+	–	–	+	+	+	–	+
Adrenal	+	+	+	+	+	+	–	●	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma								X																	
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell carcinoma																		X							
Parathyroid	+	–	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	+	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																			X						
Adenoma, NOS												X								X					
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, lymphocytic type								X																	
Malignant lymphoma, histiocytic type							X																		
Malignant lymphoma, mixed type																X									
Scrotum, NOS																									
Sarcoma, NOS																									

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	0 0 1	0 0 2	0 0 5	0 0 6	0 0 7	0 0 9	0 1 1	0 1 2	0 1 4	0 1 5	0 2 1	0 2 2	0 2 4	0 2 7	0 3 2	0 3 3	0 3 4	0 3 5	0 3 6	0 3 7	0 3 9	0 4 1	0 4 2	0 4 4	0 4 6	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Basal cell carcinoma																				X						1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																										1
Fibroma		X																	X							3
Fibrosarcoma																										3
Neurilemoma																	X									1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic																										3
Alveolar/bronchiolar adenoma			X					X																		2
Alveolar/bronchiolar carcinoma						X			X		X					X										7
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	49
Thymus	+	−	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	−	+	+	−	38
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma			X						X										X							4
Hepatocellular carcinoma				X	X	X																				16
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	N	+	+	+	N	+	N	+	+	N	+	N	+	+	+	N	+	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma		X																								2
Small intestine	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, NOS																										1
Large intestine	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	42
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma			X																							2
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell carcinoma																										1
Parathyroid	+	+	+	+	−	−	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	42
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
Adenoma, NOS																									X	3
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, lymphocytic type																										1
Malignant lymphoma, histiocytic type																										1
Malignant lymphoma, mixed type																										1
Scrotum, NOS																										
Sarcoma, NOS						X																				1

* Animals necropsied

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3: HIGH DOSE

ANIMAL NUMBER	037	038	019	036	006	016	032	004	015	014	029	034	030	031	002	007	010	033	020	023	041	001	046	028	003
WEEKS ON STUDY	025	041	047	074	075	075	075	079	081	085	085	085	086	086	087	088	091	093	093	094	095	095	097	100	101	104
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																										
Squamous cell carcinoma			X																							
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS							X										X									
Fibrosarcoma									X						X	X										
Neurilemoma												X														
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic																								X		
Alveolar/bronchiolar adenoma																										
Alveolar/bronchiolar carcinoma													X										X			
Trachea	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																										
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic															X											
Thymus	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+	+	-	-	-	+	-	+	
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma											X			X	X											
Hepatocellular carcinoma					X	X					X						X			X				X		
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	N	+	+	+	+	N	+	+	+	+	+	+	+	+	N	+	+	N	N	+	+	N	+	N	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Squamous cell papilloma																						X				
Small intestine	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	
Adenocarcinoma, NOS										X																
Large intestine	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Fibrosarcoma, metastatic															X											
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
ENDOCRINE SYSTEM																										
Pituitary	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	
Adenoma, NOS																										
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adenoma, NOS																										
Pheochromocytoma																								X		
Thyroid	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	
Follicular cell carcinoma								X																		
Parathyroid	-	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	
REPRODUCTIVE SYSTEM																										
Mammary gland	N	+	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
Adenoma, NOS														X		X					X					
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
Hemangiosarcoma																								X		
Malignant lymphoma, lymphocytic type										X																
Malignant lymphoma, histiocytic type				X																X						
Malignant lymphoma, mixed type													X													

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER	0 0 5	0 0 8	0 0 9	0 1 1	0 1 2	0 1 3	0 1 7	0 1 8	0 2 1	0 2 2	0 2 4	0 2 5	0 2 6	0 2 7	0 3 5	0 3 9	0 4 0	0 4 2	0 4 3	0 4 4	0 4 5	0 4 7	0 4 8	0 4 9	0 5 0	TOTAL:
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell papilloma						X																				1
Squamous cell carcinoma																										1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																										2
Fibrosarcoma																										3
Neurilemoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic															X											2
Alveolar/bronchiolar adenoma		X				X					X	X										X				5
Alveolar/bronchiolar carcinoma										X											X		X			5
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma											X															1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	+	–	39
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma			X																	X				X		6
Hepatocellular carcinoma					X			X			X				X											10
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	N	+	+	N	N	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	N	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										1
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenocarcinoma, NOS				X																						2
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic																										1
Urinary bladder	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	44
Adenoma, NOS									X																	1
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS												X														1
Pheochromocytoma																		X								2
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell carcinoma																										1
Parathyroid	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+	-	+	-	+	40
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	*50
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS									X									X					X			6
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Hemangiosarcoma																										1
Malignant lymphoma, lymphocytic type												X														2
Malignant lymphoma, histiocytic type																										2
Malignant lymphoma, mixed type							X																			2

* Animals necropsied

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

	Vehicle Control	125 mg/kg	250 mg/kg
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	0.0%	11.4%	0.0%
Terminal Rates (c)	0/38 (0%)	2/25 (8%)	0/26 (0%)
Week of First Observation		102	
Life Table Tests (d)	P=0.527	P=0.063	(e)
Incidental Tumor Tests (d)	P=0.642N	P=0.134	(e)
Cochran-Armitage Trend Test (d)	P=0.640		
Fisher Exact Test (d)		P=0.121	(e)
Subcutaneous Tissue: Fibrosarcoma			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted Rates (b)	2.6%	10.4%	7.8%
Terminal Rates (c)	0/38 (0%)	0/25 (0%)	0/26 (0%)
Week of First Observation	97	98	81
Life Table Tests (d)	P=0.168	P=0.220	P=0.253
Incidental Tumor Tests (d)	P=0.549	P=0.573N	P=0.605
Cochran-Armitage Trend Test (d)	P=0.238		
Fisher Exact Test (d)		P=0.309	P=0.309
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	1/50 (2%)	5/50 (10%)	3/50 (6%)
Adjusted Rates (b)	2.6%	17.6%	7.8%
Terminal Rates (c)	0/38 (0%)	2/25 (8%)	0/26 (0%)
Week of First Observation	97	98	81
Life Table Tests (d)	P=0.170	P=0.051	P=0.253
Incidental Tumor Tests (d)	P=0.490	P=0.364	P=0.605
Cochran-Armitage Trend Test (d)	P=0.264		
Fisher Exact Test (d)		P=0.102	P=0.309
Subcutaneous Tissue: Sarcoma or Fibrosarcoma			
Overall Rates (a)	2/50 (4%)	4/50 (8%)	5/50 (10%)
Adjusted Rates (b)	4.9%	12.9%	12.5%
Terminal Rates (c)	0/38 (0%)	0/25 (0%)	0/26 (0%)
Week of First Observation	90	93	75
Life Table Tests (d)	P=0.116	P=0.251	P=0.172
Incidental Tumor Tests (d)	P=0.496	P=0.351N	P=0.561
Cochran-Armitage Trend Test (d)	P=0.169		
Fisher Exact Test (d)		P=0.339	P=0.218
Subcutaneous Tissue: Fibroma, Sarcoma, or Fibrosarcoma			
Overall Rates (a)	2/50 (4%)	6/50 (12%)	5/50 (10%)
Adjusted Rates (b)	4.9%	19.9%	12.5%
Terminal Rates (c)	0/38 (0%)	2/25 (8%)	0/26 (0%)
Week of First Observation	90	93	75
Life Table Tests (d)	P=0.117	P=0.074	P=0.172
Incidental Tumor Tests (d)	P=0.452	P=0.540	P=0.561
Cochran-Armitage Trend Test (d)	P=0.187		
Fisher Exact Test (d)		P=0.134	P=0.218
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	8/50 (16%)	2/50 (4%)	5/50 (10%)
Adjusted Rates (b)	19.5%	8.0%	19.2%
Terminal Rates (c)	6/38 (16%)	2/25 (8%)	5/26 (19%)
Week of First Observation	85	104	104
Life Table Tests (d)	P=0.413N	P=0.141N	P=0.522N
Incidental Tumor Tests (d)	P=0.354N	P=0.104N	P=0.438N
Cochran-Armitage Trend Test (d)	P=0.202N		
Fisher Exact Test (d)		P=0.046N	P=0.277N

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	125 mg/kg	250 mg/kg
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	6/50 (12%)	7/50 (14%)	5/50 (10%)
Adjusted Rates (b)	14.2%	23.2%	16.8%
Terminal Rates (c)	3/38 (8%)	4/25 (16%)	3/26 (12%)
Week of First Observation	74	90	86
Life Table Tests (d)	P=0.450	P=0.295	P=0.551
Incidental Tumor Tests (d)	P=0.392N	P=0.614N	P=0.415N
Cochran-Armitage Trend Test (d)	P=0.439N		
Fisher Exact Test (d)		P=0.500	P=0.500N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	13/50 (26%)	9/50 (18%)	10/50 (20%)
Adjusted Rates (b)	29.7%	30.5%	34.9%
Terminal Rates (c)	8/38 (21%)	6/25 (24%)	8/26 (31%)
Week of First Observation	74	90	86
Life Table Tests (d)	P=0.488	P=0.543N	P=0.538
Incidental Tumor Tests (d)	P=0.343N	P=0.227N	P=0.371N
Cochran-Armitage Trend Test (d)	P=0.271N		
Fisher Exact Test (d)		P=0.235N	P=0.318N
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	7/50 (14%)	1/50 (2%)	2/50 (4%)
Adjusted Rates (b)	17.2%	2.9%	6.4%
Terminal Rates (c)	5/38 (13%)	0/25 (0%)	1/26 (4%)
Week of First Observation	85	95	86
Life Table Tests (d)	P=0.093N	P=0.085N	P=0.184N
Incidental Tumor Tests (d)	P=0.028N	P=0.015N	P=0.072N
Cochran-Armitage Trend Test (d)	P=0.036N		
Fisher Exact Test (d)		P=0.030N	P=0.080N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	8/50 (16%)	3/50 (6%)	6/50 (12%)
Adjusted Rates (b)	19.7%	7.3%	16.9%
Terminal Rates (c)	6/38 (16%)	0/25 (0%)	2/26 (8%)
Week of First Observation	85	75	74
Life Table Tests (d)	P=0.500N	P=0.219N	P=0.606N
Incidental Tumor Tests (d)	P=0.254N	P=0.063N	P=0.322N
Cochran-Armitage Trend Test (d)	P=0.318N		
Fisher Exact Test (d)		P=0.100N	P=0.387N
Circulatory System: Hemangiosarcoma			
Overall Rates (a)	6/50 (12%)	1/50 (2%)	2/50 (4%)
Adjusted Rates (b)	14.3%	3.0%	7.3%
Terminal Rates (c)	4/38 (11%)	0/25 (0%)	1/26 (4%)
Week of First Observation	41	97	100
Life Table Tests (d)	P=0.149N	P=0.123N	P=0.252N
Incidental Tumor Tests (d)	P=0.044N	P=0.038N	P=0.093N
Cochran-Armitage Trend Test (d)	P=0.070N		
Fisher Exact Test (d)		P=0.056N	P=0.134N
Circulatory System: Hemangioma or Hemangiosarcoma			
Overall Rates (a)	7/50 (14%)	1/50 (2%)	2/50 (4%)
Adjusted Rates (b)	16.8%	3.0%	7.3%
Terminal Rates (c)	5/38 (13%)	0/25 (0%)	1/26 (4%)
Week of First Observation	41	97	100
Life Table Tests (d)	P=0.092N	P=0.083N	P=0.180N
Incidental Tumor Tests (d)	P=0.024N	P=0.024N	P=0.062N
Cochran-Armitage Trend Test (d)	P=0.036N		
Fisher Exact Test (d)		P=0.030N	P=0.080N

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	125 mg/kg	250 mg/kg
Liver: Hepatocellular Adenoma			
Overall Rates (a)	16/50 (32%)	4/50 (8%)	6/50 (12%)
Adjusted Rates (b)	40.8%	15.0%	18.3%
Terminal Rates (c)	15/38 (39%)	3/25 (12%)	3/26 (12%)
Week of First Observation	78	101	85
Life Table Tests (d)	P=0.048N	P=0.031N	P=0.093N
Incidental Tumor Tests (d)	P=0.022N	P=0.015N	P=0.053N
Cochran-Armitage Trend Test (d)	P=0.006N		
Fisher Exact Test (d)		P=0.003N	P=0.014N
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	7/50 (14%)	16/50 (32%)	10/50 (20%)
Adjusted Rates (b)	17.0%	37.4%	28.4%
Terminal Rates (c)	5/38 (13%)	3/25 (12%)	4/26 (15%)
Week of First Observation	85	53	75
Life Table Tests (d)	P=0.139	P=0.011	P=0.142
Incidental Tumor Tests (d)	P=0.399	P=0.057	P=0.356
Cochran-Armitage Trend Test (d)	P=0.273		
Fisher Exact Test (d)		P=0.028	P=0.298
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	21/50 (42%)	20/50 (40%)	15/50 (30%)
Adjusted Rates (b)	50.8%	47.8%	41.5%
Terminal Rates (c)	18/38 (47%)	6/25 (24%)	7/26 (27%)
Week of First Observation	78	53	75
Life Table Tests (d)	P=0.488N	P=0.234	P=0.532N
Incidental Tumor Tests (d)	P=0.135N	P=0.530N	P=0.244N
Cochran-Armitage Trend Test (d)	P=0.128N		
Fisher Exact Test (d)		P=0.500N	P=0.149N
Forestomach: Squamous Cell Papilloma			
Overall Rates (a)	3/49 (6%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	7.9%	7.3%	3.4%
Terminal Rates (c)	3/38 (8%)	1/25 (4%)	0/26 (0%)
Week of First Observation	104	101	97
Life Table Tests (d)	P=0.356N	P=0.672N	P=0.441N
Incidental Tumor Tests (d)	P=0.205N	P=0.526N	P=0.288N
Cochran-Armitage Trend Test (d)	P=0.216N		
Fisher Exact Test (d)		P=0.490N	P=0.301N
Forestomach: Squamous Cell Papilloma or Carcinoma			
Overall Rates (a)	4/49 (8%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	10.5%	7.3%	3.4%
Terminal Rates (c)	4/38 (11%)	1/25 (4%)	0/26 (0%)
Week of First Observation	104	101	97
Life Table Tests (d)	P=0.229N	P=0.528N	P=0.305N
Incidental Tumor Tests (d)	P=0.118N	P=0.384N	P=0.187N
Cochran-Armitage Trend Test (d)	P=0.113N		
Fisher Exact Test (d)		P=0.329N	P=0.175N
Small Intestine: Adenocarcinoma			
Overall Rates (a)	3/50 (6%)	1/50 (2%)	2/48 (4%)
Adjusted Rates (b)	7.9%	2.6%	6.2%
Terminal Rates (c)	3/38 (8%)	0/25 (0%)	1/26 (4%)
Week of First Observation	104	85	85
Life Table Tests (d)	P=0.520N	P=0.437N	P=0.644N
Incidental Tumor Tests (d)	P=0.431N	P=0.389N	P=0.586N
Cochran-Armitage Trend Test (d)	P=0.415N		
Fisher Exact Test (d)		P=0.309N	P=0.520N

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	125 mg/kg	250 mg/kg
Harderian Gland: Adenoma			
Overall Rates (a)	3/50 (6%)	3/50 (6%)	6/50 (12%)
Adjusted Rates (b)	7.9%	9.5%	19.0%
Terminal Rates (c)	3/38 (8%)	1/25 (4%)	3/26 (12%)
Week of First Observation	104	85	86
Life Table Tests (d)	P=0.091	P=0.500	P=0.120
Incidental Tumor Tests (d)	P=0.193	P=0.665N	P=0.240
Cochran-Armitage Trend Test (d)	P=0.178		
Fisher Exact Test (d)		P=0.661	P=0.243
Harderian Gland: Adenoma or Carcinoma			
Overall Rates (a)	3/50 (6%)	4/50 (8%)	6/50 (12%)
Adjusted Rates (b)	7.9%	12.5%	19.0%
Terminal Rates (c)	3/38 (8%)	1/25 (4%)	3/26 (12%)
Week of First Observation	104	85	86
Life Table Tests (d)	P=0.094	P=0.334	P=0.120
Incidental Tumor Tests (d)	P=0.228	P=0.621	P=0.240
Cochran-Armitage Trend Test (d)	P=0.187		
Fisher Exact Test (d)		P=0.500	P=0.243
All Sites: Benign Tumors			
Overall Rates (a)	28/50 (56%)	13/50 (26%)	19/50 (38%)
Adjusted Rates (b)	68.0%	40.5%	54.6%
Terminal Rates (c)	25/38 (66%)	7/25 (28%)	11/26 (42%)
Week of First Observation	78	79	85
Life Table Tests (d)	P=0.382N	P=0.091N	P=0.473N
Incidental Tumor Tests (d)	P=0.084N	P=0.008N	P=0.137N
Cochran-Armitage Trend Test (d)	P=0.041N		
Fisher Exact Test (d)		P=0.003N	P=0.055N
All Sites: Malignant Tumors			
Overall Rates (a)	25/50 (50%)	29/50 (58%)	28/50 (56%)
Adjusted Rates (b)	53.0%	61.1%	62.5%
Terminal Rates (c)	16/38 (42%)	7/25 (28%)	10/26 (38%)
Week of First Observation	41	53	47
Life Table Tests (d)	P=0.084	P=0.071	P=0.088
Incidental Tumor Tests (d)	P=0.385N	P=0.362N	P=0.494N
Cochran-Armitage Trend Test (d)	P=0.308		
Fisher Exact Test (d)		P=0.274	P=0.344
All Sites: All Tumors			
Overall Rates (a)	42/50 (84%)	36/50 (72%)	39/50 (78%)
Adjusted Rates (b)	87.5%	75.0%	82.8%
Terminal Rates (c)	32/38 (84%)	13/25 (52%)	18/26 (69%)
Week of First Observation	41	53	47
Life Table Tests (d)	P=0.131	P=0.250	P=0.121
Incidental Tumor Tests (d)	P=0.144N	P=0.024N	P=0.234N
Cochran-Armitage Trend Test (d)	P=0.273N		
Fisher Exact Test (d)		P=0.114N	P=0.306N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the vehicle controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) No P value is reported because no tumors were observed in the 250 mg/kg and vehicle control groups.

TABLE C4. HISTORICAL INCIDENCE OF CIRCULATORY SYSTEM TUMORS IN MALE B6C3F$_1$ MICE ADMINISTERED CORN OIL BY GAVAGE (a)

Study	Incidence in Vehicle Controls		
	Hemangioma	Hemangiosarcoma	Hemangioma or Hemangiosarcoma
Historical Incidence at Southern Research Institute			
Ethyl acrylate	0/49	1/49	1/49
Benzyl acetate	0/50	4/50	4/50
Allyl isovalerate	0/50	1/50	1/50
HC Red No. 3	0/50	7/50	7/50
Chlorinated paraffins (C_{23}, 43% chlorine)	0/50	7/50	7/50
Allyl isothiocyanate	0/50	2/50	2/50
Geranyl acetate	1/50	2/50	3/50
Chlorinated paraffins (C_{12}, 60% chlorine)	0/50	2/50	2/50
TOTAL	1/399 (0.3%)	26/399 (6.5%)	27/399 (6.8%)
SD (b)	0.71%	4.98%	4.89%
Range (c)			
High	1/50	7/50	7/50
Low	0/50	1/50	1/50
Overall Historical Incidence			
TOTAL	19/1,743 (1.1%)	84/1,743 (4.8%)	101/1,743 (5.8%)
SD (b)	2.24%	4.20%	4.94%
Range (c)			
High	(d) 6/50	7/50	10/50
Low	0/50	0/50	0/50

(a) Data as of August 7, 1986, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.
(d) Second highest: 2/50

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Epidermal inclusion cyst			1 (2%)
Ulcer, NOS	1 (2%)	4 (8%)	4 (8%)
Abscess, NOS			1 (2%)
Inflammation, chronic	3 (6%)	15 (30%)	15 (30%)
Inflammation, granulomatous		1 (2%)	
Fibrosis		1 (2%)	
Fibrosis, focal	1 (2%)		
Infection, fungal		1 (2%)	
Hyperplasia, epithelial		1 (2%)	
Hyperplasia, basal cell		1 (2%)	
*Subcutaneous tissue	(50)	(50)	(50)
Cyst, NOS		1 (2%)	
Edema, NOS			1 (2%)
Hemorrhage		1 (2%)	
Ulcer, NOS		1 (2%)	
Abscess, NOS			1 (2%)
Inflammation, chronic	1 (2%)	2 (4%)	
Inflammation, granulomatous		1 (2%)	
Lipogranuloma			1 (2%)
Infection, fungal		1 (2%)	
Angiectasis			1 (2%)
RESPIRATORY SYSTEM			
*Nasal cavity	(50)	(50)	(50)
Foreign body, NOS	1 (2%)		
Inflammation, suppurative	5 (10%)	2 (4%)	1 (2%)
Reaction, foreign body	2 (4%)		
*Nasal mucosa	(50)	(50)	(50)
Polypoid hyperplasia	3 (6%)		
#Lung	(50)	(50)	(50)
Aspiration, foreign body		2 (4%)	
Congestion, NOS		5 (10%)	5 (10%)
Hemorrhage		3 (6%)	
Inflammation, focal	1 (2%)		
Hyperplasia, alveolar epithelium	2 (4%)	1 (2%)	
#Lung/alveoli	(50)	(50)	(50)
Histiocytosis	1 (2%)		1 (2%)
HEMATOPOIETIC SYSTEM			
#Bone marrow	(50)	(50)	(50)
Myelofibrosis			1 (2%)
#Spleen	(50)	(50)	(50)
Amyloidosis			1 (2%)
Atrophy, NOS		1 (2%)	1 (2%)
Hyperplasia, lymphoid	1 (2%)	3 (6%)	1 (2%)
Hematopoiesis	5 (10%)	10 (20%)	11 (22%)
#Mandibular lymph node	(50)	(49)	(50)
Hyperplasia, NOS		1 (2%)	
Hyperplasia, lymphoid			1 (2%)
#Bronchial lymph node	(50)	(49)	(50)
Hyperplasia, NOS		1 (2%)	
Hyperplasia, lymphoid	1 (2%)		

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Mesenteric lymph node	(50)	(49)	(50)
Congestion, NOS		1 (2%)	1 (2%)
Angiectasis	9 (18%)	6 (12%)	4 (8%)
Hyperplasia, lymphoid	2 (4%)		
Hematopoiesis			1 (2%)
#Iliac lymph node	(50)	(49)	(50)
Hyperplasia, plasma cell			1 (2%)
#Inguinal lymph node	(50)	(49)	(50)
Hyperplasia, NOS		1 (2%)	1 (2%)
Hyperplasia, lymphoid			1 (2%)
#Lung	(50)	(50)	(50)
Leukocytosis, NOS	2 (4%)		3 (6%)
Hyperplasia, lymphoid	1 (2%)		
#Salivary gland	(50)	(50)	(49)
Hyperplasia, lymphoid	1 (2%)		
#Liver	(50)	(50)	(50)
Hyperplasia, reticulum cell			1 (2%)
Hematopoiesis		2 (4%)	
#Small intestine	(50)	(50)	(48)
Hyperplasia, lymphoid		1 (2%)	
#Peyer's patch	(50)	(50)	(48)
Hyperplasia, lymphoid		2 (4%)	
#Kidney	(50)	(50)	(50)
Hyperplasia, lymphoid	18 (36%)	35 (70%)	33 (66%)
*Epididymis	(50)	(50)	(50)
Hyperplasia, lymphoid	1 (2%)		
*Spermatic cord	(50)	(50)	(50)
Hyperplasia, lymphoid	1 (2%)		
#Thymic lymphocytes	(46)	(38)	(39)
Necrosis, NOS		1 (3%)	
CIRCULATORY SYSTEM			
*Subcutaneous tissue	(50)	(50)	(50)
Lymphangiectasis	1 (2%)		
#Bone marrow	(50)	(50)	(50)
Thrombosis, NOS		1 (2%)	
#Heart	(50)	(50)	(50)
Thrombosis, NOS		1 (2%)	
Inflammation, suppurative	1 (2%)	1 (2%)	1 (2%)
#Heart/atrium	(50)	(50)	(50)
Thrombosis, NOS		1 (2%)	
#Myocardium	(50)	(50)	(50)
Inflammation, focal		2 (4%)	
*Mesenteric artery	(50)	(50)	(50)
Hypertrophy, NOS			1 (2%)
#Liver	(50)	(50)	(50)
Thrombosis, NOS	2 (4%)		1 (2%)
DIGESTIVE SYSTEM			
*Root of tooth	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)	2 (4%)	1 (2%)
Dysplasia, NOS	18 (36%)	7 (14%)	6 (12%)
*Gum of mandible	(50)	(50)	(50)
Reaction, foreign body		1 (2%)	
Hypertrophy, focal		1 (2%)	
Angiectasis		1 (2%)	
*Periodontal tissues	(50)	(50)	(50)
Inflammation, chronic	2 (4%)		1 (2%)

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Liver	(50)	(50)	(50)
Mineralization			1 (2%)
Deformity, NOS		2 (4%)	
Cyst, NOS			1 (2%)
Congestion, NOS	1 (2%)	1 (2%)	
Hemorrhage	1 (2%)		2 (4%)
Inflammation, focal	1 (2%)		
Lipogranuloma			1 (2%)
Fibrosis, focal			4 (8%)
Necrosis, focal	2 (4%)	4 (8%)	6 (12%)
Infarct, NOS	1 (2%)	2 (4%)	4 (8%)
Amyloidosis			1 (2%)
Metamorphosis, fatty	1 (2%)	1 (2%)	
Cholesterol deposit		1 (2%)	1 (2%)
Pigmentation, NOS		1 (2%)	1 (2%)
Cytoplasmic vacuolization		2 (4%)	1 (2%)
Angiectasis	3 (6%)		
#Liver/centrilobular	(50)	(50)	(50)
Necrosis, NOS		2 (4%)	
Metamorphosis, fatty	1 (2%)		
Cytoplasmic vacuolization			1 (2%)
Atrophy, NOS		1 (2%)	
#Bile duct	(50)	(50)	(50)
Hyperplasia, NOS	1 (2%)	2 (4%)	
*Common bile duct	(50)	(50)	(50)
Cystic ducts	2 (4%)		
#Pancreas	(50)	(50)	(49)
Atrophy, focal	1 (2%)		2 (4%)
#Glandular stomach	(49)	(50)	(50)
Inflammation, focal	1 (2%)	1 (2%)	
#Gastric serosa	(49)	(50)	(50)
Inflammation, chronic		1 (2%)	
#Forestomach	(49)	(50)	(50)
Epidermal inclusion cyst		1 (2%)	
Ulcer, NOS	1 (2%)	2 (4%)	
Inflammation, focal	1 (2%)	3 (6%)	
Hyperplasia, epithelial	1 (2%)	3 (6%)	
#Small intestine	(50)	(50)	(48)
Hyperplasia, adenomatous	1 (2%)		
#Duodenal gland	(50)	(50)	(48)
Cyst, NOS		1 (2%)	
#Jejunum	(50)	(50)	(48)
Hyperplasia, adenomatous	1 (2%)		
#Jejunal mucosa	(50)	(50)	(48)
Hyperplasia, adenomatous		1 (2%)	
*Rectal submucosa	(50)	(50)	(50)
Reaction, foreign body	1 (2%)		
*Anus	(50)	(50)	(50)
Cyst, NOS			1 (2%)
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Hydronephrosis	1 (2%)		2 (4%)
Inflammation, NOS		4 (8%)	10 (20%)
Inflammation, suppurative	1 (2%)		2 (4%)
Fibrosis		5 (10%)	19 (38%)
Adhesion, NOS			1 (2%)
Nephrosis, NOS	47 (94%)	47 (94%)	45 (90%)
Necrosis, focal			1 (2%)
Necrosis, medullary			6 (12%)
Atrophy, NOS			2 (4%)
Metaplasia, osseous			1 (2%)

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
URINARY SYSTEM (Continued)			
#Renal papilla	(50)	(50)	(50)
Degeneration, NOS		4 (8%)	18 (36%)
#Kidney/tubule	(50)	(50)	(50)
Mineralization	31 (62%)	20 (40%)	25 (50%)
Dilatation, NOS	2 (4%)	39 (78%)	33 (66%)
#Kidney/pelvis	(50)	(50)	(50)
Inflammation, NOS			1 (2%)
Inflammation, chronic			1 (2%)
Hyperplasia, epithelial			1 (2%)
Hyperplasia, papillary			1 (2%)
#Urinary bladder	(49)	(49)	(50)
Inflammation, NOS			3 (6%)
Hyperplasia, epithelial			1 (2%)
ENDOCRINE SYSTEM			
#Anterior pituitary	(44)	(42)	(44)
Embryonal duct cyst		1 (2%)	2 (5%)
#Adrenal/capsule	(49)	(49)	(50)
Hypertrophy, focal			1 (2%)
Hyperplasia, focal	3 (6%)		1 (2%)
#Adrenal cortex	(49)	(49)	(50)
Amyloidosis			1 (2%)
Focal cellular change	1 (2%)		
Clear cell change	1 (2%)		
Atrophy, NOS		1 (2%)	
Hypertrophy, focal	1 (2%)		
Hyperplasia, focal			1 (2%)
Metaplasia, osseous			1 (2%)
#Adrenal medulla	(49)	(49)	(50)
Ectopia			1 (2%)
Fibrosis	1 (2%)		
Hyperplasia, focal		2 (4%)	4 (8%)
Hyperplasia, adenomatous		1 (2%)	
#Thyroid	(50)	(50)	(49)
Cystic follicles	4 (8%)	1 (2%)	1 (2%)
Degeneration, cystic	6 (12%)	4 (8%)	5 (10%)
Pigmentation, NOS			1 (2%)
Hyperplasia, follicular cell	3 (6%)		1 (2%)
#Parathyroid	(44)	(42)	(40)
Multiple cysts			1 (3%)
#Pancreatic islets	(50)	(50)	(49)
Hyperplasia, NOS	1 (2%)		
REPRODUCTIVE SYSTEM			
*Prepuce	(50)	(50)	(50)
Epidermal inclusion cyst			1 (2%)
Inflammation, chronic			1 (2%)
Ulcer, chronic			1 (2%)
Fibrosis		1 (2%)	
*Preputial gland	(50)	(50)	(50)
Inflammation, chronic	5 (10%)	6 (12%)	4 (8%)
Degeneration, cystic	3 (6%)	5 (10%)	4 (8%)
#Prostate	(50)	(49)	(50)
Inflammation, suppurative		1 (2%)	3 (6%)
*Seminal vesicle	(50)	(50)	(50)
Dilatation, NOS	4 (8%)	1 (2%)	
Hemorrhage		1 (2%)	
Inflammation, suppurative			1 (2%)

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
REPRODUCTIVE SYSTEM (Continued)			
#Periprostatic tissue	(50)	(49)	(50)
Inflammation, NOS	1 (2%)		
*Coagulating gland	(50)	(50)	(50)
Dilatation, NOS	2 (4%)	2 (4%)	
*Epididymis	(50)	(50)	(50)
Inflammation, chronic focal		1 (2%)	
*Spermatic cord	(50)	(50)	(50)
Mineralization	1 (2%)		
Inflammation, suppurative	2 (4%)		
Necrosis, fat	2 (4%)		1 (2%)
*Scrotum	(50)	(50)	(50)
Edema, NOS		1 (2%)	
Inflammation, NOS		1 (2%)	
Necrosis, fat		1 (2%)	
NERVOUS SYSTEM			
#Brain	(50)	(50)	(50)
Hemorrhage	1 (2%)	1 (2%)	
Inflammation, focal		1 (2%)	
Inflammation, suppurative	1 (2%)		
#Cerebral cortex	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)	
#Brain/thalamus	(50)	(50)	(50)
Mineralization	26 (52%)	21 (42%)	22 (44%)
SPECIAL SENSE ORGANS			
*Eye/cornea	(50)	(50)	(50)
Inflammation, NOS	1 (2%)		
*Nasolacrimal duct	(50)	(50)	(50)
Inflammation, NOS		1 (2%)	2 (4%)
Hyperplasia, epithelial	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*Knee joint	(50)	(50)	(50)
Dysplasia, NOS	1 (2%)		
BODY CAVITIES			
*Peritoneum	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)	1 (2%)
*Pelvic peritoneal cavity	(50)	(50)	(50)
Foreign body, NOS		1 (2%)	
Inflammation, suppurative		1 (2%)	
*Mesentery	(50)	(50)	(50)
Inflammation, suppurative			2 (4%)
Necrosis, focal		1 (2%)	
Necrosis, fat		1 (2%)	1 (2%)
*Tunica vaginalis	(50)	(50)	(50)
Fibrosis		1 (2%)	

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
ALL OTHER SYSTEMS			
Tail			
Inflammation, chronic			1
Diaphragm			
Mineralization	1		
Foot			
Inflammation, NOS	1		
Soft tissue			
Fibrosis		1	
Necrosis, fat		1	
SPECIAL MORPHOLOGY SUMMARY			
None			

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX D

SUMMARY OF LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Subcutaneous tissue	(50)	(50)	(50)
Sarcoma, NOS	1 (2%)	1 (2%)	
Fibrosarcoma	1 (2%)		
Neurilemoma, malignant	†1 (2%)		
RESPIRATORY SYSTEM			
#Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		1 (2%)	3 (6%)
Alveolar/bronchiolar carcinoma	2 (4%)	1 (2%)	
Sarcoma, NOS, metastatic		1 (2%)	
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Malignant lymphoma, NOS	1 (2%)	1 (2%)	1 (2%)
Malignant lymphoma, lymphocytic type	3 (6%)	2 (4%)	
Malignant lymphoma, histiocytic type	2 (4%)		
Malignant lymphoma, mixed type	8 (16%)	4 (8%)	5 (10%)
#Spleen	(50)	(50)	(50)
Malignant lymphoma, mixed type		2 (4%)	2 (4%)
#Pancreatic lymph node	(50)	(50)	(50)
Malignant lymphoma, mixed type			1 (2%)
#Liver	(50)	(50)	(50)
Malignant lymphoma, histiocytic type	1 (2%)		
CIRCULATORY SYSTEM			
*Multiple organs	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)	
#Liver	(50)	(50)	(50)
Angiolipoma		1 (2%)	
Hemangioma		1 (2%)	
#Uterus	(50)	(50)	(50)
Hemangioma			1 (2%)
DIGESTIVE SYSTEM			
*Lip	(50)	(50)	(50)
Neurilemoma		1 (2%)	
#Liver	(50)	(50)	(50)
Hepatocellular adenoma	3 (6%)	2 (4%)	3 (6%)
Hepatocellular carcinoma		2 (4%)	1 (2%)
#Forestomach	(50)	(50)	(50)
Squamous cell papilloma	4 (8%)		
#Jejunum	(50)	(49)	(49)
Adenomatous polyp, NOS			1 (2%)
*Rectum	(50)	(50)	(50)
Adenocarcinoma, NOS	1 (2%)		
URINARY SYSTEM			
#Urinary bladder	(50)	(49)	(50)
Squamous cell carcinoma		1 (2%)	

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM			
#Anterior pituitary	(45)	(46)	(45)
Carcinoma, NOS			1 (2%)
Adenoma, NOS	13 (29%)	10 (22%)	9 (20%)
#Adrenal	(50)	(50)	(50)
Cortical adenoma			1 (2%)
#Adrenal/capsule	(50)	(50)	(50)
Adenoma, NOS			1 (2%)
#Adrenal medulla	(50)	(50)	(50)
Pheochromocytoma	1 (2%)		
#Thyroid	(50)	(50)	(49)
Follicular cell adenoma	3 (6%)	2 (4%)	2 (4%)
Follicular cell carcinoma	1 (2%)	3 (6%)	
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Adenocarcinoma, NOS			1 (2%)
#Uterus	(50)	(50)	(50)
Leiomyosarcoma	1 (2%)		
Endometrial stromal polyp	2 (4%)		1 (2%)
Endometrial stromal sarcoma	2 (4%)		2 (4%)
#Uterus/endometrium	(50)	(50)	(50)
Adenocarcinoma, NOS	1 (2%)		
#Fallopian tube	(50)	(50)	(50)
Papillary cystadenocarcinoma, NOS		1 (2%)	
#Ovary	(49)	(48)	(48)
Papillary cystadenoma, NOS		1 (2%)	
Granulosa cell tumor			1 (2%)
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(50)	(50)	(50)
Adenoma, NOS	1 (2%)	3 (6%)	
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
None			
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	12	11	12
Moribund sacrifice	15	16	15
Terminal sacrifice	23	23	23

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	35	27	23
Total primary tumors	55	41	37
Total animals with benign tumors	22	15	19
Total benign tumors	27	22	22
Total animals with malignant tumors	23	17	14
Total malignant tumors	28	19	14
Total animals with secondary tumors##		1	
Total secondary tumors		1	
Total animals with tumors uncertain-- benign or malignant			1
Total uncertain tumors			1

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ
† Multiple occurrence of morphology in the same organ; tissue counted only once.

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY C.I. ACID ORANGE 3: VEHICLE CONTROL

ANIMAL NUMBER	0040	0161	0004	0499	0115	0117	0331	0446	0110	0229	0330	0334	0226	0445	0006	0336	0111	0447	0119	0221	0550	0001	0005	0002	0288
WEEKS ON STUDY	071	073	076	076	078	079	079	080	082	083	083	084	085	085	086	087	088	088	090	090	092	093	093	096	097
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Sarcoma, NOS																									
Fibrosarcoma																					X				
Neurilemoma, malignant											@X														
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma			X																			X			
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+
Spleen	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	-	-	+	+	-
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma					X																				
Malignant lymphoma, histiocytic type								X																	
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	+	N	N	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																						X			
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rectum	+	+	+	+	+	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																			X						
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+
Adenoma, NOS							X																X		
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																									X
Follicular cell carcinoma																									
Parathyroid	+	-	+	+	-	-	-	-	+	+	+	+	-	+	-	+	+	+	+	-	+	-	+	+	-
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																									
Leiomyosarcoma										X															
Endometrial stromal polyp																							X		
Endometrial stromal sarcoma																			X				X		
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, NOS															X										
Malignant lymphoma, lymphocytic type					X																				
Malignant lymphoma, histiocytic type						X						X													
Malignant lymphoma, mixed type																					X				

+: Tissue examined microscopically
-: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
@: Multiple occurrence of morphology
: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: VEHICLE CONTROL
(Continued)

ANIMAL NUMBER	038	008	003	007	009	012	013	014	018	020	022	023	024	025	027	032	035	036	037	039	041	042	043	045	048	TOTAL:
WEEKS ON STUDY	101	102	104	104	104	104	104	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS												X														1
Fibrosarcoma																										1
Neurilemoma, malignant																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																										2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	−	+	+		41
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																		X		X						3
Malignant lymphoma, histiocytic type																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	N	+	N	+	+	N	+	+	+	+	N	+	+	N	+	N	N	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma				X							X							X								4
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	N	+		*50
Adenocarcinoma, NOS																										1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	45
Adenoma, NOS	X			X	X			X				X	X			X	X			X	X	X				13
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma							X																			1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma									X						X											3
Follicular cell carcinoma					X																					1
Parathyroid	+	+	+	+	−	+	+	−	+	−	+	−	−	+	+	+	−	+	+	+	+	+	+	+	+	34
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, NOS			X																							1
Leiomyosarcoma																										1
Endometrial stromal polyp																									X	2
Endometrial stromal sarcoma																										2
Ovary	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS				X																						1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, NOS																										1
Malignant lymphoma, lymphocytic type	X						X																			3
Malignant lymphoma, histiocytic type																										2
Malignant lymphoma, mixed type		X	X											X	X	X		X						X		8

* Animals necropsied

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3: LOW DOSE

ANIMAL NUMBER	0040	0045	0046	0090	0093	0041	0220	0191	0006	0048	0040	0010	0222	0143	0333	0490	0266	0334	0133	0055	0244	0394	0446	0166	0336
WEEKS ON STUDY	0556	0661	0664	0665	0668	0770	0744	0755	0777	0778	0833	0888	0900	0933	0933	0935	0966	0966	0967	0988	0998	0998	0998	0999	0999
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS																									X
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																			X						
Alveolar/bronchiolar carcinoma																									X
Sarcoma, NOS, metastatic																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type												X													
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	−	+	+	+	+	+	−	−	−	+	−	−	−	+	+	−	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Neurilemoma																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Hepatocellular carcinoma																			X						
Angiolipoma																		X							
Hemangioma																									X
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	N	+	+	N	+	+	+	N	+	+	+	N	N	N	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	−	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																									
Follicular cell carcinoma																						X			
Parathyroid	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papillary cystadenocarcinoma, NOS																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papillary cystadenoma, NOS																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hemangiosarcoma							X																		
Malignant lymphoma, NOS																				X					
Malignant lymphoma, lymphocytic type																									
Malignant lymphoma, mixed type													X	X											

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	0 1 1	0 3 1	0 0 1	0 0 2	0 0 7	0 0 8	0 1 2	0 1 5	0 1 7	0 1 8	0 2 1	0 2 3	0 2 5	0 2 7	0 2 8	0 2 9	0 3 0	0 3 2	0 3 5	0 3 7	0 3 8	0 4 2	0 4 3	0 4 7	0 5 0	TOTAL:
WEEKS ON STUDY	1 0 0	1 0 3	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma						X																				1
Alveolar/bronchiolar carcinoma																										1
Sarcoma, NOS, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Malignant lymphoma, mixed type																									X	2
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	−	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	39
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Neurilemoma																					X					1
Salivary gland	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma			X															X								2
Hepatocellular carcinoma	X																									2
Angiolipoma																										1
Hemangioma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	N	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell carcinoma									X																	1
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Adenoma, NOS			X								X		X			X		X	X	X	X		X	X		10
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma			X																					X		2
Follicular cell carcinoma											X												X			3
Parathyroid	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	45
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Papillary cystadenocarcinoma, NOS									X																	1
Ovary	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	48
Papillary cystadenoma, NOS																					X					1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS						X						X		X												3
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Hemangiosarcoma																										1
Malignant lymphoma, NOS																										1
Malignant lymphoma, lymphocytic type															X							X				2
Malignant lymphoma, mixed type						X													X							4

* Animals necropsied

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3: HIGH DOSE

ANIMAL NUMBER	0 2 8	0 1 8	0 2 5	0 4 3	0 3 3	0 4 4	0 0 2	0 1 4	0 3 6	0 1 1	0 2 9	0 3 4	0 4 5	0 1 2	0 2 0	0 3 9	0 1 0	0 2 7	0 3 5	0 4 9	0 0 4	0 1 5	0 2 1	0 2 4	0 1 3
WEEKS ON STUDY	0 4 7	0 7 0	0 7 2	0 7 3	0 7 8	0 7 8	0 8 5	0 8 5	0 8 6	0 8 9	0 9 1	0 9 3	0 9 3	0 9 5	0 9 5	0 9 5	0 9 6	0 9 8	0 9 8	0 9 8	0 9 9	0 9 9	0 9 9	0 9 9	1 0 1
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma									X								X								
Trachea	+	+	+	–	–	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type															X										
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type																									
Thymus	+	+	+	–	+	+	+	+	–	+	+	+	+	+	–	–	–	+	+	+	–	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																								X	
Hepatocellular carcinoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	N	N	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+
Pancreas	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenomatous polyp, NOS															X										
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS												X													
Adenoma, NOS													X	X			X								
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Cortical adenoma																									
Thyroid	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																									
Parathyroid	+	+	+	–	+	+	+	–	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	N	+	+
Adenocarcinoma, NOS																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp																									
Endometrial stromal sarcoma													X						X						
Hemangioma																			X						
Ovary	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, NOS									X																
Malignant lymphoma, mixed type																									

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER	0032	0001	0003	0005	0006	0007	0008	0009	0016	0017	0019	0022	0023	0026	0030	0031	0037	0038	0040	0041	0042	0046	0047	0048	0050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	102	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma								X																		3
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Malignant lymphoma, mixed type																		X								2
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Malignant lymphoma, mixed type					X																					1
Thymus	+	−	+	+	−	−	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	38
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma					X					X																3
Hepatocellular carcinoma															X											1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	N	+	+	N	+	+	+	N	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenomatous polyp, NOS																										1
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	45
Carcinoma, NOS																										1
Adenoma, NOS				X												X		X		X				X	X	9
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS																				X						1
Cortical adenoma													X													1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell adenoma							X					X														2
Parathyroid	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	−	+	40
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Adenocarcinoma, NOS												X														1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrial stromal polyp					X																					1
Endometrial stromal sarcoma																										2
Hemangioma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Granulosa cell tumor																						X				1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, NOS																										1
Malignant lymphoma, mixed type							X									X					X	X			X	5

* Animals necropsied

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

	Vehicle Control	250 mg/kg	500 mg/kg
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted Rates (b)	0.0%	4.3%	9.2%
Terminal Rates (c)	0/23 (0%)	1/23 (4%)	1/24 (4%)
Week of First Observation		104	86
Life Table Tests (d)	P=0.074	P=0.500	P=0.150
Incidental Tumor Tests (d)	P=0.046	P=0.500	P=0.097
Cochran-Armitage Trend Test (d)	P=0.060		
Fisher Exact Test (d)		P=0.500	P=0.121
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	2/50 (4%)	2/50 (4%)	3/50 (6%)
Adjusted Rates (b)	5.5%	7.4%	9.2%
Terminal Rates (c)	0/23 (0%)	1/23 (4%)	1/24 (4%)
Week of First Observation	76	98	86
Life Table Tests (d)	P=0.467	P=0.663N	P=0.560
Incidental Tumor Tests (d)	P=0.468	P=0.472N	P=0.568
Cochran-Armitage Trend Test (d)	P=0.406		
Fisher Exact Test (d)		P=0.691	P=0.500
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted Rates (b)	10.2%	8.7%	0.0%
Terminal Rates (c)	1/23 (4%)	2/23 (9%)	0/24 (0%)
Week of First Observation	78	104	
Life Table Tests (d)	P=0.081N	P=0.514N	P=0.119N
Incidental Tumor Tests (d)	P=0.053N	P=0.366N	P=0.072N
Cochran-Armitage Trend Test (d)	P=0.082N		
Fisher Exact Test (d)		P=0.500N	P=0.121N
Hematopoietic System: Malignant Lymphoma, Histiocytic Type			
Overall Rates (a)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted Rates (b)	6.9%	0.0%	0.0%
Terminal Rates (c)	0/23 (0%)	0/23 (0%)	0/24 (0%)
Week of First Observation	79		
Life Table Tests (d)	P=0.038N	P=0.136N	P=0.117N
Incidental Tumor Tests (d)	P=0.202N	P=0.518N	P=0.380N
Cochran-Armitage Trend Test (d)	P=0.037N		
Fisher Exact Test (d)		P=0.121N	P=0.121N
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	8/50 (16%)	6/50 (12%)	8/50 (16%)
Adjusted Rates (b)	31.5%	19.7%	31.1%
Terminal Rates (c)	6/23 (26%)	3/23 (13%)	7/24 (29%)
Week of First Observation	92	88	95
Life Table Tests (d)	P=0.482N	P=0.347N	P=0.547N
Incidental Tumor Tests (d)	P=0.515	P=0.531N	P=0.545N
Cochran-Armitage Trend Test (d)	P=0.556		
Fisher Exact Test (d)		P=0.387N	P=0.607
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	15/50 (30%)	9/50 (18%)	9/50 (18%)
Adjusted Rates (b)	45.2%	30.1%	32.7%
Terminal Rates (c)	7/23 (30%)	5/23 (22%)	7/24 (29%)
Week of First Observation	78	88	86
Life Table Tests (d)	P=0.068N	P=0.123N	P=0.096N
Incidental Tumor Tests (d)	P=0.198N	P=0.292N	P=0.199N
Cochran-Armitage Trend Test (d)	P=0.092N		
Fisher Exact Test (d)		P=0.121N	P=0.121N

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. *ACID ORANGE 3* (Continued)

	Vehicle Control	250 mg/kg	500 mg/kg
Liver: Hepatocellular Adenoma			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	3/50 (6%)
Adjusted Rates (b)	10.7%	8.7%	11.4%
Terminal Rates (c)	2/23 (9%)	2/23 (9%)	2/24 (8%)
Week of First Observation	78	104	99
Life Table Tests (d)	P=0.562N	P=0.511N	P=0.635N
Incidental Tumor Tests (d)	P=0.529N	P=0.438N	P=0.582N
Cochran-Armitage Trend Test (d)	P=0.588		
Fisher Exact Test (d)		P=0.500N	P=0.661
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	3/50 (6%)	4/50 (8%)	4/50 (8%)
Adjusted Rates (b)	10.7%	15.1%	15.4%
Terminal Rates (c)	2/23 (9%)	2/23 (9%)	3/24 (13%)
Week of First Observation	78	97	99
Life Table Tests (d)	P=0.462	P=0.507	P=0.531
Incidental Tumor Tests (d)	P=0.559	P=0.636N	P=0.584
Cochran-Armitage Trend Test (d)	P=0.424		
Fisher Exact Test (d)		P=0.500	P=0.500
Forestomach: Squamous Cell Papilloma			
Overall Rates (a)	4/50 (8%)	0/50 (0%)	0/50 (0%)
Adjusted Rates (b)	16.0%	0.0%	0.0%
Terminal Rates (c)	3/23 (13%)	0/23 (0%)	0/24 (0%)
Week of First Observation	93		
Life Table Tests (d)	P=0.012N	P=0.056N	P=0.051N
Incidental Tumor Tests (d)	P=0.008N	P=0.039N	P=0.034N
Cochran-Armitage Trend Test (d)	P=0.015N		
Fisher Exact Test (d)		P=0.059N	P=0.059N
Pituitary Gland: Adenoma			
Overall Rates (a)	13/45 (29%)	10/46 (22%)	9/45 (20%)
Adjusted Rates (b)	52.5%	45.5%	34.3%
Terminal Rates (c)	10/21 (48%)	10/22 (45%)	6/21 (29%)
Week of First Observation	79	104	93
Life Table Tests (d)	P=0.152N	P=0.251N	P=0.186N
Incidental Tumor Tests (d)	P=0.114N	P=0.233N	P=0.106N
Cochran-Armitage Trend Test (d)	P=0.192N		
Fisher Exact Test (d)		P=0.294N	P=0.231N
Pituitary Gland: Adenoma or Carcinoma			
Overall Rates (a)	13/45 (29%)	10/46 (22%)	10/45 (22%)
Adjusted Rates (b)	52.5%	45.5%	36.0%
Terminal Rates (c)	10/21 (48%)	10/22 (45%)	6/21 (29%)
Week of First Observation	79	104	93
Life Table Tests (d)	P=0.220N	P=0.251N	P=0.254N
Incidental Tumor Tests (d)	P=0.162N	P=0.233N	P=0.140N
Cochran-Armitage Trend Test (d)	P=0.269N		
Fisher Exact Test (d)		P=0.294N	P=0.315N
Thyroid Gland: Follicular Cell Adenoma			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	2/49 (4%)
Adjusted Rates (b)	12.2%	8.7%	8.3%
Terminal Rates (c)	2/23 (9%)	2/23 (9%)	2/24 (8%)
Week of First Observation	97	104	104
Life Table Tests (d)	P=0.376N	P=0.481N	P=0.463N
Incidental Tumor Tests (d)	P=0.342N	P=0.426N	P=0.405N
Cochran-Armitage Trend Test (d)	P=0.415N		
Fisher Exact Test (d)		P=0.500N	P=0.510N

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	250 mg/kg	500 mg/kg
Thyroid Gland: Follicular Cell Carcinoma			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	0/49 (0%)
Adjusted Rates (b)	4.3%	11.6%	0.0%
Terminal Rates (c)	1/23 (4%)	2/23 (9%)	0/24 (0%)
Week of First Observation	104	98	
Life Table Tests (d)	P=0.347N	P=0.325	P=0.492N
Incidental Tumor Tests (d)	P=0.306N	P=0.378	P=0.492N
Cochran-Armitage Trend Test (d)	P=0.384N		
Fisher Exact Test (d)		P=0.309	P=0.505N
Thyroid Gland: Follicular Cell Adenoma or Carcinoma			
Overall Rates (a)	4/50 (8%)	5/50 (10%)	2/49 (4%)
Adjusted Rates (b)	16.4%	20.1%	8.3%
Terminal Rates (c)	3/23 (13%)	4/23 (17%)	2/24 (8%)
Week of First Observation	97	98	104
Life Table Tests (d)	P=0.243N	P=0.530	P=0.302N
Incidental Tumor Tests (d)	P=0.198N	P=0.614	P=0.254N
Cochran-Armitage Trend Test (d)	P=0.292N		
Fisher Exact Test (d)		P=0.500	P=0.349N
Harderian Gland: Adenoma			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	4.3%	13.0%	0.0%
Terminal Rates (c)	1/23 (4%)	3/23 (13%)	0/24 (0%)
Week of First Observation	104	104	
Life Table Tests (d)	P=0.364N	P=0.302	P=0.492N
Incidental Tumor Tests (d)	P=0.364N	P=0.302	P=0.492N
Cochran-Armitage Trend Test (d)	P=0.378N		
Fisher Exact Test (d)		P=0.309	P=0.500N
All Sites: Benign Tumors			
Overall Rates (a)	22/50 (44%)	15/50 (30%)	19/50 (38%)
Adjusted Rates (b)	75.0%	59.4%	59.1%
Terminal Rates (c)	16/23 (70%)	13/23 (57%)	12/24 (50%)
Week of First Observation	73	96	86
Life Table Tests (d)	P=0.189N	P=0.071N	P=0.214N
Incidental Tumor Tests (d)	P=0.106N	P=0.022N	P=0.087N
Cochran-Armitage Trend Test (d)	P=0.303N		
Fisher Exact Test (d)		P=0.107N	P=0.343N
All Sites: Malignant Tumors			
Overall Rates (a)	23/50 (46%)	17/50 (34%)	14/50 (28%)
Adjusted Rates (b)	59.7%	50.7%	45.4%
Terminal Rates (c)	9/23 (39%)	8/23 (35%)	9/24 (38%)
Week of First Observation	76	74	86
Life Table Tests (d)	P=0.028N	P=0.160N	P=0.037N
Incidental Tumor Tests (d)	P=0.053N	P=0.242N	P=0.062N
Cochran-Armitage Trend Test (d)	P=0.038N		
Fisher Exact Test (d)		P=0.154N	P=0.049N
All Sites: All Tumors			
Overall Rates (a)	35/50 (70%)	27/50 (54%)	23/50 (46%)
Adjusted Rates (b)	89.2%	80.9%	70.1%
Terminal Rates (c)	19/23 (83%)	17/23 (74%)	15/24 (63%)
Week of First Observation	76	74	86
Life Table Tests (d)	P=0.006N	P=0.086N	P=0.009N
Incidental Tumor Tests (d)	P=0.004N	P=0.101N	P=0.004N
Cochran-Armitage Trend Test (d)	P=0.010N		
Fisher Exact Test (d)		P=0.075N	P=0.013N

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the vehicle controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

TABLE D4a. HISTORICAL INCIDENCE OF FORESTOMACH SQUAMOUS CELL TUMORS IN FEMALE B6C3F$_1$ MICE ADMINISTERED CORN OIL BY GAVAGE (a)

Study	No. Examined	No. of Tumors	Diagnosis
Historical Incidence at Southern Research Institute			
Ethyl acrylate	50	1	Squamous cell papilloma
Allyl isovalerate	50	1	Squamous cell papilloma
		1	Adenoma, NOS
Geranyl acetate	50	1	Adenomatous polyp
Chlorinated paraffins (C_{12}, 60% chlorine)	50	2	Squamous cell papilloma
All others	196	0	
TOTAL	396	6 (1.5%)	
Overall Historical Incidence			
		2	Papilloma, NOS
		14	Squamous cell papilloma
		1	Adenoma, NOS
		1	Adenomatous polyp
TOTAL	1,709	18 (1.1%)	
Range			
High	47	4	
Low	50	0	

(a) Data as of August 7, 1986, for studies of at least 104 weeks

TABLE D4b. HISTORICAL INCIDENCE OF URINARY BLADDER SQUAMOUS CELL TUMORS IN FEMALE B6C3F$_1$ MICE ADMINISTERED CORN OIL BY GAVAGE (a)

	No. Examined	No. of Tumors
Historical Incidence at Southern Research Institute		
	400	0
Overall Historical Incidence		
	1,665	0

(a) Data as of August 7, 1986, for studies of at least 104 weeks

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Inflammation, chronic	1 (2%)	2 (4%)	
Hyperplasia, NOS		1 (2%)	
*Subcutaneous tissue	(50)	(50)	(50)
Inflammation, chronic	1 (2%)		
Fibrosis			1 (2%)
Necrosis, fat		1 (2%)	
RESPIRATORY SYSTEM			
*Nasal cavity	(50)	(50)	(50)
Foreign body, NOS	1 (2%)	1 (2%)	
Inflammation, suppurative	3 (6%)	2 (4%)	
Reaction, foreign body	5 (10%)	3 (6%)	
#Lung	(50)	(50)	(50)
Congestion, NOS	3 (6%)	1 (2%)	1 (2%)
Inflammation, focal			1 (2%)
Hyperplasia, alveolar epithelium	1 (2%)		1 (2%)
#Lung/alveoli	(50)	(50)	(50)
Histiocytosis	2 (4%)		
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Myeloproliferative disorder	1 (2%)		
Leukocytosis, NOS		1 (2%)	
Hyperplasia, lymphoid	2 (4%)		
Hematopoiesis		1 (2%)	
#Bone marrow	(50)	(50)	(50)
Angiectasis		1 (2%)	
Myelofibrosis	2 (4%)		2 (4%)
Hyperplasia, granulocytic	1 (2%)	2 (4%)	
#Spleen	(50)	(50)	(50)
Pigmentation, NOS			1 (2%)
Atrophy, NOS		1 (2%)	1 (2%)
Hyperplasia, lymphoid	2 (4%)	3 (6%)	4 (8%)
Mastocytosis			1 (2%)
Hematopoiesis	21 (42%)	21 (42%)	19 (38%)
#Splenic red pulp	(50)	(50)	(50)
Atrophy, NOS		1 (2%)	
#Lymph node	(50)	(50)	(50)
Angiectasis	1 (2%)		1 (2%)
#Mandibular lymph node	(50)	(50)	(50)
Hyperplasia, NOS	1 (2%)	1 (2%)	
Hyperplasia, lymphoid	3 (6%)	2 (4%)	
#Bronchial lymph node	(50)	(50)	(50)
Hyperplasia, lymphoid			1 (2%)
#Mediastinal lymph node	(50)	(50)	(50)
Hyperplasia, NOS	6 (12%)	3 (6%)	5 (10%)
Angiectasis			1 (2%)
Hyperplasia, lymphoid		1 (2%)	
#Pancreatic lymph node	(50)	(50)	(50)
Hyperplasia, NOS		1 (2%)	1 (2%)

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Mesenteric lymph node	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)		
Necrosis, focal	1 (2%)		
Amyloidosis			1 (2%)
Hyperplasia, NOS	2 (4%)		1 (2%)
Angiectasis	1 (2%)	2 (4%)	1 (2%)
Hyperplasia, lymphoid	1 (2%)		
#Renal lymph node	(50)	(50)	(50)
Hyperplasia, NOS	9 (18%)	7 (14%)	10 (20%)
Hyperplasia, lymphoid		1 (2%)	
#Iliac lymph node	(50)	(50)	(50)
Hyperplasia, NOS	7 (14%)	4 (8%)	5 (10%)
Angiectasis	1 (2%)		
#Inguinal lymph node	(50)	(50)	(50)
Hyperplasia, lymphoid	1 (2%)		
#Lung	(50)	(50)	(50)
Leukocytosis, NOS	2 (4%)	1 (2%)	2 (4%)
Hyperplasia, lymphoid	1 (2%)	4 (8%)	1 (2%)
#Salivary gland	(49)	(48)	(48)
Hyperplasia, lymphoid		1 (2%)	
#Liver	(50)	(50)	(50)
Leukocytosis, NOS	4 (8%)	4 (8%)	8 (16%)
Hyperplasia, lymphoid		1 (2%)	
Mastocytosis			1 (2%)
Hematopoiesis	11 (22%)	4 (8%)	8 (16%)
#Omentum	(50)	(50)	(50)
Hyperplasia, lymphoid		1 (2%)	
#Peyer's patch	(50)	(49)	(49)
Hyperplasia, lymphoid	1 (2%)		
#Kidney	(50)	(50)	(50)
Hyperplasia, lymphoid	20 (40%)	24 (48%)	29 (58%)
Mastocytosis			1 (2%)
#Urinary bladder	(50)	(49)	(50)
Hyperplasia, lymphoid		1 (2%)	
#Adrenal	(50)	(50)	(50)
Hematopoiesis			1 (2%)
#Adrenal cortex	(50)	(50)	(50)
Hematopoiesis		1 (2%)	
#Thymus	(41)	(39)	(38)
Embryonal duct cyst		1 (3%)	
Hyperplasia, epithelial	1 (2%)		
Hyperplasia, lymphoid		1 (3%)	
CIRCULATORY SYSTEM			
*Head	(50)	(50)	(50)
Periarteritis	1 (2%)		
#Mesenteric lymph node	(50)	(50)	(50)
Thrombosis, NOS			1 (2%)
#Heart	(50)	(50)	(50)
Thrombosis, NOS		1 (2%)	
#Myocardium	(50)	(50)	(50)
Inflammation, NOS		1 (2%)	
*Aorta	(50)	(50)	(50)
Inflammation, NOS		1 (2%)	
*Mesentery	(50)	(50)	(50)
Periarteritis	1 (2%)	1 (2%)	
#Ovary	(49)	(48)	(48)
Thrombosis, NOS	1 (2%)		

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM			
*Root of tooth	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)	
Dysplasia, NOS	1 (2%)	1 (2%)	
*Periodontal tissues	(50)	(50)	(50)
Periodontal cyst	1 (2%)		
Reaction, foreign body	1 (2%)		
#Salivary gland	(49)	(48)	(48)
Inflammation, chronic		1 (2%)	1 (2%)
#Liver	(50)	(50)	(50)
Inflammation, focal	1 (2%)		1 (2%)
Fibrosis, focal			3 (6%)
Cholangiofibrosis			1 (2%)
Necrosis, focal	3 (6%)	1 (2%)	2 (4%)
Necrosis, coagulative	1 (2%)		
Metamorphosis, fatty			3 (6%)
Angiectasis		1 (2%)	
#Liver/centrilobular	(50)	(50)	(50)
Necrosis, NOS	1 (2%)		
Cytoplasmic vacuolization	1 (2%)		
Atrophy, NOS	1 (2%)		
*Gallbladder	(50)	(50)	(50)
Fibrosis			1 (2%)
Degeneration, hyaline			1 (2%)
#Bile duct	(50)	(50)	(50)
Hyperplasia, NOS			2 (4%)
*Common bile duct	(50)	(50)	(50)
Hyperplasia, cystic			1 (2%)
#Pancreas	(50)	(50)	(49)
Cyst, NOS			1 (2%)
Cystic ducts		1 (2%)	
Edema, NOS		1 (2%)	
Inflammation, chronic		1 (2%)	
Fibrosis		1 (2%)	
Atrophy, focal		3 (6%)	1 (2%)
#Pancreatic duct	(50)	(50)	(49)
Hyperplasia, cystic	1 (2%)		
#Esophagus	(50)	(50)	(50)
Inflammation, chronic		1 (2%)	
#Glandular stomach	(50)	(50)	(50)
Inflammation, focal	1 (2%)		
Pigmentation, NOS			1 (2%)
#Forestomach	(50)	(50)	(50)
Ulcer, NOS	2 (4%)		
Inflammation, focal	5 (10%)	1 (2%)	
Hyperplasia, epithelial	4 (8%)	3 (6%)	2 (4%)
#Small intestine	(50)	(49)	(49)
Fibrosis			1 (2%)
Amyloidosis			1 (2%)
#Jejunum	(50)	(49)	(49)
Hyperplasia, adenomatous			1 (2%)
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Hydronephrosis			2 (4%)
Polycystic kidney			1 (2%)
Inflammation, NOS	5 (10%)	4 (8%)	18 (36%)
Inflammation, focal	1 (2%)		
Inflammation, suppurative	1 (2%)	3 (6%)	4 (8%)
Glomerulonephritis, chronic	1 (2%)		

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
URINARY SYSTEM			
#Kidney (Continued)	(50)	(50)	(50)
Fibrosis	4 (8%)	9 (18%)	31 (62%)
Nephrosis, NOS	13 (26%)	42 (84%)	50 (100%)
Necrosis, medullary	2 (4%)	5 (10%)	8 (16%)
Pigmentation, NOS	2 (4%)		
Atrophy, NOS			8 (16%)
Metaplasia, osseous	2 (4%)		2 (4%)
#Kidney/medulla	(50)	(50)	(50)
Degeneration, hyaline			1 (2%)
#Renal papilla	(50)	(50)	(50)
Degeneration, NOS		3 (6%)	19 (38%)
#Kidney/glomerulus	(50)	(50)	(50)
Amyloidosis	1 (2%)		1 (2%)
#Kidney/tubule	(50)	(50)	(50)
Mineralization	3 (6%)	15 (30%)	22 (44%)
Dilatation, NOS	2 (4%)	35 (70%)	42 (84%)
#Urinary bladder	(50)	(49)	(50)
Inflammation, focal			1 (2%)
Fibrosis			1 (2%)
Hyperplasia, epithelial		1 (2%)	3 (6%)
ENDOCRINE SYSTEM			
#Anterior pituitary	(45)	(46)	(45)
Hemorrhagic cyst			1 (2%)
Focal cellular change	1 (2%)		
Hyperplasia, NOS			1 (2%)
Hyperplasia, focal	7 (16%)	4 (9%)	9 (20%)
Angiectasis	3 (7%)	1 (2%)	2 (4%)
#Adrenal	(50)	(50)	(50)
Congestion, NOS		1 (2%)	
#Adrenal/capsule	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)		1 (2%)
#Adrenal cortex	(50)	(50)	(50)
Cyst, NOS		1 (2%)	1 (2%)
Depletion, lipid	2 (4%)		
#Adrenal medulla	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)	1 (2%)	
#Thyroid	(50)	(50)	(49)
Embryonal duct cyst		1 (2%)	
Cystic follicles	1 (2%)		3 (6%)
Inflammation, focal			1 (2%)
Degeneration, cystic	8 (16%)	7 (14%)	4 (8%)
Hyperplasia, follicular cell	5 (10%)	4 (8%)	7 (14%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Dilatation, NOS	10 (20%)	8 (16%)	10 (20%)
Hyperplasia, focal	1 (2%)		
*Preputial gland	(50)	(50)	(50)
Inflammation, chronic	1 (2%)		
Degeneration, cystic	1 (2%)		
*Vagina	(50)	(50)	(50)
Inflammation, NOS			1 (2%)
#Uterus	(50)	(50)	(50)
Hydrometra		1 (2%)	
Hemorrhage			1 (2%)
Inflammation, suppurative	2 (4%)	6 (12%)	3 (6%)
Decidual alteration, NOS			1 (2%)

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
REPRODUCTIVE SYSTEM (Continued)			
#Uterus/endometrium	(50)	(50)	(50)
Hyperplasia, cystic	42 (84%)	46 (92%)	45 (90%)
#Ovary/parovarian	(49)	(48)	(48)
Hemorrhagic cyst	1 (2%)		
Inflammation, suppurative		1 (2%)	
#Ovary	(49)	(48)	(48)
Follicular cyst, NOS	17 (35%)	23 (48%)	23 (48%)
Inflammation, suppurative	3 (6%)	2 (4%)	1 (2%)
Fibrosis		1 (2%)	
Angiectasis		2 (4%)	
#Mesovarium	(49)	(48)	(48)
Inflammation, suppurative		1 (2%)	
Necrosis, fat			1 (2%)
NERVOUS SYSTEM			
#Brain	(50)	(50)	(50)
Deformity, NOS			1 (2%)
Hemorrhage	1 (2%)		
#Cerebral basal surface	(50)	(50)	(50)
Displacement, NOS	3 (6%)		
#Brain/thalamus	(50)	(50)	(50)
Mineralization	20 (40%)	26 (52%)	20 (40%)
*Cauda equina	(50)	(50)	(50)
Status spongiosus	1 (2%)		
SPECIAL SENSE ORGANS			
*Eye/cornea	(50)	(50)	(50)
Inflammation, NOS	1 (2%)		
*Nasolacrimal duct	(50)	(50)	(50)
Inflammation, NOS			3 (6%)
*Harderian gland	(50)	(50)	(50)
Degeneration, cystic			1 (2%)
MUSCULOSKELETAL SYSTEM			
*Skull	(50)	(50)	(50)
Abscess, NOS		1 (2%)	
*Femur	(50)	(50)	(50)
Fracture, NOS			1 (2%)
BODY CAVITIES			
*Mediastinum	(50)	(50)	(50)
Lymphocytic inflammatory infiltration	1 (2%)		
Inflammation, suppurative			1 (2%)
*Abdominal cavity	(50)	(50)	(50)
Hemorrhage	1 (2%)		
*Peritoneum	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)	
*Pleura	(50)	(50)	(50)
Inflammation, NOS		1 (2%)	
*Mesentery	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)	2 (4%)	
Inflammation, chronic		1 (2%)	
Lipogranuloma			1 (2%)
Necrosis, fat	4 (8%)	1 (2%)	1 (2%)

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF C.I. ACID ORANGE 3 (Continued)

	Vehicle Control	Low Dose	High Dose
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Inflammation, suppurative	13 (26%)	11 (22%)	13 (26%)
Broad ligament			
Necrosis, focal		1	
Necrosis, fat	2	3	
SPECIAL MORPHOLOGY SUMMARY			
None			

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX E

MUTAGENICITY OF C.I. ACID ORANGE 3 IN *SALMONELLA TYPHIMURIUM*

MUTAGENICITY OF C.I. ACID ORANGE 3 IN *SALMONELLA TYPHIMURIUM* (a)

Strain	Dose (µg/plate)	Revertants/plate (b)					
		−S9		+S9 (hamster)		+S9 (rat)	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TA100	0	105 ± 6.8	96 ± 4.0	85 ± 6.7	90 ± 1.2	96 ± 7.6	94 ± 8.0
	10	90 ± 4.5	92 ± 1.9	--	--	--	--
	33	108 ± 2.5	108 ± 9.0	84 ± 2.7	--	114 ± 6.3	--
	100	120 ± 7.3	103 ± 1.5	105 ± 6.7	99 ± 7.6	103 ± 6.1	116 ± 4.1
	333	259 ± 10.5	182 ± 7.9	118 ± 10.0	103 ± 8.9	124 ± 7.4	109 ± 9.9
	500	(c) 407 ± 29.2	215 ± 4.4	--	--	--	--
	667	--	--	--	153 ± 10.9	--	175 ± 6.6
	1,000	--	--	414 ± 18.8	391 ± 9.8	(c) 458 ± 22.8	(c) 346 ± 18.8
	2,000	--	--	(c) 849 ± 31.6	(c) 993 ± 25.8	(c) 1,024 ± 41.5	(c) 1,120 ± 58.7
	Trial summary	Positive	Positive	Positive	Positive	Positive	Positive
	Positive control (d)	1,118 ± 10.7	956 ± 83.1	1,476 ± 84.1	617 ± 7.4	2,189 ± 38.4	1,229 ± 20.7
TA1535	0	24 ± 2.6	--	13 ± 1.0	--	11 ± 1.2	--
	10	23 ± 3.8	--	--	--	--	
	33	25 ± 2.4	--	9 ± 1.5	--	11 ± 1.2	--
	100	27 ± 5.3	--	14 ± 1.7	--	9 ± 0.9	--
	333	(c) 29 ± 0.9	--	10 ± 1.2	--	9 ± 0.6	--
	500	(c) 23 ± 2.1	--	--	--	--	--
	1,000	--	--	(c) 16 ± 2.0	--	(c) 10 ± 1.8	--
	2,000	--	--	(c) 20 ± 1.7	--	(c) 15 ± 1.2	--
	Trial summary	Negative	--	Negative	--	Negative	--
	Positive control (d)	806 ± 16.9	--	120 ± 8.3	--	124 ± 10.0	--
TA97	0	80 ± 2.2	109 ± 5.7	92 ± 6.0	124 ± 3.8	141 ± 3.7	140 ± 5.2
	10	68 ± 4.8	96 ± 5.2	--	--	--	--
	33	81 ± 16.5	96 ± 3.4	112 ± 8.0	--	131 ± 9.8	--
	100	108 ± 7.5	122 ± 12.4	108 ± 6.8	117 ± 5.8	151 ± 10.1	137 ± 6.0
	333	(c) 189 ± 5.0	(c) 201 ± 2.9	152 ± 1.5	146 ± 4.9	166 ± 2.1	161 ± 3.8
	500	(c) 162 ± 11.5	(c) 200 ± 6.9	--	--	--	--
	667	--	--	--	170 ± 6.6	--	187 ± 7.5
	1,000	--	--	(c) 201 ± 2.8	(c) 209 ± 13.9	(c) 174 ± 6.0	(c) 200 ± 8.7
	2,000	--	--	(c) 233 ± 8.8	(c) 293 ± 10.3	(c) 246 ± 7.1	(c) 311 ± 9.6
	Trial summary	Positive	Equivocal	Positive	Positive	Weak Positive	Positive
	Positive control (d)	520 ± 39.7	735 ± 84.2	947 ± 22.1	685 ± 4.8	1,289 ± 35.6	891 ± 46.9
TA98	0	19 ± 1.7	16 ± 0.6	32 ± 2.4	37 ± 3.4	30 ± 4.6	36 ± 1.0
	10	18 ± 0.0	24 ± 2.7	--	--	--	--
	33	20 ± 2.0	25 ± 3.0	27 ± 2.3	--	34 ± 2.3	--
	100	34 ± 1.2	34 ± 3.5	34 ± 2.2	40 ± 2.8	40 ± 4.0	43 ± 1.7
	333	72 ± 13.5	61 ± 8.5	62 ± 3.3	63 ± 0.9	58 ± 4.1	58 ± 8.0
	500	94 ± 1.0	84 ± 7.3	--	--	--	--
	667	--	--	--	79 ± 6.9	--	82 ± 3.5
	1,000	--	--	93 ± 5.5	134 ± 37.0	97 ± 11.8	95 ± 5.6
	2,000	--	--	175 ± 2.6	228 ± 11.2	(c) 243 ± 13.7	248 ± 9.3
	Trial summary	Positive	Positive	Positive	Positive	Positive	Positive
	Positive control (d)	1,804 ± 39.0	1,566 ± 34.7	1,169 ± 65.8	718 ± 7.6	1,692 ± 63.1	1,240 ± 38.4

(a) Study performed at Microbiological Associates. The detailed protocol is presented in Haworth et al. (1983). Cells and study compound or solvent (dimethyl sulfoxide) were incubated in the absence of exogenous metabolic activation (−S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague Dawley rat liver. High dose was limited by toxicity or solubility but did not exceed 10 mg/plate; 0 µg/plate dose is the solvent control.
(b) Revertants are presented as mean ± standard error from three plates.
(c) Slight toxicity
(d) Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation 4-nitro-*o*-phenylenediamine was used with TA98, sodium azide was used with TA100 and TA1535, and 9-aminoacridine was used with TA97.

APPENDIX F

SENTINEL ANIMAL PROGRAM

APPENDIX F. SENTINEL ANIMAL PROGRAM

I. 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 viral serology on sera from extra (sentinel) animals in the study rooms. These animals are untreated, and these animals and the study animals are both 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.

Fifteen $B6C3F_1$ mice and 15 F344/N rats of each sex are selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group are killed at 6, 12, and 18 months on study. Data from animals surviving 24 months are collected from 5/50 randomly selected control animals of each sex and species. The blood from each animal is collected and clotted, and the serum is separated. The serum is cooled on ice and shipped to Microbiological Associates' Comprehensive Animal Diagnostic Service for determination of the viral antibody titers. The following tests are performed:

	Hemagglutination Inhibition	Complement Fixation	ELISA
Mice	PVM (pneumonia virus of mice) Reo 3 (reovirus type 3) GDVII (Theiler's encephalomyelitis virus) Poly (polyoma virus) MVM (minute virus of mice) Ectro (infectious ectromelia) Sendai (6, 12 mo)	M. Ad. (mouse adenovirus) LCM (lymphocytic choriomeningitis virus) MHV (6 mo) Sendai (18, 24 mo)	MHV (mouse hepatitis virus) (12, 18, 24 mo)
Rats	PVM KRV (Kilham rat virus) H-1 (Toolan's H-1 virus) Sendai (6, 12 mo)	RCV (rat coronavirus) Sendai (18, 24 mo)	

II. Results

Results are presented in Table F1.

TABLE F1. MURINE VIRUS ANTIBODY DETERMINATIONS FOR RATS AND MICE IN THE TWO-YEAR GAVAGE STUDIES OF C.I. ACID ORANGE 3 (a)

Interval (months)	No. of Animals	Positive Serologic Reaction for
RATS		
6	--	None positive
12	3/10	RCV
18	--	None positive
24	--	None positive
MICE		
6	--	None positive
12	--	None positive
18	--	None positive
24	1/10	PVM

(a) Blood samples were taken from sentinel animals at 6, 12, and 18 months after the start of dosing and from the vehicle control animals just before they were killed; samples were sent to Microbiological Associates (Bethesda, MD) for determination of antibody titers.

APPENDIX G

INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Pelleted Diet: September 1980 to October 1982

(Manufactured by Zeigler Bros., Inc., Gardners, PA)

TABLE G1. INGREDIENTS OF NIH 07 RAT AND MOUSE RATION (a)

Ingredients (b)	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Dried brewer's yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

(a) NIH, 1978; NCI, 1976
(b) Ingredients ground to pass through a U.S. Standard Screen No. 16 before being mixed

TABLE G2. VITAMINS AND MINERALS IN NIH 07 RAT AND MOUSE RATION (a)

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 µg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

(a) Per ton (2,000 lb) of finished product

TABLE G3. NUTRIENT COMPOSITION OF NIH 07 RAT AND MOUSE RATION (a)

Nutrients	Mean ± Standard Deviation	Range	Number of Samples
Crude protein (percent by weight)	23.91 ± 0.79	22.7-25.3	24
Crude fat (percent by weight)	4.99 ± 0.43	4.2-5.7	24
Crude fiber (percent by weight)	3.32 ± 0.23	2.9-3.8	24
Ash (percent by weight)	6.49 ± 0.47	5.7-7.43	24
Amino Acids (percent of total diet)			
Arginine	1.260	1.21-1.31	2
Cystine	0.395	0.39-0.40	2
Glycine	1.175	1.15-1.20	2
Histidine	0.553	0.530-0.576	2
Isoleucine	0.908	0.881-0.934	2
Leucine	1.905	1.85-1.96	2
Lysine	1.250	1.20-1.30	2
Methionine	0.310	0.306-0.314	2
Phenylalanine	0.967	0.960-0.974	2
Threonine	0.834	0.827-0.840	2
Tryptophan	0.175	0.171-0.178	2
Tyrosine	0.587	0.566-0.607	2
Valine	1.085	1.05-1.12	2
Essential Fatty Acids (percent of total diet)			
Linoleic	2.37		1
Linolenic	0.308		1
Arachidonic	0.008		1
Vitamins			
Vitamin A (IU/kg)	10,920 ± 1,824	8,300-15,000	24
Vitamin D (IU/kg)	6,300		1
α-Tocopherol (ppm)	37.6	31.1-44.0	2
Thiamine (ppm)	17.2 ± 1.8	14.0-21.0	(b) 23
Riboflavin (ppm)	6.9	6.1-7.4	2
Niacin (ppm)	75	65-85	2
Pantothenic acid (ppm)	30.2	29.8-30.5	2
Pyridoxine (ppm)	7.2	5.6-8.8	2
Folic acid (ppm)	2.1	1.8-2.4	2
Biotin (ppm)	0.24	0.21-0.27	2
Vitamin B_{12} (ppb)	12.8	10.6-15.0	2
Choline (ppm)	3,315	3,200-3,430	2
Minerals			
Calcium (percent)	1.28 ± 0.18	1.08-1.69	24
Phosphorus (percent)	0.99 ± 0.06	0.88-1.10	24
Potassium (percent)	0.809	0.772-0.846	2
Chloride (percent)	0.557	0.479-0.635	2
Sodium (percent)	0.304	0.258-0.349	2
Magnesium (percent)	0.172	0.166-0.177	2
Sulfur (percent)	0.278	0.270-0.285	2
Iron (ppm)	418	409-426	2
Manganese (ppm)	90.8	86.0-95.5	2
Zinc (ppm)	55.1	54.2-56.0	2
Copper (ppm)	12.68	9.65-15.70	2
Iodine (ppm)	2.58	1.52-3.64	2
Chromium (ppm)	1.86	1.79-1.93	2
Cobalt (ppm)	0.57	0.49-0.65	2

(a) One or two batches of feed analyzed for nutrients reported in this table were manufactured in January and/or April 1983.
(b) One batch (7/22/81) not analyzed for thiamine

TABLE G4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Contaminants	Mean ± Standard Deviation	Range	Number of Samples
Arsenic (ppm)	0.44 ± 0.19	<0.05-1.06	24
Cadmium (ppm) (a)	<0.10		24
Lead (ppm)	1.00 ± 0.73	0.42-3.37	24
Mercury (ppm) (a)	< 0.05		24
Selenium (ppm)	0.31 ± 0.07	0.14-0.52	24
Aflatoxins (ppb) (a,b)	<10	<5.0-<10.0	24
Nitrate nitrogen (ppm) (c)	8.70 ± 3.67	2.1-17.0	24
Nitrite nitrogen (ppm) (c)	2.20 ± 1.59	0.4-6.9	24
BHA (ppm) (d,e)	6.02 ± 4.57	<0.5-16.0	24
BHT (ppm) (d)	3.03 ± 1.82	0.8-7.0	24
Aerobic plate count (CFU/g)	35,950 ± 27,857	4,900-88,000	24
Coliform (MPN/g) (f)	27.4 ± 52.6	<3-240	22
Coliform (MPN/g) (g)	90.0 ± 237.9	<3-1,100	24
E. coli (MPN/g) (h)	<3		24
Total nitrosamines (ppb) (i, j)	6.48 ± 5.82	<0.8-18.5	21
Total nitrosamines (ppb) (i,k)	28.76 ± 64.88	<0.8-273.2	24
N-Nitrosodimethylamine (ppb) (i, j)	5.24 ± 5.66	<0.8-16.5	21
N-Nitrosodimethylamine (ppb) (i,k)	27.29 ± 64.45	<0.8-272	24
N-Nitrosopyrrolidine (ppb)	1.23 ± 0.79	0.3-3.5	24
Pesticides (ppm)			
α-BHC (a,l)	<0.01		24
β-BHC (a)	<0.02		24
γ-BHC-Lindane (a)	<0.01		24
δ-BHC (a)	<0.01		24
Heptachlor (a)	<0.01		24
Aldrin (a)	<0.01		24
Heptachlor epoxide (a)	<0.01		24
DDE (a)	<0.01		24
DDD (a)	<0.01		24
DDT (a)	<0.01		24
HCB (a)	<0.01		24
Mirex (a)	<0.01		24
Methoxychlor (m)	<0.05	0.09; 8/26/81	24
Dieldrin (a)	<0.01		24
Endrin (a)	<0.01		24
Telodrin (a)	<0.01		24
Chlordane (a)	<0.05		24
Toxaphene (a)	<0.1		24
Estimated PCBs (a)	<0.2		24
Ronnel (a)	<0.01		24
Ethion (a)	<0.02		24
Trithion (a)	<0.05		24
Diazinon (m)	<0.1	0.2; 4/27/81	24
Methyl parathion (a)	<0.02		24
Ethyl parathion (a)	<0.02		24
Malathion (n)	0.09 ± 0.06	<0.05-0.27	24
Endosulfan I (a)	<0.01		24
Endosulfan II (a)	<0.01		24
Endosulfan sulfate (a)	<0.03		24

TABLE G4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION (Continued)

(a) All values were less than the detection limit, given in the table as the mean.
(b) The detection limit was reduced from 10 ppb to 5 ppb after 7/81.
(c) Source of contamination: alfalfa, grains, and fish meal
(d) Source of contamination: soy oil and fish meal
(e) Two batches contained less than 0.5 ppm.
(f) Mean, standard deviation, and range exclude one very high value of 1,100 obtained for the batch produced on 12/16/80 and one high value of 460 obtained for the batch produced on 9/23/82 (MPN = most probable number).
(g) Mean, standard deviation, and range include the high values listed in footnote (f).
(h) All values were less than 3 MPN/g.
(i) All values were corrected for percent recovery.
(j) Mean, standard deviation, and range exclude three very high values in the range of 115-273.2 ppb for batches produced on 1/26/81, 2/23/81, and 4/27/81.
(k) Mean, standard deviation, and range include the very high values given in footnote (j).
(l) BHC = hexachlorocyclohexane or benzene hexachloride.
(m) There was one observation above the detection limit; the value and date it was obtained are given under the range.
(n) Ten batches contained more than 0.05 ppm.

APPENDIX H

AUDIT SUMMARY

APPENDIX H. AUDIT SUMMARY

The experimental data, documents, and pathology materials for the 2-year studies of C.I. Acid Orange 3 in rats and mice were audited for accuracy, consistency, completeness, and compliance with Good Laboratory Practice (GLP) regulations of the Food and Drug Administration (implemented by the NTP beginning on October 1, 1981). The laboratory studies were conducted for the NTP by Southern Research Institute, Birmingham, Alabama, under a subcontract with Tracor Jitco, Inc., until May 31, 1982, and then under contract with the NIEHS. Exposure to C.I. Acid Orange 3 by gavage in corn oil began on October 16, 1980, for rats and on December 9, 1980, for mice. The retrospective audit was conducted at the NTP Archives in July 1986 and March 1987 by Argus Research Laboratories, Inc. (Paul A. Wennerberg, D.V.M., M.S., Principal Investigator). The individuals who conducted the audit are listed in the full audit report, which is on file at the NIEHS. The audit included a review of:

(1) All records concerning animal receipt, quarantine, randomization, and disposition prior to study start.
(2) Clinical observations recorded during the last 3 months of life and all body weights for a random 10% sample of the study animals.
(3) All inlife records concerning environmental conditions, palpable masses, mortality, animal identification, and correlation of final inlife observation of masses, date of death, and disposition with necropsy records.
(4) All chemistry records, including spectra, MRI reports, chemical use and dose preparation records, analytical records, and correspondence.
(5) Pathology tables and all post mortem records for individual animals concerning identification, disposition codes, condition codes, and correlation between gross observations and microscopic diagnoses.
(6) Inventory and labeling for all wet tissue bags.
(7) Wet tissues from a random 20% sample plus those from animals that had a gross observation without a corresponding microscopic diagnosis to verify animal identification and to examine for untrimmed lesions.
(8) Blocks and slides of tissues from a random 20% sample of animals to examine for proper match and inventory.
(9) The Staff Review Draft of the NTP Technical Report (during September and December 1986).

The audit showed that inlife procedures were documented adequately by archival records with the exception of information on the exact number of animals received, the disposition of extra animals, and animal randomization. The audit findings were not considered to have major significance on the interpretation of the studies. For example, a 100% review of masses recorded among the last clinical observations for each animal showed that all but 4/85 masses noted in 69 rats and 4/60 masses noted in 57 mice were correlated with histopathologic observations. The wet tissues for these four rats and four mice were examined and found to contain no masses or other untrimmed potential lesions. The time to necropsy exceeded 8 hours for 11 rats and 15 mice; however, tissue accountability was good for the kidney (target organ) and good or fair for all other tissues except the gallbladder in some groups of mice (vehicle control, low dose, and high dose males and vehicle control females).

The audit of the pathology data clarified that all of the accidental deaths were related to error in gavage administration technique. Forms used earlier in these studies did not distinguish between dosing error and accidental deaths by other causes. Inspection of wet tissues for individual animal identifiers showed that all but 5/91 rats and 10/97 mice were identified correctly. Extensive followup on all identification ambiguities suggested that they were the result of tears in the ear punch holes rather than animal mixup. The audit also identified a variety of untrimmed potential lesions and gross observations that lacked corresponding microscopic diagnoses which, when evaluated by NTP staff, were judged to be relatively minor and to not adversely affect interpretation of the pathology

data. Full details about these and other audit findings are presented in the audit report on file at the NIEHS.

In conclusion, the documents and materials at the NTP Archives support the data and results presented in the NTP Technical Report.

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PRINTED AS OF OCTOBER 1988

TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-p-dioxin (Dermal)
206	Dibromochloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane (Inhalation)
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butylbenzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-ethylhexyl)phthalate
219	2,6-Dichloro-*p*-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
229	Guar Gum
230	Agar
231	Stannous Chloride
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	D-Mannitol
238	Ziram
239	Bis(2-chloro-1-methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
247	L-Ascorbic Acid
248	4,4'-Methylenedianiline Dihydrochloride
249	Amosite Asbestos
250	Benzyl Acetate
251	Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
266	Monuron
267	Propylene Oxide
269	Telone II®
271	HC Blue No. 1
272	Propylene
273	Trichloroethylene (Four strains of rats)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
281	H.C. Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethy)phosphonium Sulfate and Tetrakis(hydroxymethy)phosphonium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	o-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane
307	Ephedrine Sulfate
308	Chlorinated Paraffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
320	Rotenone
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
328	Methyl Carbamate
329	1,2-Epoxybutane
330	4-Hexylresorcinol
331	Malonaldehyde, Sodium Salt
332	Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
336	Penicillin VK
337	Nitrofurazone
339	2-Amino-4-nitrophenol

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