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NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 366

TOXICOLOGY AND CARCINOGENESIS STUDIES OF HYDROQUINONE

(CAS NO. 123-31-9)

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 HYDROQUINONE

(CAS NO. 123-31-9)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

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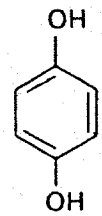

HYDROQUINONE

CAS No. 123-31-9

$C_6H_6O_2$ Molecular weight 110.1

Synonyms: 1,4-benzenediol; *p*-benzenediol; benzohydroquinone; benzoquinol; 1,4-dihydroxybenzene; *p*-dihydroxybenzene; *p*-dioxobenzene; *p*-dioxybenzene; hydroquinol; hydroquinole; α-hydroquinone; *p*-hydroquinone; *p*-hydroxyphenol; quinol; β-quinol

ABSTRACT

Hydroquinone is used as an antioxidant in the rubber industry and as a developing agent in photography. It is also an intermediate in the manufacture of rubber and food antioxidants and monomer inhibitors. Hydroquinone and products containing hydroquinone are used as depigmenting agents to lighten skin. Toxicology and carcinogenesis studies were conducted by administering hydroquinone (greater than 99% pure) in corn oil or water by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Additionally, genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, Chinese hamster ovary (CHO) cells, and *Drosophila melanogaster*.

Preliminary 3-day dermal studies were conducted with rats and mice using sufficient hydroquinone in 95% ethanol to crystallize on the skin (4 or 40 mg per animal); conjugated metabolites of hydroquinone were detected in the urine. Fourteen-day dermal studies were conducted at doses up to 3,840 mg/kg for rats and 4,800 mg/kg for mice. No toxic effects were seen in the 3- or 14-day dermal studies. Therefore, in further evaluations of hydroquinone, the gavage route of administration was used.

Results of Fourteen-Day and Thirteen-Week Studies: Fourteen-day gavage studies were conducted by administering hydroquinone in corn oil to rats at doses ranging from 63 to 1,000 mg/kg body weight and to mice at doses ranging from 31 to 500 mg/kg. All rats receiving 1,000 mg/kg and 1/5 male and 4/5 female rats receiving 500 mg/kg died before the end of the 14 days. Compound-related clinical signs in rats included tremors lasting up to 30 minutes after each dosing at 500 and 1,000 mg/kg. In the 14-day gavage studies with mice, 4/5 male mice and 5/5 female mice receiving 500 mg/kg and 3/5 males receiving 250 mg/kg died before the end of the studies. Tremors followed by convulsions were seen at 250 and 500 mg/kg.

In the 13-week studies, doses for rats and mice ranged from 25 to 400 mg/kg. All rats receiving 400 mg/kg and 3/10 female rats receiving 200 mg/kg died before the end of the studies. The mean body weight at necropsy of male rats administered 100 or 200 mg/kg was about 8%-9% lower than that of vehicle controls. Mean body weights of vehicle control and dosed female rats at necropsy were similar. Tremors and convulsions were observed after dosing in most rats receiving 400 mg/kg and in several female rats receiving 200 mg/kg. Inflammation and/or epithelial hyperplasia (acanthosis) of the forestomach were seen in 4/10 male rats and 1/10 female rats receiving 200 mg/kg. Toxic nephropathy, characterized by tubular cell degeneration in the renal cortex, was seen in 7/10 male and 6/10 female rats receiving 200 mg/kg and in 1/10 females receiving 100 mg/kg.

In the 13-week studies in mice, 8/10 males and 8/10 females receiving 400 mg/kg and 2/10 male mice receiving 200 mg/kg died early. Mean body weights of dosed and vehicle control mice at necropsy were similar. Liver weight to body weight ratios for dosed male mice were significantly greater than for vehicle controls. Ulceration, inflammation, or epithelial hyperplasia of the forestomach was found in 3/10 male and 2/10 female mice receiving 400 mg/kg and 1/10 females receiving 200 mg/kg.

Based on these collective results, 2-year studies were conducted by administering 0, 25, or 50 mg/kg hydroquinone in deionized water by gavage to groups of 65 rats of each sex, 5 days per week. Groups of 65 mice of each sex were administered 0, 50, or 100 mg/kg on the same schedule. Ten rats and 10 mice from each group were killed after 15 months for an interim evaluation.

Observations at Fifteen Months: In the rats killed at 15 months, the relative kidney weight for high dose male rats was greater than that for vehicle controls. The hematocrit value, hemoglobin concentration, and erythrocyte count for high dose female rats were decreased. Compound-related increased severity of nephropathy was observed in male rats. In mice killed at 15 months, the relative liver weights for high dose male and female mice were significantly greater than those for vehicle controls. Lesions seen in the liver of male mice included increased syncytial cells and diffuse cytomegaly.

Body Weights, Organ Weights, and Survival in the Two-Year Studies: Mean body weights of high dose male rats were 5%-13% lower than those of vehicle controls after week 73, and those of low dose male rats were 5%-9% lower than those of vehicle controls after week 89. Mean body weights of dosed female rats were similar to those of vehicle controls throughout the study. The relative kidney and liver weights for high dose male rats were higher than those for vehicle controls. Mean body weights of high dose male mice were 5%-8% lower than those of vehicle controls after week 93, and those of high dose female mice were 5%-14% lower after week 20. Relative liver weights were increased for dosed male and high dose female mice. No significant differences in survival were observed between any groups of rats or mice of either sex after 2 years (male rats: vehicle control, 27/55; low dose, 18/55; high dose, 18/55; female rats: 40/55; 27/55; 32/55; male mice: 33/55; 37/54; 36/55; female mice: 37/55; 39/55; 36/55).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Nearly all male rats and most female rats in all vehicle control and dosed groups had nephropathy. The severity of this disease was judged to be greater in high dose male rats. Hyperplasia of the renal pelvic transitional epithelium and renal cortical cysts, changes observed with advanced renal disease, were increased in male rats. Renal tubular hyperplasia was seen in 2 high dose male rats, and renal tubular adenomas were seen in 4/55 low dose and 8/55 high dose male rats; none was seen in vehicle controls.

Mononuclear cell leukemia in female rats occurred with a positive trend, and the incidences in the dosed groups were greater than that in the vehicle controls (vehicle control, 9/55; low dose, 15/55; high dose, 22/55). The historical incidence of leukemia in water gavage vehicle control female F344/N rats is 25% ± 15% and in untreated controls is 19% ± 7%.

Compound-related lesions observed in the liver of high dose male mice included anisokaryosis (0/55; 2/54; 12/55), syncytial alteration (5/55; 3/54; 25/55), and basophilic foci (2/55; 5/54; 11/55). The incidences of hepatocellular adenomas were increased in dosed male mice (9/55; 21/54; 20/55), but these increases were offset by decreases in the incidences of hepatocellular carcinomas (13/55; 11/54; 7/55). The incidences of hepatocellular neoplasms, primarily adenomas, were increased in dosed female mice (3/55; 16/55; 13/55).

Follicular cell hyperplasia of the thyroid gland was increased in dosed mice (male: 5/55; 15/53; 19/54; female: 13/55; 47/55; 45/55). Follicular cell adenomas were seen in 2/55 vehicle control, 1/53 low dose, and 2/54 high dose male mice and in 3/55 vehicle control, 5/55 low dose, and 6/55 high dose female

mice; a follicular cell carcinoma was seen in a seventh high dose female mouse. The highest observed incidence of follicular cell adenomas or carcinomas (combined) in historical water gavage vehicle control female $B6C3F_1$ mice is 3/48 (6%).

Genetic Toxicology: Hydroquinone was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation. It induced trifluorothymidine (Tft) resistance in mouse L5178Y/TK lymphoma cells in the presence or absence of metabolic activation. An equivocal response was obtained in tests for induction of sex-linked recessive lethal mutations in Drosophila administered hydroquinone by feeding. Hydroquinone induced sister chromatid exchanges (SCEs) in CHO cells both with or without exogenous metabolic activation and caused chromosomal aberrations in the presence of activation.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity** of hydroquinone for male F344/N rats, as shown by marked increases in tubular cell adenomas of the kidney. There was *some evidence of carcinogenic activity* of hydroquinone for female F344/N rats, as shown by increases in mononuclear cell leukemia. There was *no evidence of carcinogenic activity* of hydroquinone for male $B6C3F_1$ mice administered 50 or 100 mg/kg in water by gavage. There was *some evidence of carcinogenic activity* of hydroquinone for female $B6C3F_1$ mice, as shown by increases in hepatocellular neoplasms, mainly adenomas.

Administration of hydroquinone was associated with thyroid follicular cell hyperplasia in both male and female mice and anisokaryosis, multinucleated hepatocytes, and basophilic foci of the liver in male mice.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 7.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on pages 10-11.

SUMMARY OF THE TWO-YEAR GAVAGE AND GENETIC TOXICOLOGY STUDIES OF HYDROQUINONE

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses			
0, 25, or 50 mg/kg hydroquinone in water, 5 d/wk	0, 25, or 50 mg/kg hydroquinone in water, 5 d/wk	0, 50, or 100 mg/kg hydroquinone in water, 5 d/wk	0, 50, or 100 mg/kg hydroquinone in water, 5 d/wk
Body weights in the 2-year study			
Dosed groups lower than vehicle controls	Dosed and vehicle control groups similar	High dose group lower than vehicle controls	High dose group lower than vehicle controls
Survival rates in the 2-year study			
27/55; 18/55; 18/55	40/55; 27/55; 32/55	33/55; 37/54; 36/55	37/55; 39/55; 36/55
Nonneoplastic effects			
		Thyroid gland follicular cell hyperplasia; hepatic proliferative lesions	Thyroid gland follicular cell hyperplasia
Neoplastic effects			
Renal tubular cell adenomas (0/55; 4/55; 8/55);	Mononuclear cell leukemia (9/55; 15/55; 22/55)	None	Hepatocellular adenomas or carcinomas (combined) (3/55; 16/55; 13/55)
Level of evidence of carcinogenic activity			
Some evidence	Some evidence	No evidence	Some evidence
Genetic toxicology			
Salmonella Gene Mutation: Negative with and without S9	Mouse L5178Y/TK Tft Resistance: Positive with and without S9	CHO Cells in Vitro SCE: Positive with and without S9; CHO Cells in Vitro Aberration: Negative without S9; positive with S9	Drosophila Sex-Linked Rec. Lethals: Equivocal

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results ("Clear Evidence" and "Some Evidence"); one category for uncertain findings ("Equivocal Evidence"); one category for no observable effects ("No Evidence"); and one category for experiments that 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 to neoplastic lesions;
- Some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- Combining benign and malignant tumor 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.

CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of Hydroquinone is based on 13-week studies that began in June 1981 and ended in September 1981 and on 2-year studies that began in August 1982 and ended in September 1984 at Bioassay Systems Corporation (Woburn, MA).

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PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on hydroquinone on October 3, 1988, 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 HYDROQUINONE

On October 3, 1988, the draft Technical Report on the toxicology and carcinogenesis studies of hydroquinone received public 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, NC.

Dr. F.W. Kari, NIEHS, began the discussion by reviewing the experimental design, results, and proposed conclusions (clear evidence of carcinogenic activity for male rats, some evidence of carcinogenic activity for female rats, no evidence of carcinogenic activity for male mice, some evidence of carcinogenic activity for female mice).

Dr. Popp, a principal reviewer, agreed with the conclusions, although he thought that the conclusion for male rats was a borderline call between clear evidence of carcinogenic activity and some evidence of carcinogenic activity. He said that a better rationale was needed as to why the oral route of administration rather than the dermal route was chosen. Dr. Kari replied that practical limitations of how much chemical could be applied dermally and the lack of toxicity in the short-term studies justified the use of gavage for optimizing the potential for observing systemic toxicity and carcinogenicity. Dr. Popp stated that the relationship between nephropathy and renal carcinogenicity in male rats needed to be clarified in the Discussion. He said that the likelihood of finding hyaline droplets was dependent on the interval between the time the animals were killed and the examination for droplets. Dr. Kari said that 72 hours elapsed between cessation of exposure and necropsy in the short-term studies; however, no other indices of hyaline droplet formation, such as granular cast formation in the loop of Henle or mineralization in the renal papilla, were seen. Dr. J. Huff, NIEHS, pointed out that in the NTP studies on *d*-limonene, the levels of hyaline droplets in the kidney of exposed male rats were still clearly increased after 72 hours.

Dr. Gallo, the second principal reviewer, agreed with the conclusions for female rats and for male and female mice but disagreed with the conclusion for male rats, suggesting that it be changed to some evidence of carcinogenic activity. He based this opinion on the presence of nephropathy in nearly all male and most female rats in all dosed and vehicle control groups, on the possibility of products of reduction/oxidation cycling in the kidney as a function of pH and high renal concentrations of hydroquinone, and on the activity of cysteine lyase in the kidney and the role of thiol adducts in acute nephrotic syndrome as a precursor to hyperplasia. Dr. Gallo questioned the use of the oral route of exposure in view of the fact that the major route of human exposure appears to be dermal. He said that a complete absorption, distribution, metabolism, and excretion profile should have been developed before 2-year studies were begun. He asked that the Report be deferred until chemical disposition data could be incorporated. Dr. Kari agreed that such data would be meaningful for interpretation but said that the lack of these data does not detract from the validity of the information obtained when the oral route was used. Further, he said that there was no indication that the route of exposure would influence the overall outcome.

Dr. Mirer, the third principal reviewer, agreed with the conclusions for male rats and male mice. He argued for changing the conclusion for female mice to clear evidence of carcinogenic activity, based on highly significant dose-related increased incidences of hepatocellular neoplasms in both low and high dose groups. Dr. Kari mentioned that there was no clear dose-response relationship, the numbers were not overwhelming, and there was no supporting evidence in the other sex or the other species. Dr. Ashby commented that the high and quite variable historical vehicle control incidence of mononuclear cell leukemia was not supportive of a higher level of evidence in female rats. Dr. Mirer

noted that dermal absorption had been observed in preliminary animal studies, a finding of importance for drawing public health conclusions.

Ms. Susan Murphy, Goodyear Tire and Rubber Company, and Chairperson, Toxicology Research Task Group of the Hydroquinone Program Panel, Chemical Manufacturers Association, asked the Peer Review Panel to consider inclusion of more discussion on the role of nephrotoxicity in tumor formation in the kidney, while noting the high incidence of spontaneous nephrotoxicity in all rat groups, and to consider changing the conclusion for female rats to equivocal evidence of carcinogenic activity, based on the high and variable historical vehicle control incidences for mononuclear cell leukemia. Dr. Caroline English, Eastman Kodak Company, expressed concern that changes in feed consumption, water consumption, body weight, and the virologic status of study animals may have contributed to the observed nephrotoxic responses and consequently were associated with the production of renal tumors in male rats. She asked that results of recent hydroquinone metabolism studies be considered before the Report is finalized, because metabolism in F344 rats produces a cysteine conjugate that may be a nephrotoxin. Dr. Huff pointed out that these data have not been published and that the NTP ordinarily does not cite unpublished studies.

Dr. Ashby thought that the discussion about the role of hydroquinone in the carcinogenicity of benzene was overstated, pointing out the differences in the physicochemical characteristics of the two chemicals. Dr. Mirer thought that the metabolic connection between hydroquinone and benzene lent support for changing the conclusion for female rats to clear evidence of carcinogenic activity, since benzene is a potent leukemogen. Dr. J. Haseman, NIEHS, commented that for tumors with quite variable incidences, such as mononuclear cell leukemia, the concurrent control incidence is most appropriate.

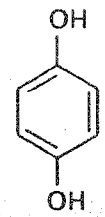
There was considerable discussion among Panel members and staff regarding the degree of correlation between toxicity (nephropathy) and carcinogenicity (renal tubular adenomas) in male rats. Dr. Popp noted that the definition of clear evidence of carcinogenic activity called for dose-related increased incidences in malignant neoplasms or a combination of malignant and benign neoplasms or in a marked increase in benign neoplasms. He questioned whether eight adenomas in the top dose group constituted a marked increase.

Dr. Gallo moved that the conclusion for male rats be changed to some evidence of carcinogenic activity. Dr. Popp seconded the motion, which was approved by five affirmative votes (Drs. Gallo, Garman, Klaassen, Newberne, and Popp) to four negative votes (Drs. Ashby, Gold, McKnight, and Mirer). Dr. Gallo moved that the conclusion be accepted as written for female rats, some evidence of carcinogenic activity. Dr. Popp seconded the motion, which was approved by seven affirmative votes to two negative votes (Drs. McKnight and Mirer). Dr. Gallo moved that the conclusion be accepted as written for male mice, no evidence of carcinogenic activity. Dr. Popp seconded the motion, which was approved unanimously by the Panel. Dr. Gallo moved that the conclusion be accepted as written for female mice, some evidence of carcinogenic activity. Dr. Garman seconded the motion, which was approved by eight affirmative votes to one negative vote (Dr. Mirer).

I. INTRODUCTION

Properties
Synthesis, Production, and Use
Toxicity in Animals
Interference with Melanogenesis
Evaluation of Reproductive Function and Teratogenicity
Absorption, Metabolism, and Excretion
Genetic Toxicology
Long-Term Toxicity and Carcinogenicity
Study Rationale

I. INTRODUCTION



HYDROQUINONE

CAS No. 123-31-9

$C_6H_6O_2$ Molecular weight 110.1

Synonyms: 1,4-benzenediol; *p*-benzenediol; benzohydroquinone; benzoquinol; 1,4-dihydroxybenzene; *p*-dihydroxybenzene; *p*-dioxobenzene; *p*-dioxybenzene; hydroquinol; hydroquinole; α-hydroquinone; *p*-hydroquinone; *p*-hydroxyphenol; quinol; β-quinol

Properties

At room temperature, hydroquinone exists as white crystals that melt at 173° C. It has a boiling point of 285° C at 730 mm mercury and a vapor pressure of 4 mm mercury at 150° C. Hydroquinone is slightly soluble in benzene, soluble in ether and in water (9.4 g/100 ml at 28.5° C), and very soluble in ethanol, acetone, and carbon tetrachloride (CRC, 1976).

Synthesis, Production, and Use

Hydroquinone is manufactured in the United States primarily by the aniline-oxidation process (Varagnat, 1981). The process involves the oxidation of aniline with manganese dioxide to quinone followed by iron-catalyzed reduction to hydroquinone. Quinone formed in the first step of the process is removed from the oxidation solution by steam stripping. The quinone-steam mixture can be reduced with an aqueous suspension of iron or by catalytic hydrogenation. Technical-grade hydroquinone is prepared from the reaction solution by crystallization, centrifugation, and drying. Alternatively, hydroquinone is manufactured by the hydroperoxidation of diisopropylbenzene; the *para* isomer is isolated, oxidized to the dihydroperoxide, and treated with sulfuric acid to produce hydroquinone and acetone. In 1984, annual U.S. production capacity was estimated to be 34 million pounds (SRI, 1984).

Hydroquinone is used as a developer in black-and-white photography and as an antioxidant by the rubber industry (Varagnat, 1981). Hydroquinone serves as an intermediate in the manufacture of rubber antioxidants and food antioxidants (BHA [butylated hydroxyanisole] and *t*-butyl hydroquinone) and as a polymerization inhibitor of unsaturated monomers. Hydroquinone and products containing hydroquinone are used as depigmenting agents to lighten small areas of hyperpigmented skin in the treatment of melasma, freckles, senile lentigines, and post-inflammatory hyperpigmentation (Fed. Regist., 1978; Findlay and De Beer, 1980; Engasser and Maibach, 1981).

Hydroquinone has been identified and quantitated in mainstream smoke of nonfiltered cigarettes in amounts ranging from 88 to 155 µg per cigarette (Wynder and Hoffmann, 1967; Ishiguro et al., 1976).

The National Institute for Occupational Safety and Health recommends that exposure to hydroquinone be limited to a ceiling concentration of 2 mg/m^3 (0.44 ppm) during a 15-minute sampling period (NIOSH, 1978). The American Conference of Governmental Industrial Hygienists currently recommends a threshold limit value/time-weighted average of 2 mg/m^3 (ACGIH, 1987).

Toxicity in Animals

Short-term oral toxicity of hydroquinone has been studied in rats, mice, guinea pigs, rabbits, dogs, cats, and swine (Lehman et al., 1951; Patty's, 1981; Stuart et al., 1981). The LD_{50} values in these species ranged from 0.2 to 0.5 g/kg, except for cats, which had greater sensitivity, with LD_{50} values of 0.07 g/kg body weight. Hyperexcitability, tremors, convulsions, salivation, and emesis were observed within 90 minutes of administration of lethal doses, and death occurred after several hours.

When fed to rats at 5% of the diet (50,000 ppm) for 9 weeks, hydroquinone caused severe body weight loss, aplastic anemia, bone marrow depletion, liver atrophy, and ulceration and hemorrhage of the gastric mucosa (Carlson and Brewer, 1953). Hydroquinone-induced immunotoxicity is well documented, and the toxicity of hydroquinone to bone marrow and lymphoid organs correlates well with its accumulation in these tissues (Greenlee et al., 1981).

The skin-sensitization potential of hydroquinone for guinea pigs has been investigated (Draize et al., 1944; Draize, 1951; Goodwin et al., 1981). Generally, little potential for skin sensitization was observed. For example, a 2% solution of hydroquinone in dimethyl phthalate was given by intradermal injection to guinea pigs three times per week for 10 weeks. Two weeks later, a challenge injection was made. Evaluations made 24 hours later revealed no dermal sensitization (Draize et al., 1944; Draize, 1951). However, guinea pigs sensitized to *p*-methoxyphenol cross-reacted to a challenge with hydroquinone (Van der Walle et al., 1982).

Interference with Melanogenesis

The utility of hydroquinone as a skin-bleaching chemical stems from its ability to inhibit the production and accumulation of melanin when applied topically to skin. The biochemical basis for hydroquinone-induced disruption of melanogenesis is not completely understood, but several hypotheses are being investigated. Hydroquinone has been shown to inhibit tyrosine-mediated conversion of tyrosine to dopa and dopa to dopaquinone, thereby decreasing the concentration of melanin precursors (Usami et al., 1980). However, in other investigations, both activation and depression of tyrosinase activity have been demonstrated in melanoma explants, depending on the source of the cells and the concentrations of hydroquinone (Abramowitz and Chavin, 1980). Other investigators showed that hydroquinone causes inhibition of both DNA and RNA synthesis and found greatly different sensitivities between melanocytic and nonmelanocytic cell lines, suggesting that the depigmenting effect of hydroquinone may be exerted by selective toxicity to melanocytic cells (Penney et al., 1984) rather than by direct effects on melanin biosynthetic pathways. Since hydroquinone is a substrate for tyrosinase, it is conceivable that cells containing tyrosinase are more capable of producing toxic metabolites of hydroquinone.

Evaluation of Reproductive Function and Teratogenicity

No chemical-related effects on reproduction, as shown by gestation length, mean litter size, fetal viability, and lactation index, were seen in two groups of 10 female rats fed diets containing 30 or 3,000 ppm hydroquinone (Ames et al., 1956).

Groups of 10 nulliparous female rats (Walter Reed-Carworth Farms) were mated and then given a total of 0.5 g of hydroquinone in feed during pregnancy (Telford et al., 1962). Hydroquinone was not toxic for the dams. The rats were killed 22 days after mating, and uteri were examined. One or more resorptions were observed in 100% of the dosed rats, and 27% of all implantations terminated in resorption. Corresponding control values in untreated pregnant rats were 41% of the dams with resorptions and 11% of the total implantations resorbed.

Absorption, Metabolism, and Excretion

Early investigations of the metabolism and disposition of hydroquinone in humans and experimental animals showed that the chemical is readily absorbed from the gastrointestinal tract and is eliminated primarily as sulfate and glucuronide conjugates in the urine. Male volunteers ingesting up to 0.5 g of hydroquinone per day excreted 8%-15% of the dose unchanged and about

40% as urinary conjugates (Fassett and Roudabush, 1952). In rabbits, less than 1% of the dose was excreted unchanged, and about 80% of the dose was recovered as conjugates in the urine (Garton and Williams, 1949; Bray et al., 1952). Hydroquinone has also been determined to be absorbed after application to mouse skin in vitro and in vivo and to human skin in vitro (Marty et al., 1981).

Mass balance studies in which a single dose of radiolabeled hydroquinone (200 mg/kg) was given orally to rats revealed that, within 48 hours, approximately 90% of the label was excreted in the urine, approximately 4% was excreted in the feces, 1.2% remained in the carcass, and 0.4% was trapped in expired air (Divincenzo et al., 1984). The major radiolabeled species in the urine were identified as hydroquinone monoglucuronide (50%-60%), hydroquinone monosulfate (25%-42%), and unchanged parent compound (1%-8.6%). The excretion pattern was similar for rats dosed once or once per day for 5 days. In these studies, repeated dosing did not alter absolute or relative liver weights, hepatic microsomal protein content, or cytochrome b5 or cytochrome c reductase activity; hepatic P450 content decreased slightly.

A complete perspective of hydroquinone biotransformation must include consideration of hydroquinone as a metabolite of benzene and phenol. The schematic in Figure 1 depicts the stepwise conversion of benzene to benzene oxide via epoxidation (Daly et al., 1972), the spontaneous or protein-catalyzed rearrangement of benzene oxide to phenol (Jerina et al., 1968; Tunek et al., 1978), and the subsequent conversion of phenol to hydroquinone, catechol, and other hydroxylated benzenes.

Observations that benzene must be metabolized to exert its characteristic toxicity in bone marrow, combined with evidence that hydroquinone and catechol (or metabolites of these compounds) are taken up in bone marrow and lymphoid organs against a concentration gradient (Greenlee et al., 1981), implicate hydroquinone and other hydroxylated benzenes as possible contributors to the hemotoxicity of benzene (Parke and Williams, 1953b; Tunek et al., 1978; Sawahata and Neal, 1983). Consistent with this idea are the observations that manipulation of benzene metabolism alters benzene-induced hematotoxicity. Andrews et al. (1977) showed that toluene is a competitive inhibitor of benzene metabolism and protects dosed animals against benzene-induced bone marrow depression. Furthermore, differing genetic susceptibility to benzene-induced bone marrow toxicity in mice is paralleled by altered metabolism of benzene. Multiple-dose studies revealed that the more resistant C57BL/6 mice had lower levels of water-soluble benzene metabolites in bone marrow, liver, kidney, blood, spleen, and lung and of covalently bound metabolites in bone marrow, blood, spleen, and muscle than did DBA/2 mice (Longacre et al., 1981).

Evidence obtained in vivo and from various in vitro preparations demonstrate that hydroquinone and other metabolites of benzene are capable of binding covalently to DNA and other macromolecules. For example, radiolabeled benzene administered in vivo yielded covalently bound metabolites in mouse bone marrow (Gill and Ahmed, 1981) and to rat liver DNA (Lutz and Schlatter, 1977). Additionally, mitochondrial preparations from rabbit bone marrow metabolize benzene to products that bind to mitochondrial DNA (Rushmore et al., 1984). Subsequent analysis following digestion of this mitochondrial DNA to nucleosides revealed the presence of at least six different adducts to guanosine. Cytochrome P450-dependent (Sawahata and Neal, 1983) and cytochrome P450-independent (Wallin et al., 1985) metabolism of hydroquinone to reactive metabolites has also been shown. In vitro incubation of hydroquinone with polyguanosine yielded several adducts (Jowa et al., 1986).

Although many studies implicate hydroquinone as a toxic metabolite of benzene, other evidence suggests that metabolites other than hydroquinone contribute to the toxicity and genotoxicity of benzene. Pellack-Walker and Blumer (1986) used a mouse lymphoma cell line to evaluate the DNA-damaging ability of benzene and a variety of hydroxylated metabolites over a 1,000-fold concentration range. Benzene, phenol, or catechol at concentrations as high as 1.0 mM or

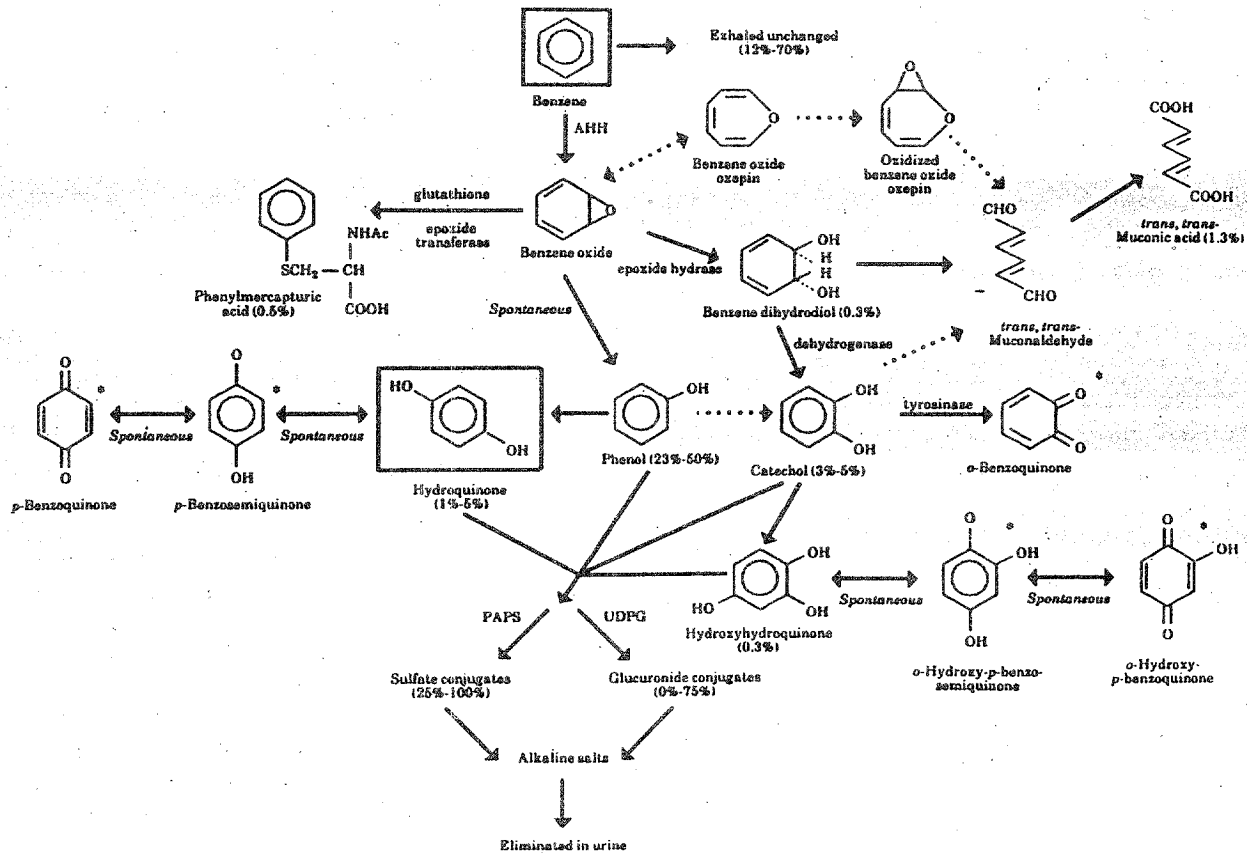


FIGURE 1. RELATIONSHIP BETWEEN BENZENE METABOLISM AND HYDROQUINONE METABOLISM

Adapted from Parke and Williams, 1953a; Laskin and Goldstein, 1977; Goldstein et al., 1982; Irons and Pfeifer, 1982; Pfeifer and Irons, 1983; Erexson et al., 1985; Sawahata et al., 1985. Values in parentheses are percentages of metabolic products detected in urine of animals (rabbits, rats, mice, dogs) or humans. Asterisks (*) denote putative or demonstrated alkylating activity toward intracellular nucleophiles. AHH = aryl hydrocarbon hydroxylase; UDPG = uridine diphosphate glucuronyl transferase; PAPS = 3′-phospho-adenosine-5′-phosphosulfate. Dashed lines indicate putative pathways. *trans, trans*-Muconaldehyde is a postulated intermediate (Gad-El-Karim et al., 1985; Latriano et al., 1986).

hydroquinone at concentrations up to 0.1 mM did not increase the percentage of single-stranded DNA as evidenced by alkaline elution. In contrast, micromolar concentrations of *p*-benzoquinone produced strand breaks, suggesting that quinones and semiquinones may be responsible in part for the damage associated with benzene. This is conceivable, since benzoquinones serve as substrates for DT-diaphorase and lipoamide dehydrogenase (Smart and Zannoni, 1984) and can therefore undergo one- or two-electron reductions to their respective semiquinone radicals. Autoxidation of these semiquinones back to quinones would be expected to form superoxide anion radicals, which may be clastogenic. Although it is generally agreed that the cytotoxicity, genotoxicity, and covalent binding of benzene are dependent on its further metabolism, the complexity of its metabolism currently precludes a clear understanding of the requisite pathways (and hence the role of hydroquinone) in causing these toxic effects.

Genetic Toxicology

The genetic toxicity of hydroquinone has been extensively investigated in a variety of assays. Results of these are presented in Table 1.

Hydroquinone was generally negative in mutagenicity tests conducted in several strains of Salmonella with or without exogenous metabolic activation (Florin et al., 1980; Rapson et al., 1980; Haworth et al., 1983; Sakai et al., 1985; see Table 34). However, Gocke et al. (1981) reported mutagenic activity in the absence of S9 in *Salmonella typhimurium* TA1535A, a strain that the authors suggested might harbor an undefined genetic alteration from strain TA1535 because of differences in length of storage. In addition, the mutagenic response was observed with the ZLM medium only, not with the standard Vogel Bonner minimal medium. Hydroquinone did not induce sex-linked recessive lethal mutations when fed to adult male *Drosophila melanogaster* at concentrations of 50 or 100 mM (0.5-1 mg/ml) (Gocke et al., 1981), nor did it induce gene mutations in somatic cells of mice as measured in the mouse spot test (Gocke et al., 1983).

Hydroquinone has been shown to induce sister chromatid exchanges (SCEs) in both the presence and absence of S9 in Chinese hamster ovary (CHO) cells (Galloway et al., 1987; see Table 36), Chinese hamster Don cells (Shimada et al., 1988), and human lymphocytes (Morimoto et al., 1983). Hydroquinone, with or without S9, induced a significant increase in trifluorothymidine-resistant mouse L5178Y/TK lymphoma cells (McGregor et al., 1988; see Table 35). It has also been shown to cause inhibition of DNA synthesis in mouse lymphoma cells and HeLa cells in the presence and absence of S9 (Painter and Howard, 1982; Pellack-Walker et al., 1985). Although Pellack-Walker and Blumer (1986) did not demonstrate DNA strand breakage in mouse lymphoma cells in vitro, Shimada et al. (1988) reported increased DNA strand breaks in DDY mouse bone marrow cells after exposure to hydroquinone.

There is extensive evidence for the clastogenicity of hydroquinone in a variety of cells. Induction of mitotic segregation in *Aspergillus nidulans* diploid strain 19 by 1-3 mM hydroquinone (up to 333 µg/ml) without S9 was reported by Crebelli et al. (1987). Characterization of the damage by complementarity studies with haploid strain 35 indicated that this effect resulted from induction of structural chromosomal aberrations rather than from aneuploidy. Induction of chromosomal fragmentation and chromatid bridge formation in the nuclei of antheridial cells of the plant *Chara zeylanica* was reported after treatment with 1-50 mM hydroquinone (up to 5.5 mg/ml) for 3 or 24 hours (Chatterjee and Sharma, 1972).

There are numerous examples of hydroquinone-induced clastogenicity in mammalian cells both in vitro and in vivo. Galloway et al. (1987) demonstrated the induction of chromosomal aberrations in CHO cells by hydroquinone in the presence of S9 (see Table 37), and induction of micronuclei in bone marrow cells in vivo has been demonstrated in NMRI mice (Gocke et al., 1981; Tunek et al., 1982), CD®-1 mice (Gad-El-Karim et al., 1986), and DDY mice (Shimada et al., 1988).

TABLE 1. SUMMARY OF RESULTS OF GENETIC TOXICOLOGY STUDIES OF HYDROQUINONE

Test System/References	Endpoint	Results
Bacteria		
Salmonella typhimurium		
Epler et al., 1978	Gene mutation	Negative
Florin et al., 1980		Negative
Rapson et al., 1980		Negative
Gocke et al., 1981		Positive (a)
Haworth et al., 1983 (NTP)		Negative
Sakai et al., 1985		Negative
Fungi		
Aspergillus nidulans		
Crebelli et al., 1987	Chromosomal aberrations	Positive
Higher plants		
Allium cepa		
Krogulevich and Stom, 1969	Chromosomal aberrations	Negative
	Chromosomal thickening	Positive
Chara zeylanica		
Chatterjee and Sharma, 1972	Chromosomal breaks	Positive
Callisia fragrans		
Roy, 1973	Polyploidy	Negative
Insects		
Drosophila melanogaster		
Gocke et al., 1981	Sex-linked recessive lethal mutations	Negative
Mammalian cells (in vitro)		
Mouse lymphoma cells		
Pellack-Walker and Blumer, 1986	DNA strand breaks	Negative
Pellack-Walker et al., 1985	Inhibition of DNA synthesis	Positive
McGregor et al., 1988 (NTP)	Trifluorothymidine resistance	Positive
Chinese hamster ovary cells		
Galloway et al., 1987 (NTP)	Sister chromatid exchanges	Positive
	Chromosomal aberrations	Positive
Chinese hamster Don cells		
Shimada et al., 1988	Sister chromatid exchanges	Positive
Human HeLa cells		
Painter and Howard, 1982	Inhibition of DNA synthesis	Positive
Human lymphocytes		
Morimoto et al., 1983	Sister chromatid exchanges	Positive
Knadle, 1985		Positive
Mammalian cells (in vivo)		
Mice (NMRI)		
Gocke et al., 1981	Micronuclei	Positive
Mice (CD®-1)		
Gad-El-Karim et al., 1986	Micronuclei	Positive
Mice (DDY)		
Shimada et al., 1988	Micronuclei	Positive
	DNA strand breaks	Positive
Mice (C57BL)		
Gocke et al., 1983	Gene mutation	Negative

(a) Positive result was obtained with genetically uncharacterized strain in nonstandard medium.

The mutagenic responses seen with hydroquinone parallel those observed with benzene, which is known to be metabolized to hydroquinone and which generally requires metabolic activation to produce its effects. With the exception of in vivo mammalian studies, results with benzene in genotoxicity assays are mixed. Results of bacterial gene mutation studies were negative (Florin et al., 1980; Ho et al., 1981; Zeiger and Haworth, 1985), but results of tests for DNA damage in bacteria induced by benzene were positive (McCarroll et al., 1981a,b). Benzene did not induce gene mutations in mouse lymphoma cells (Lebowitz et al., 1979; Myhr et al., 1985) or sex-linked recessive lethal mutations in *D. melanogaster* (NTP unpublished results). There was one report of gene mutation in Tradescantia plants after exposure to benzene vapors (Schairer and Sautkulis, 1982). Positive responses were observed in in vitro mammalian cell assays for cytogenetic damage including induction of SCEs (Morimoto et al., 1983; Gulati et al., 1985, 1989) and chromosomal aberrations (Koizumi et al., 1974; Morimoto, 1974).

Numerous in vivo studies indicate that benzene is clearly clastogenic and that it must be metabolized to produce its effects (NTP, 1986). In vivo assays with benzene in both mice and rats, including induction of micronuclei (Lyon, 1975; Diaz et al., 1980; Hite et al., 1980; Meyne and Legator, 1980; Siou et al., 1981; Tunek et al., 1982; NTP, 1986) and chromosomal aberrations (Dean, 1969; Lyon, 1975; Meyne and Legator, 1980; Tice et al., 1980, 1982; Anderson and Richardson, 1981; Siou et al., 1981), were uniformly positive. Benzene genotoxicity was reviewed in detail (NTP, 1986; Huff et al., 1989).

Long-Term Toxicity and Carcinogenicity

A 2-year study of the effects of hydroquinone given in the diet was conducted with weanling (24-day-old) Sprague Dawley rats (Carlson and Brewer, 1953). Groups of 10 rats of each sex were given diets containing hydroquinone at three concentrations ranging from 1,000 to 10,000 ppm (plus controls) and examined for hematologic and pathologic changes at unspecified times up to 103 weeks. Approximately 13 tissues were examined histologically. No chemical-related chronic effects or tumors were observed.

In studies designed to evaluate the carcinogenicity of hydroquinone in the urinary bladder of mice, an unspecified number of mice were implanted with a 10-mg cholesterol/20% hydroquinone pellet (2 mg hydroquinone per mouse) and were observed for 25 weeks (Boyland et al., 1964). At 25 weeks, the incidence of urinary bladder carcinomas in survivors of the dosed group (6/19) was significantly greater ($P=0.03$) than the incidence of bladder carcinomas in mice receiving only a cholesterol pellet (5/77).

Twenty-four male mice of the "*S*" strain (7-9 weeks old) received a single application of 20 mg hydroquinone (in acetone) as an initiator on the clipped back (Roe and Salaman, 1955). Three weeks later, the promoter croton oil was applied to the same area of the skin (0.3 ml of a 0.5% croton oil solution in acetone), one time per week for 18 weeks. One week after the final application of promoter, 22 survivors were killed and examined. One dosed mouse had a skin tumor. In the control group receiving only croton oil, 1 of the 20 surviving mice had three skin tumors. The authors concluded that no evidence of tumor-initiating activity due to hydroquinone was observed.

The cocarcinogenic potential of hydroquinone has been investigated by comparing mouse skin tumorigenicity in groups receiving topical applications of benzo[*a*]pyrene (BP) alone, hydroquinone alone, or BP plus hydroquinone (Van Duuren and Goldschmidt, 1976). Fifty female ICR/Ha Swiss mice were given dermal applications of 5 µg BP and 5 mg hydroquinone three times per week. Control animals received only BP or only hydroquinone. After 368 days on study, 11/50 mice receiving only BP had 16 papillomas. In the group receiving BP and hydroquinone, seven mice had a total of 11 papillomas (three mice had squamous carcinomas). No papillomas were seen in mice dosed with hydroquinone alone. It was concluded that under these conditions, hydroquinone had weak inhibitory action on the carcinogenicity of BP.

Study Rationale

The National Toxicology Program studied hydroquinone to assess its long-term toxicity and potential carcinogenicity in F344/N rats and $B6C3F_1$ mice. Hydroquinone was nominated for study by the National Cancer Institute, National Institute for Occupational Safety and Health, and Occupational Safety and Health Administration, based on high levels of production, potential for exposure, and the lack of adequate carcinogenicity data. Since dermal exposure is a major route of contact of this chemical with humans, preliminary studies were undertaken to compare the efficacy of dermal and oral routes of administration in assessing the systemic toxicity and carcinogenicity of hydroquinone. Based on the results of these studies, gavage was selected for evaluation in 13-week and 104-week studies.

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF HYDROQUINONE

PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES

PRELIMINARY QUALITATIVE DERMAL ABSORPTION STUDY

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

FIFTEEN-MONTH AND TWO-YEAR STUDIES

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

GENETIC TOXICOLOGY

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF HYDROQUINONE

Hydroquinone (Techquincol 1G) was obtained in one lot (lot no. 56978) as a colorless, crystalline solid from Callahan Chemicals (Palmyra, NJ); Eastman Kodak Company, Eastman Chemical Products, Inc. (Kingsport, TN) was the manufacturer. Purity and identity analyses were conducted at Midwest Research Institute (MRI) (Kansas City, MO). MRI reports on analyses performed in support of the hydroquinone studies are on file at the National Institute of Environmental Health Sciences.

The study chemical was identified as hydroquinone by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The infrared spectrum (Figure 2) was identical to the literature spectrum, and the nuclear magnetic resonance spectrum (Figure 3) was consistent with the literature spectrum (Sadtler Standard Spectra). The λ_{max} and λ_{min} of the ultraviolet/visible spectrum were similar to those of the literature spectrum, but the determined ε value was 90% of the literature value at 292 nm and 75% of the literature value at 224 nm; the difference in molar absorptivity was attributed to the instrumental parameters used and not considered to be significant in the absence of the use of a standard of known purity.

Purity of lot no. 56978 was determined to be greater than 99% by elemental analysis, Karl Fischer water analysis, titration with ceric (Ce^{4+}) ion to oxidize both phenol groups, potentiometric titration with tetrabutylammonium hydroxide of one phenolic hydrogen, thin-layer chromatography, and high-performance liquid chromatography. Thin-layer chromatography was performed on silica gel plates with two solvent systems: toluene:acetone (70:30) and toluene:dioxane:glacial acetic acid (70:25:4), with visualization at 254 nm and with 0.4% methanolic 2,6-dibromoquinonechlorimide spray followed by a 10% aqueous sodium carbonate spray and exposure to ammonia vapor. High-performance liquid chromatography was performed with detection at 280 nm, a μBondapak C_{18} column, and a solvent system of water:acetonitrile (95:5).

The results of elemental analysis for carbon, hydrogen, and oxygen were in agreement with the theoretical values. Oxidation of both phenolic groups by ceric ions indicated a purity of 101.3%, and potentiometric titration of one phenolic hydrogen with tetrabutylammonium hydroxide indicated a purity of 101.2%. Only the major component was detected by thin-layer chromatography and high-performance liquid chromatography.

Stability studies were conducted on the hydroquinone study material. The stability was monitored by an oxidative titration with ceric ion (Ce^{4+}) and indicated that hydroquinone is stable as the bulk chemical when stored in the dark for 2 weeks at temperatures up to 60° C. Periodic reanalysis by the study laboratory of the bulk chemical by infrared spectroscopy (replaced by ultraviolet spectroscopy at the end of 1980) and by oxidative titration indicated no degradation of the study material throughout the studies. The bulk chemical was stored at room temperature under an inert atmosphere of nitrogen or argon throughout the studies.

PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES

The dose mixtures were prepared by mixing appropriate amounts of hydroquinone and vehicle to give the desired concentrations (Table 2). For the 14-day studies, dose mixtures were prepared in 95% ethanol for dermal application and in corn oil for gavage administration. The highest dose mixture of hydroquinone in ethanol (480 mg/ml) and all dose mixtures in corn oil formed suspensions, rather than true solutions, and were homogenized in a blender to reduce particle size. The 480 mg/ml ethanol mixture and the corn oil resuspensions used during the 13-week studies were stirred continuously during dosing.

Stability of hydroquinone in corn oil and 95% ethanol was determined by performing flame ionization detection gas chromatography with a 3% SP2100 column with *n*-heptanol as an internal standard after extracting the corn oil samples or diluting the ethanol solutions with acetonitrile and preparing a hydroquinone derivative

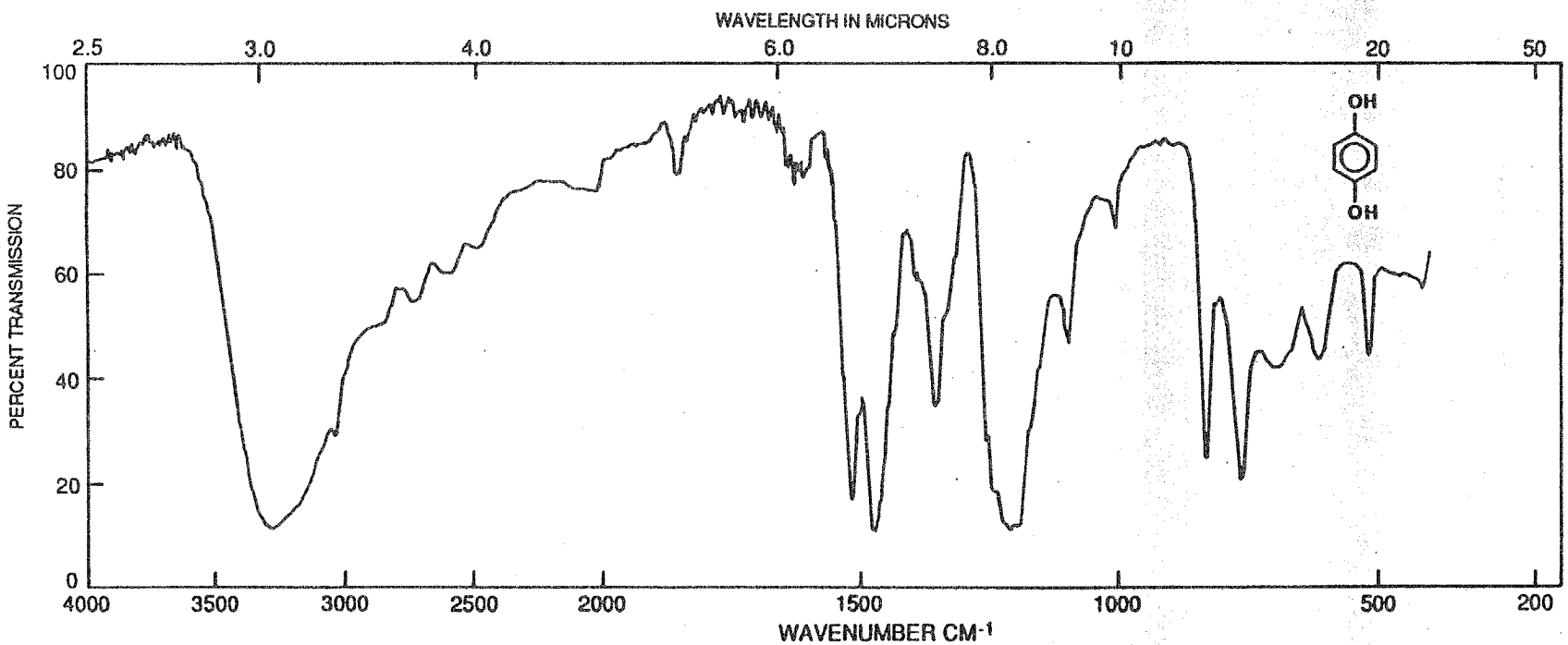


FIGURE 2. INFRARED ABSORPTION SPECTRUM OF HYDROQUINONE (LOT NO. 56978)

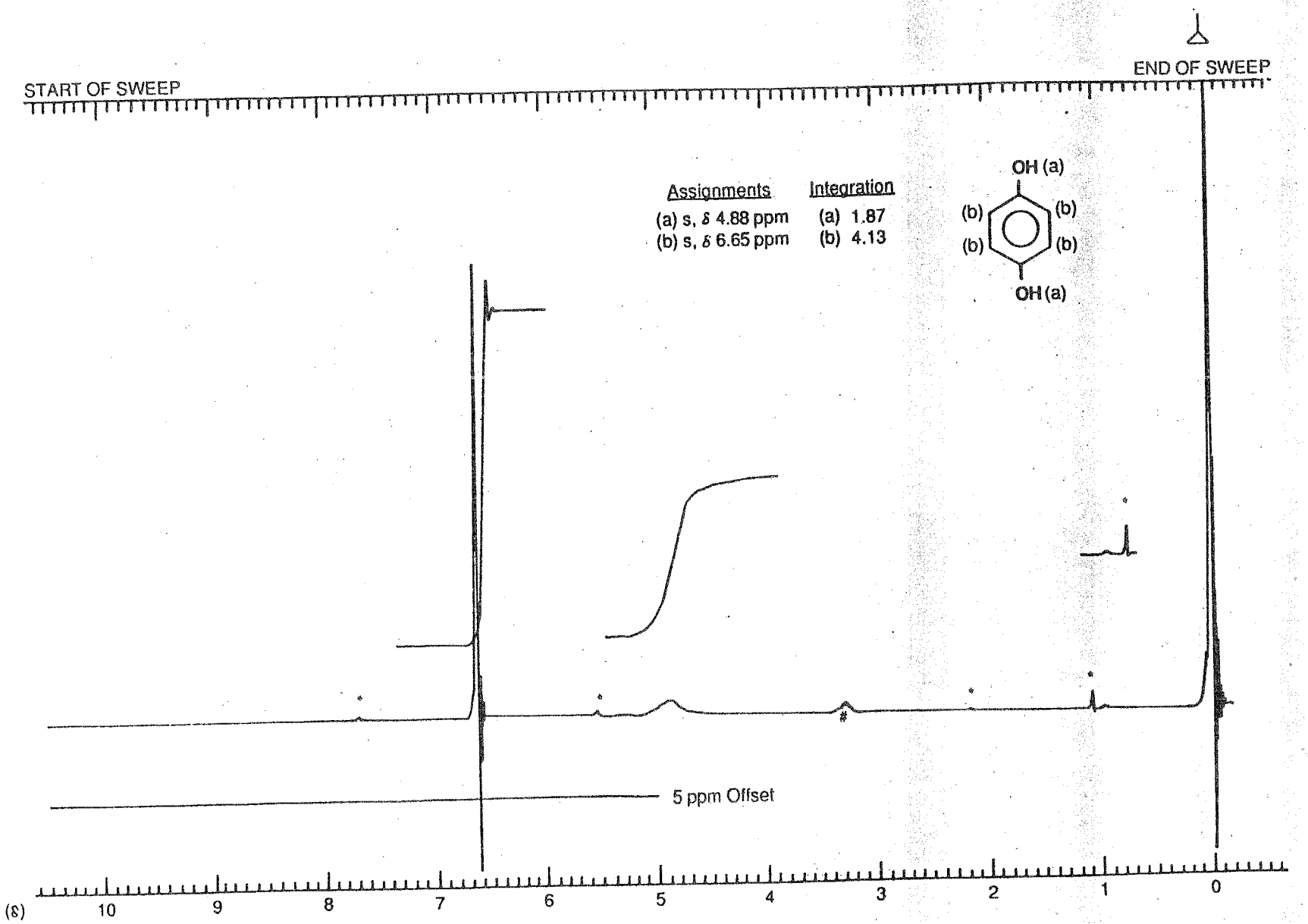


FIGURE 3. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF HYDROQUINONE (LOT NO. 56978)

TABLE 2. PREPARATION AND STORAGE OF DOSE MIXTURES IN THE STUDIES OF HYDROQUINONE

Fourteen-Day Dermal Studies	Fourteen-Day Gavage Studies	Thirteen-Week Studies	Two-Year Studies
Preparation			
Appropriate weight of hydroquinone was dissolved in appropriate volume of of 95% ethanol. Highest dose mixture was homogenized in a Waring Blender.	Appropriate weight of hydroquinone was mixed with appropriate volume of corn oil. Suspensions were homogenized in a Waring Blender.	Appropriate weight of hydroquinone was mixed with appropriate volume of corn oil up to 2 min with a Polytron® homogenizer. Air was removed under vacuum. Solutions were sealed under argon.	Appropriate weight of hydroquinone was placed in a volumetric flask and dissolved in deionized water by stirring with a magnetic stir bar. Solutions were diluted to volume with deionized water and mixed with a magnetic stir bar.
Maximum Storage Time			
	7 d	11 d	21 d
Storage Conditions			
Room temperature in tinfoil-wrapped flasks in a closed box	Room temperature in tinfoil-wrapped flasks in a closed box	Room temperature in the dark in amber serum vials with Teflon®-lined seals	Room temperature in amber serum vials with Teflon®-lined seals; sparged with argon or nitrogen before sealing

with *N-O*-bis-(trimethylsilyl)-trifluoroacetamide and a 1% trimethylchlorosilane catalyst. Hydroquinone in a corn oil suspension at 50 mg/ml or a 33% solution in ethanol was found to be stable at room temperature both in the dark and under normal lighting conditions for 7 days. During storage, the ethanol solutions turned reddish brown, indicative of the formation of oxidation products. However, analysis of these solutions showed no decrease in hydroquinone concentrations, indicating that the concentration of the decomposition products was below the 1% detection limit of the method. The stability of hydroquinone in deionized water was also determined. The study material was mixed with water and stored for up to 21 days at room temperature or 5° C. The samples were analyzed by dilution of aliquots with an aqueous resorcinol solution (an internal standard), filtration, and high-performance liquid chromatography with a Brownlee RP-18 column and a mobile phase of water:acetonitrile (95:5). Hydroquinone at 5 mg/ml in deionized water was found to be stable at room temperature in the dark for 21 days and for 3 hours at room temperature when exposed to light and air. Initially colorless solutions stored for 14 days or longer at room temperature turned pale brown, but no notable decrease in the hydroquinone content was observed.

Once before and once during the 13-week studies, analysis of hydroquinone dose mixtures by ultraviolet spectroscopy was conducted at the study laboratory by measuring the absorbance of acetonitrile extracts at 295 nm and at the analytical chemistry laboratory by measuring the absorbance of methanol extracts at 293 nm (Table 3). During the 2-year studies, approximately every seventh preparation was analyzed; for all of these samples, the hydroquinone mixtures were formulated within ±10% of the target concentrations (Table 4). Results of periodic referee analysis performed by the analytical chemistry laboratory indicated generally good agreement with the results from the study laboratory (Table 5).

TABLE 3. RESULTS OF ANALYSIS OF DOSE MIXTURES IN THE THIRTEEN-WEEK GAVAGE STUDIES OF HYDROQUINONE

Date Mixed	Concentration of Hydroquinone in Corn Oil (mg/ml) Target	Determined (a)	Determined as a Percent of Target
06/23/81--Mouse	80.0	(b) 82.9	103.6
06/24/81--Rat	5.0	(c) 4.81	96.2
		(d) 5.02	100.4
		(e) 4.87	97.4
	10.0	(c) 10.20	102.0
		(d) 9.28	92.8
		(e) 10.72	107.2
	20.0	(c) 18.10	90.5
		(d) 22.17	110.8
		(e) 20.36	101.8
	40.0	(c) 38.63	96.6
		(d) 39.83	99.6
		(e) 38.65	96.6
	80.0	(c) 87.97	110.0
		(d) 84.11	105.1
		(e) 84.90	106.1
06/24/81--Mouse	2.5	(c) 2.64	105.6
		(d) 2.73	109.2
		(e) 2.81	112.5
	5.0	(c) 5.14	102.8
		(d) 5.45	109.0
		(e) 5.15	103.0
	10.0	(c) 9.82	98.2
		(d) 8.02	80.2
		(e) 9.37	93.7
	20.0	(c) 20.38	101.9
		(d) 18.62	93.1
		(e) 26.38	131.9
	40.0	(c) 37.47	93.7
		(d) 35.69	89.2
		(e) 35.30	88.2
08/17/81--Rat	5.0	(c) 4.98	99.6
		(d) 4.93	98.6
		(e) 5.07	101.4
	10.0	(c) 9.57	95.7
		(d) 9.60	96.0
		(e) 9.67	96.7
	20.0	(c) 20.00	100.0
		(d) 19.91	99.5
		(e) 19.56	97.8
	40.0	(c) 40.00	100.0
		(d) 39.57	98.9
		(e) 39.66	99.2
	80.0	(c) 79.47	99.3
		(d) 78.34	97.9
		(e) 77.37	96.7
08/17/81--Mouse	2.5	(c) 2.46	98.4
		(d) 2.43	97.2
		(e) 2.58	103.2
	5.0	(c) 4.58	91.6
		(d) 4.59	91.8
		(e) 4.50	90.0
	10.0	(c) 9.73	97.3
		(d) 9.80	98.0
		(e) 9.83	98.3
	20.0	(c) 20.11	100.6
		(d) 20.27	101.4
		(e) 20.03	100.2
	40.0	(c) 38.49	96.2
		(d) 39.57	98.9
		(e) 40.69	101.7

(a) Results of single analysis unless otherwise specified
(b) Sample removed from top of dose mixture while it was being stirred
(c) Sample removed from middle of dose mixture while it was being stirred
(d) Sample removed from bottom of dose mixture while it was being stirred
(e) Referee sample; results of triplicate analysis.

TABLE 4. RESULTS OF ANALYSIS OF DOSE MIXTURES IN THE TWO-YEAR GAVAGE STUDIES OF HYDROQUINONE

Date Mixed	Concentration of Hydroquinone in Deionized Water for Target Concentration (mg/ml) (a)	
	5	10
08/27/82	4.75	9.39
09/10/82	4.91	10.19
10/29/82	4.95	9.61
	(b) 5.01	(b) 10.06
12/17/82	4.92	10.02
03/25/83	5.09	10.25
06/30/83	4.72	10.01
	(b) 4.89	(b) 10.01
10/07/83	4.94	9.75
01/13/84	4.74	9.61
04/20/84	4.91	9.42
	(b) 5.14	(b) 9.61
07/27/84	4.89	9.59
Mean (mg/ml)	4.88	9.78
Standard deviation	0.115	0.312
Coefficient of variation (percent)	2.4	3.2
Range (mg/ml)	4.72-5.09	9.39-10.25
Number of samples	10	10

(a) Results of duplicate analysis
(b) Animal room samples taken after dosing; not included in the mean.

TABLE 5. RESULTS OF REFEREE ANALYSIS OF DOSE MIXTURES IN THE TWO-YEAR GAVAGE STUDIES OF HYDROQUINONE

Date Mixed	Target Concentration (mg/ml)	Determined Concentration (mg/ml)	
		Study Laboratory (a)	Referee Laboratory (b)
08/27/82	5.0	4.75	4.98
03/25/83	10.0	10.25	9.93
10/07/83	5.0	4.94	5.03
04/20/84	5.0	4.91	5.04

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

PRELIMINARY QUALITATIVE DERMAL ABSORPTION STUDY

Groups of six 5- to 6-week-old male F344/N rats and $B6C3F_1$ mice (obtained from Charles River Breeding Laboratories, Kingston, NY) were given dermal applications of 0.2 ml of 0%, 2%, or 20% solutions of hydroquinone (4 or 40 mg per animal) in 95% ethanol on the clipped interscapular region for 3 consecutive days. The animals were housed in individual metabolism cages after the start of dosing, and urine samples were collected at 2, 8, 24, 48, and 72 hours after the initial dose. Urine and urine treated with β-glucuronidase and aryl sulfatase were extracted with ether and analyzed by thin-layer chromatography with silica gel plates and a chloroform: ethyl acetate:acetic acid (60:30:10) solvent system; visualization was by ultraviolet light at 254 nm and iodine vapor. The R_f values of the spots observed with the urine samples were compared with those of known standards of hydroquinone.

FOURTEEN-DAY STUDIES

Male and female F344/N rats and $B6C3F_1$ mice were obtained from Charles River Breeding Laboratories and were held for 13 days for the dermal studies or 14 days for the gavage studies before the studies began. The rats were 6 weeks old when placed on study, and the mice were 6-8 weeks old.

Groups of five rats of each sex were administered 0, 240, 480, 960, 1,920, or 3,840 mg hydroquinone/kg in 95% ethanol by dermal application to the clipped scapular area for 12 doses over 14 days. Groups of five mice of each sex were administered 0, 300, 600, 1,200, 2,400, or 4,800 mg/kg on the same schedule. The 3,840 and 4,800 mg/kg doses were administered in two portions, with a 15- to 30-minute interval to allow the applied material to dry.

Groups of five rats of each sex were administered 0, 63, 125, 250, 500, or 1,000 mg hydroquinone/kg in corn oil by gavage 5 days per week for 12 doses over 14 days. Groups of five mice of each sex were administered 0, 31, 63, 125, 250, or 500 mg/kg on the same schedule.

Animals were housed five per cage. Water and feed were available ad libitum. The rats and mice were observed once per day and were weighed on days 0, 7, and 14. Animals were fasted overnight after the last hydroquinone dose and before the final weighing; blood was taken by cardiac puncture for determination of the hydroquinone concentration. A necropsy was performed on all animals. Details of animal maintenance are presented in Table 6.

THIRTEEN-WEEK STUDIES

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

Four- to five-week-old male and female F344/N rats and 5- to 6-week-old male and female $B6C3F_1$ mice were obtained from Charles River Breeding Laboratories, observed for 22 days (rats) or 21 days (mice), distributed to weight classes, and then assigned to dose groups according to a table of random numbers. Rats were 7-8 weeks old when placed on study, and mice were 8-9 weeks old.

Groups of 10 rats and 10 mice of each sex were administered 0, 25, 50,100, 200, or 400 mg hydroquinone/kg in corn oil by gavage, 5 days per week for 13 weeks. Animals were observed two times per day; moribund animals were killed. Individual animal weights were recorded initially and once per week thereafter.

At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Histologic examinations were performed on vehicle controls, rats and mice at 200 and 400 mg/kg, and animals dying before the end of the studies. Selected tissues were examined in rats at 100 mg/kg. Further experimental details and tissues and groups examined are given in Table 6.

FIFTEEN-MONTH AND TWO-YEAR STUDIES

Study Design

Groups of 65 rats of each sex were administered 0, 25, or 50 mg hydroquinone/kg in deionized water by gavage 5 days per week for up to 103 weeks. Groups of 64 or 65 mice of each sex were administered 0, 50, or 100 mg/kg on the same schedule.

At 15 months, 10 animals from each group were selected by a table of random numbers and anesthetized with methoxyflurane; blood was collected by cardiac puncture. A Coulter Counter (Model ZF) was used to measure erythrocyte and leukocyte counts and hematocrit values. Hemoglobin concentration was measured on a Coulter Hemoglobinometer. Differential leukocyte counts and reticulocyte counts were read from slides. Analyses of blood urea nitrogen, creatinine, total protein, albumin, alkaline phosphatase, and alanine aminotransferase were determined on an Olympus Demand System, and sorbitol dehydrogenase was analyzed on an Abbott ABA-100 Bichromatic Analyzer. At necropsy, the brain, liver, and kidney were weighed.

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE AND DERMAL STUDIES OF HYDROQUINONE

Fourteen-Day Studies	Thirteen-Week Studies	Fifteen-Month and 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	64 or 65 males and 65 females of each species
Doses: Gavage		
Rats--0, 63, 125, 250, 500, or 1,000 mg hydroquinone/kg in corn oil by gavage; mice--0, 31, 63, 125, 250, or 500 mg/kg	0, 25, 50, 100, 200, or 400 mg hydroquinone/kg in corn oil by gavage; dose vol--5 ml/kg (rats) or 10 ml/kg (mice)	Rats--0, 25, or 50 mg hydroquinone/kg in deionized water by gavage; mice--0, 50, or 100 mg/kg; dose vol--5 ml/kg (rats) or 10 ml/kg (mice)
Doses: Dermal		
Rats--0, 240, 480, 960, 1,920, or 3,840 mg hydroquinone/kg in 95% ethanol to the clipped scapular area; mice--0, 300, 600, 1,200, 2,400, or 4,800 mg/kg; dose vol--0.2 ml (rats) or 0.1 ml (mice); high dose animals received 1,920 or 2,400 mg/kg 2 × d		
Date of First Dose		
Gavage--8/2/79; dermal--8/1/79	Rats--6/26/81; mice--6/25/81	Rats--9/14/82 (male) or 9/21/82 (female); mice--8/30/82 (male) or 9/7/82 (female)
Date of Last Dose		
Gavage--8/15/79; dermal--8/14/79	9/24/81	Rats--8/31/84 (male) or 9/10/84 (female); mice--8/17/84 (male) or 8/27/84 (female)
Duration of Dosing		
12 doses over 14 d	5 d/wk for 13 wk	5 d/wk for 65 or 103 wk
Type and Frequency of Observation		
Observed 1 × d; weighed 1 × wk	Observed 2 × d; weighed initially and 1 × wk thereafter	Observed 2 × d; weighed initially, 1 × wk for 13 wk, and then 1 × mo
Necropsy, Histologic Examinations, and Supplemental Analyses		
Necropsy performed on all animals	Necropsy performed on all animals; histologic exams performed on all vehicle controls, animals receiving 200 or 400 mg/kg, and animals dying before the end of the studies; tissues examined include adrenal glands, brain, colon, esophagus, gallbladder (mice), gross lesions and tissue masses, heart, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular or mesenteric lymph nodes, pancreas, parathyroid glands, pituitary gland, prostate/testes or ovaries/uterus, salivary glands, skin, small intestine, spinal cord (rats), spleen, sternebrae and vertebrae including marrow, stomach, thymus, thyroid gland, trachea, and urinary bladder. Tissues examined in 100 mg/kg groups included liver, kidneys, and stomach of male rats and kidneys of female rats	Necropsy performed on all animals; histologic exams performed on all rats (except for preputial gland and thyroid gland for low dose male rats) and vehicle control and high dose mice; tissues examined include adrenal glands, brain, cecum, colon, duodenum, epididymis/prostate/testes or ovaries/uterus, esophagus, gallbladder (mice), gross lesions and tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular or mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pituitary gland, preputial or clitoral gland (rats only), rectum, salivary glands, skin, spleen, sternebrae and vertebrae including marrow, stomach, thymus, thyroid gland, trachea, and urinary bladder. Tissues examined for low dose mice include adrenal glands, gross lesions, liver, spleen, and thyroid gland for males and gross lesions, liver, lungs, ovaries, salivary glands, and thyroid gland for females. Hematologic and clinical chemical analyses performed at 15 mo; organ weights recorded at 15 mo and 2 y

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE AND DERMAL STUDIES OF HYDROQUINONE (Continued)

Fourteen-Day Studies	Thirteen-Week Studies	Fifteen-Month and Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE		
Strain and Species F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice
Animal Source Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Kingston, NY)	Charles River Breeding Laboratories (Kingston, NY)
Study Laboratory SRI International	Bioassay Systems Corporation	Bioassay Systems Corporation
Method of Animal Identification Ear punch	Ear punch and individual number	Ear punch and individual number
Time Held Before Study Dermal--13 d; gavage--14 d	Rats--22 d; mice--21 d	Rats--21 d (male) or 28 d (female); mice--18 d (male) or 26 d (female)
Age When Placed on Study Rats--6 wk; mice--6-8 wk	Rats--7-8 wk; mice--8-9 wk	Rats--7-8 wk (male) or 8-9 wk (female); mice--8-9 wk (male) or 9-10 wk (female)
Age When Killed Rats--8 wk; mice--8-10 wk	Rats--20-21 wk; mice--21-22 wk	Rats--111-113 wk; mice--112-114 wk
Necropsy Dates Dermal--8/15/79; gavage--8/16/79	Rats--9/25/81-9/28/81; mice--9/24/81-9/25/81	2-y--rats: 9/13/84-9/20/84; mice: 8/27/84-9/12/84; 15-mo--male rats: 12/16/83; female rats: 12/22/83; male mice: 11/30/83; female mice: 12/7/83
Method of Animal Distribution Animals distributed to weight classes and then assigned to cages by one table of random numbers and to groups by another table of random numbers	Same as 14-d studies	Assigned to cages by one table of random numbers and then to groups by another table of random numbers
Feed Purina Rodent Laboratory Chow® pellets; available ad libitum	NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum	Same as 13-wk studies
Bedding Hardwood chips (P.W.I., Inc., Loweville, NY)	Sani-Chips heat-treated hardwood chips (Old Mother Hubbard, Lowell, MA)	Same as 13-wk studies
Water Automatic watering system; available ad libitum	Automatic watering system (Hardco, Cincinnati, OH)	Same as 13-wk studies
Cages Polycarbonate	Polycarbonate (Hazleton Systems, Inc., Aberdeen, MD)	Same as 13-wk studies
Cage Filters	Nonwoven fiber filters (Snow Filtration, Cincinnati, OH)	Same as 13-wk studies
Animals per Cage 5	5	5

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE AND DERMAL STUDIES OF HYDROQUINONE (Continued)

Fourteen-Day Studies	Thirteen-Week Studies	Fifteen-Month and Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)		
Other Chemicals on Study in the Same Room		
	None	None
Animal Room Environment		
Temp--64°-80° F; hum--50%-65%; fluorescent light 12 h/d; 12-15 room air changes/h	Temp--66°-76° F; hum--44%-84%; fluorescent light 12 h/d; 10-12 room air changes/h	Temp--65°-80° F; hum--40%-79%; fluorescent light 12 h/d; 6-13 room air changes/h

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. 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 animals were quarantined at the study laboratory for 3-4 weeks. Thereafter, a complete necropsy was performed on five or six animals of each sex and species to assess their health status. Rats were placed on study at 7-9 weeks of age and mice at 8-10 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 E).

Animal Maintenance

Animals were housed five per cage. Feed and water were available ad libitum. Cages and racks were rotated during these studies. Further details of animal maintenance are given in Table 6.

Clinical Examinations and Pathology

All animals were observed two times per day. Body weights were recorded once per week for the first 13 weeks of the study 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, except for tissues that were excessively autolyzed or 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. Brain, liver, kidneys, thymus, lung, heart, and testes were weighed. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histopathologic examination of tissues of mice was performed according to an "inverse pyramid" design (McConnell, 1983a,b). Complete histopathologic examinations (Table 6) were performed on all rats and on all high dose and vehicle control mice and on low dose mice dying before the end of the study. In addition, histopathologic examinations were performed on all grossly visible lesions in all dose groups. Potential target organs for chemically related neoplastic and nonneoplastic effects were identified from the short-term studies or the literature and were determined by examination of the pathology data; these target organs/tissues in the lower dose group were examined histopathologically. If mortality in the highest dose group exceeded that in the vehicle control group by 15%, complete histopathologic examinations were performed on all animals in the second highest dose group in addition to those in the high dose group.

When the pathology evaluation was completed by the laboratory pathologist and the pathology data entered into the Toxicology Data Management System, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were 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 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

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: The majority of tumors in this study were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was a logistic regression analysis, which assumed that the diagnosed tumors were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, tumor prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The dosed and vehicle control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When

tumors are incidental, this comparison of the time-specific tumor prevalences also provides a comparison of the time-specific tumor incidences (McKnight and Crowley, 1984).

In addition to logistic regression, alternative methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal tumors, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart et al., 1979), procedures based on the overall proportion of tumor-bearing animals.

Tests of significance include pairwise comparisons of each dosed group with vehicle controls and a test for an overall dose-response trend. Continuity-corrected tests were used in the analysis of tumor incidence, and reported P values are one-sided. The procedures described above also were used to evaluate selected nonneoplastic lesions. (For further discussion of these statistical methods, see Haseman, 1984.)

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.

Analysis of Continuous Variables: The statistical analysis of organ weight, hematologic, and clinical chemical data was carried out by using the nonparametric multiple comparison procedures of Dunn (1964) or Shirley (1977) to assess the significance of pairwise comparisons between dosed and vehicle control groups. Jonckheere's test (Jonckheere, 1954) was used to evaluate the significance of dose-response trends.

GENETIC TOXICOLOGY

Salmonella Protocol: Testing was performed as reported by Ames et al. (1975) with modifications listed below and described by Haworth et al. (1983). Chemicals were sent to the laboratories as coded aliquots from Radian Corporation (Austin, TX). The study chemical was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver) for 20 minutes at 37° C before the addition of soft agar supplemented with L-histidine and D-biotin and subsequent plating on minimal glucose agar plates. Incubation was continued for an additional 48 hours. Chemicals were tested in four strains; if all results were negative, the chemical was retested in all strains.

Each test consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of the study chemical. The high dose was limited by toxicity or solubility but did not exceed 10 mg/plate. All negative assays were repeated, and all positive assays were repeated under the conditions that elicited the positive response.

A positive response was defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response was defined as an increase in revertants which was not dose related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A response was considered negative when no increase in revertant colonies was observed after chemical treatment.

Mouse Lymphoma Protocol: The experimental protocol is presented in detail by McGregor et al. (1988) and follows the basic format of Clive et al. (1979). All study chemicals were supplied as coded aliquots from Radian Corporation (Austin, TX). The highest dose of the study compound was determined by solubility or toxicity and did not exceed 5 mg/ml. Mouse lymphoma L5178Y cells were maintained at 37° C as suspension cultures in Fischer's medium supplemented with 2 mM L-glutamine, 110 µg/ml sodium pyruvate, 0.05% pluronic F68, antibiotics, and heat-inactivated horse serum; normal cycling time was about 10 hours. To reduce the number of spontaneously occurring trifluorothymidine (Tft)-resistant cells, subcultures were exposed once to

medium containing thymidine, hypoxanthine, methotrexate, and glycine for 1 day, to thymidine, hypoxanthine, and glycine for 1 day, and to normal medium for 3-5 days. For cloning, horse serum content was increased and Noble agar was added. Freshly prepared S9 metabolic activation factors were obtained from the liver of either Aroclor 1254-induced or noninduced male F344 rats.

All doses within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6×10^6 cells in 10 ml of medium. This volume included the S9 fraction in those experiments performed with metabolic activation. Incubation with the study chemical continued for 4 hours, after which time the medium plus chemical was removed and the cells were resuspended in 20 ml of fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, 3×10^6 cells were plated in medium and soft agar supplemented with Tft for selection of Tft-resistant cells ($TK^{+/+}$), and 600 cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C under 5% carbon dioxide for 10-12 days. All data were evaluated statistically for both trend and peak response. Both responses had to be significant ($P<0.05$) for a chemical to be considered capable of inducing Tft resistance; a single significant response led to an "equivocal" conclusion, and the absence of both a trend and a peak response resulted in a "negative" call.

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented in Myhr et al. (1985). This assay was initially performed without S9; if a clearly positive response was not obtained, the experiment was repeated with induced S9.

Chinese Hamster Ovary Cytogenetics Assays: Testing was performed as reported by Galloway et al. (1985, 1987) and is described briefly below. Chemicals were sent to the laboratories as coded aliquots from Radian Corporation (Austin, TX). Chemicals were tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations both in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine (BrdU)-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of the study chemical; the high dose was limited by toxicity or solubility but did not exceed 5 mg/ml.

In the SCE test without S9, CHO cells were incubated for 26 hours with the study chemical in McCoy's 5A medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and antibiotics. BrdU was added 2 hours after culture initiation. After 26 hours, the medium containing the study chemical was removed and replaced with fresh medium plus BrdU and colcemid, and incubation was continued for 2 more hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with the chemical, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing BrdU and no study chemical; incubation proceeded for an additional 26 hours, with colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9.

In the chromosomal aberration test without S9, cells were incubated in McCoy's 5A medium with the study chemical for 8 hours; colcemid was added, and incubation was continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the chromosomal aberration test with S9, cells were treated with the study chemical and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10 hours in fresh medium, with colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

For the SCE test, if significant chemical-induced cell cycle delay was seen, incubation time was lengthened to ensure a sufficient number of scorable cells. The harvest time for the chromosomal aberration test was based on the cell cycle information obtained in the SCE test; if cell

cycle delay was anticipated, the incubation period was extended approximately 5 hours.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind, and those from a single test were read by the same person. For the SCE test, 50 second-division metaphase cells were usually scored for frequency of SCEs per cell from each dose; 100 first-division metaphase cells were scored at each dose for the chromosomal aberration test. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. Chromosomal aberration data are presented as percentage of cells with aberrations. As with SCEs, both the dose-response curve and individual dose points were statistically analyzed. A statistically significant ($P < 0.003$) effect on the slope of the curve or on a dose point ($P < 0.05$) was sufficient for a conclusion of positive for a test.

Drosophila Protocol: The assays for gene mutation and chromosomal translocation induction were performed as described by Zimmering et al. (1985). Study chemicals were supplied as coded aliquots from Radian Corporation (Austin, TX). Initially, study chemicals were assayed in the sex-linked recessive lethal (SLRL) test by feeding for 3 days to adult Canton-S wild-type males that were no more than 24 hours old at the beginning of the treatment. If no response was obtained, the chemical was retested by injection into adult males. If either route of administration produced a positive result, the chemical was assayed for induction of reciprocal translocations (RTs) by using the same method of exposure. If, because of the physical nature of the chemical, feeding experiments were not possible, injection was selected as the method of study chemical administration, and a positive result was followed by an RT test.

Toxicity tests attempted to set concentrations of study chemical at a level that would produce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. For the SLRL test, exposure by feeding was done by allowing Canton-S males (10-20 flies per vial) to feed for 72 hours on a solution of the study chemical in 5% sucrose. In the injection experiments, 24- to 72-hour-old Canton-S males were given a solution of the chemical dissolved in 0.7% saline or peanut oil and allowed 24 hours to recover. Exposed males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days; sample sperm from successive matings were treated as successively earlier postmeiotic stages. F_1 heterozygous females were allowed to mate with their siblings and then were placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution.) If a cluster was identified, all data from the male in question were discarded. After 17 days, presumptive lethal mutations were identified as vials containing no wild-type males; these were retested. At least two experiments were performed for each study chemical, resulting in the testing of some 5,000 treated and 5,000 control chromosomes. The only exceptions occurred when the results of the first experiment were clearly positive (induced frequency of recessive lethal mutations equal to or greater than 1%); then, the second trial was run.

Recessive lethal data were analyzed by the normal approximation to the bionomial test (Margolin et al., 1983). A test result was considered to be positive if the P value was less than 0.01 and the mutation frequency in the tested group was greater than 0.10% or if the P value was less than 0.05 and the frequency in the treatment group was greater than 0.15%. A test

was considered to be inconclusive if (a) the P value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15% or (b) the P value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A result was considered to be negative if the P value was greater than 0.10 or if the frequency in the treatment group was less than 0.10%.

For the RT test, the exposure regimen was the same as that for the SLRL test except that small mass matings were used (10 males and 20 females). Exposed males were mated to bw;st or bw;e females for 3 days and discarded. The females were transferred to fresh medium every 3-4 days for a period of about 3 weeks to produce a total of six broods. The results of the SLRL test were used to narrow the germ-cell stage most likely to be affected by the chemical; for example, if earlier germ-cell stages seemed to exhibit increased sensitivity, mating of the males was continued and translocation tests were carried out from the offspring derived from these earlier germ cell stages. F_1 males were mated individually to bw;st females and the progeny were examined for missing classes, which indicate the occurrence of a translocation in the parental male. Suspected RTs were retested. The translocation data were analyzed according to the conditional binomial test (Kastenbaum and Bowman, 1970).

III. RESULTS

RATS

PRELIMINARY QUALITATIVE DERMAL ABSORPTION STUDY

FOURTEEN-DAY STUDIES

Dermal

Gavage

THIRTEEN-WEEK STUDIES

FIFTEEN-MONTH STUDIES

TWO-YEAR STUDIES

Body Weights, Organ Weights, and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

MICE

PRELIMINARY QUALITATIVE DERMAL ABSORPTION STUDY

FOURTEEN-DAY STUDIES

Dermal

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THIRTEEN-WEEK STUDIES

FIFTEEN-MONTH STUDIES

TWO-YEAR STUDIES

Body Weights, Organ Weights, and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

GENETIC TOXICOLOGY

PRELIMINARY QUALITATIVE DERMAL ABSORPTION STUDY

Hydroquinone was qualitatively detected in the urine of male rats by thin-layer chromatography at both doses (4 or 40 mg per animal) as soon as 2 hours and as long as 72 hours after dosing. The intensity of the spot increased after enzymatic hydrolysis by β-glucuronidase and aryl sulfatase. Crystals of hydroquinone were observed on skin following application of the high dose. Contamination of the urine by crystals of hydroquinone from the skin of dosed animals could not be ruled out, especially at the higher dose.

FOURTEEN-DAY STUDIES

Dermal

All rats survived to the end of the studies (Table 7). The final mean body weight of male rats that received 3,840 mg/kg was 6% lower than that of the vehicle controls. Crystals were seen on the skin and fur of animals at 3,840 mg/kg. Tissues were not examined histologically.

TABLE 7. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE FOURTEEN-DAY DERMAL STUDIES OF HYDROQUINONE

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	104 ± 3	159 ± 4	+55 ± 1	
240	5/5	102 ± 3	157 ± 3	+55 ± 1	99
480	5/5	104 ± 1	151 ± 3	+47 ± 2	95
960	5/5	112 ± 2	162 ± 4	+50 ± 2	102
1,920	5/5	111 ± 2	163 ± 3	+52 ± 2	103
3,840	5/5	106 ± 4	150 ± 4	+44 ± 1	94
FEMALE					
0	5/5	89 ± 2	108 ± 3	+19 ± 1	
240	5/5	94 ± 2	114 ± 2	+20 ± 1	106
480	5/5	90 ± 2	108 ± 2	+18 ± 2	100
960	5/5	89 ± 2	111 ± 2	+22 ± 1	103
1,920	5/5	86 ± 1	108 ± 2	+22 ± 2	100
3,840	5/5	91 ± 2	107 ± 3	+16 ± 2	99

(a) Number surviving/number initially in 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

Gavage

All rats receiving 1,000 mg/kg and 1/5 males and 4/5 females receiving 500 mg/kg died before the end of the studies (Table 8). The final mean body weight of rats receiving 500 mg/kg was 9% lower than that of the vehicle controls for males and 18% lower for females. Compound-related clinical signs included tremors lasting up to 30 minutes after each dose administration of 500 and 1,000 mg/kg. In males receiving 1,000 mg/kg, tremors were followed by convulsion and death. Tissues were not examined histologically.

THIRTEEN-WEEK STUDIES

All rats receiving 400 mg/kg and 3/10 female rats receiving 200 mg/kg died before the end of the studies, with most deaths occurring before week 7 (Table 9). Males receiving 200 mg/kg were noted to be lethargic after 10 weeks of dosing, and females receiving this dose exhibited tremors and sometimes convulsions. Three females receiving 200 mg/kg died during week 11, and the rest showed signs of lethargy for the duration of the 13-week study. No remarkable clinical signs were seen in the lower dose groups. Mean body weights of dosed and vehicle control female rats were similar at necropsy. The liver weight to body weight ratios for dosed male rats were lower than that for vehicle controls; liver weight to body weight ratios for the three highest dose groups of female rats were significantly greater than that for the vehicle controls (Table 10). Tremors and convulsions followed by death were common observations, and a clear orange fluid or orange staining was reported around the mouth in most cases.

TABLE 8. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE FOURTEEN-DAY GAVAGE STUDIES OF HYDROQUINONE

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	109 ± 4	172 ± 5	+63 ± 3	
63	5/5	109 ± 4	179 ± 5	+70 ± 2	104
125	5/5	114 ± 2	172 ± 5	+58 ± 3	100
250	5/5	107 ± 6	169 ± 6	+62 ± 2	98
500	(d) 4/5	104 ± 3	157 ± 3	+51 ± 5	91
1,000	(e) 0/5	110 ± 4	(f)	(f)	(f)
FEMALE					
0	5/5	95 ± 3	129 ± 2	+34 ± 2	
63	5/5	90 ± 2	126 ± 2	+36 ± 0	98
125	5/5	92 ± 2	114 ± 3	+22 ± 2	88
250	5/5	96 ± 2	127 ± 3	+31 ± 1	98
500	(g) 1/5	98 ± 3	106	+14	82
1,000	(h) 0/5	94 ± 2	(f)	(f)	(f)

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on 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: 10
(e) Day of death: 1,1,1,4; one death accidental.
(f) No data are presented due to 100% mortality in this group.
(g) Day of death: 3,5,5,13
(h) Day of death: all 2

TABLE 9. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF HYDROQUINONE

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Necropsy	Change (c)	Necropsy Weight Relative to Vehicle Controls (percent)
MALE					
0	10/10	159 ± 1	367 ± 7	+208 ± 6	
25	10/10	162 ± 1	365 ± 3	+203 ± 3	99
50	10/10	159 ± 2	347 ± 6	+188 ± 5	95
100	10/10	159 ± 2	338 ± 6	+179 ± 4	92
200	10/10	156 ± 2	333 ± 5	+177 ± 4	91
400	(d) 0/10	160 ± 2	(e)	(e)	(e)
FEMALE					
0	10/10	115 ± 2	201 ± 3	+86 ± 3	
25	10/10	116 ± 1	202 ± 2	+86 ± 2	100
50	10/10	115 ± 2	200 ± 3	+85 ± 3	100
100	10/10	117 ± 1	195 ± 3	+78 ± 3	97
200	(f) 7/10	113 ± 1	196 ± 3	+81 ± 3	98
400	(g) 0/10	114 ± 1	(e)	(e)	(e)

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on 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: 2,2,2,2,2,2,2,2,7,13
(e) No data are reported due to 100% mortality in this group.
(f) Week of death: all 11
(g) Week of death: 1,1,2,2,4,5,5,6,7,7

TABLE 10. LIVER WEIGHTS FOR RATS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF HYDROQUINONE (a)

Dose (mg/kg)	Number Weighed	Necropsy Body Weight (grams)	Liver Weight (mg)	Liver Weight/ Necropsy Body Weight (mg/g)
MALE				
0	10	367 ± 7.1	15,708 ± 652	42.9 ± 1.77
25	10	365 ± 3.4	**12,319 ± 342	**33.7 ± 0.76
50	10	**347 ± 5.6	**12,331 ± 490	*35.5 ± 1.41
100	10	**338 ± 6.2	**11,227 ± 271	**33.2 ± 0.69
200	10	**333 ± 5.3	**13,653 ± 456	40.9 ± 1.06
FEMALE				
0	10	201 ± 3.4	6,845 ± 218	34.0 ± 0.68
25	10	202 ± 2.4	6,924 ± 203	34.2 ± 0.93
50	10	200 ± 2.8	*7,611 ± 247	**38.0 ± 1.06
100	10	195 ± 2.9	*7,551 ± 224	**38.8 ± 1.12
200	7	196 ± 3.1	**7,990 ± 110	**40.9 ± 0.89

(a) Mean ± standard error; P values vs. the vehicle controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
*P<0.05
**P<0.01

Gross pathologic examinations revealed that 4/10 males and 5/10 females receiving 400 mg/kg had red-to-brown perioral staining, 1/10 males and 2/10 females had reddened mucosa in the stomach, and 1/10 males had meningeal hemorrhage. At doses of 200 mg/kg, 2/10 males had evidence of intra-abdominal bleeding. In females, 1/10 had blood in the stomach, and 2/10 had perioral staining. Microscopically, inflammation and epithelial hyperplasia (mild to moderate severity) of the forestomach were seen in 4/10 males and 1/10 females receiving 200 mg/kg. Similar lesions were not observed in any other groups.

Toxic nephropathy was seen in 7/10 males and 6/10 females receiving 200 mg/kg and in 1/10 females receiving 100 mg/kg. The kidney lesions in males were judged to be of moderate to marked severity and consisted of tubular cell degeneration and regeneration in the renal cortex. Lesions in the kidney of female rats were similar to those in males but of lesser (minimal to mild) severity. Titers to rat coronavirus were seen at the beginning and end of the studies, but no microscopic lesions, suggestive of an active infection, were observed.

Dose Selection Rationale: Because of deaths, reductions in body weight gain, and forestomach and kidney lesions at higher doses in the 13-week studies, doses selected for rats for the 2-year studies were 25 and 50 mg/kg hydroquinone, administered in water by gavage 5 days per week.

FIFTEEN-MONTH STUDIES

At the 15-month interim kill for the 2-year studies, the mean relative kidney weight for the 10 high dose male rats was significantly higher than for the vehicle controls (Table 11). Compound-related increased severity of nephropathy was observed in male but not female rats (Table 12). Decreased incidences of hyperplasia and neoplasms of the pituitary gland were seen in female rats. Compound-related toxic effects were not observed in other organs. The hematocrit value, hemoglobin concentration, and erythrocyte count for high dose female rats were decreased compared with those for vehicle controls (Table 13).

TWO-YEAR STUDIES

Body Weights, Organ Weights, and Clinical Signs

Mean body weights of high dose male rats were 5%-9% lower than those of vehicle controls between week 73 and week 93 and 10%-13% lower thereafter (Table 14 and Figure 4). Mean body weights of low dose male rats were 5%-9% lower than those of vehicle controls after week 89. Mean body weights of dosed female rats were within 4% of those of vehicle controls throughout the studies. The relative brain, kidney, and liver weights for high dose male rats were significantly greater than those for vehicle controls (Table 15). No compound-related clinical signs were observed.

TABLE 11. RELATIVE ORGAN WEIGHTS FOR RATS IN THE FIFTEEN-MONTH GAVAGE STUDIES OF HYDROQUINONE (a)

Organ	Vehicle Control	25 mg/kg	50 mg/kg
MALE			
Body weight (grams)	492 ± 9.6	504 ± 8.1	466 ± 10.3
Brain	4.4 ± 0.07	4.2 ± 0.10	4.6 ± 0.10
Kidney	6.2 ± 0.12	6.6 ± 0.20	**6.8 ± 0.14
Liver	33.6 ± 0.65	33.7 ± 0.78	36.8 ± 1.29
FEMALE			
Body weight (grams)	312 ± 11.6	307 ± 8.1	303 ± 7.9
Brain	6.3 ± 0.18	6.1 ± 0.17	6.3 ± 0.17
Kidney	6.6 ± 0.52	6.0 ± 0.19	6.0 ± 0.12
Liver	29.8 ± 1.16	30.8 ± 0.79	31.6 ± 0.73

(a) Mean ± standard error in milligrams of organ per gram body weight for groups of 10 animals
**$P<0.01$ by Shirley's test (Shirley, 1977)

TABLE 12. NUMBERS OF RATS WITH SELECTED LESIONS IN THE FIFTEEN-MONTH GAVAGE STUDIES OF HYDROQUINONE (a)

Site/Lesion	Male			Female		
	0 mg/kg	25 mg/kg	50 mg/kg	0 mg/kg	25 mg/kg	50 mg/kg
Kidney						
Minimal nephropathy	0	0	0	4	1	3
Mild nephropathy	10	5	4	1	4	4
Moderate nephropathy	0	5	6	1	0	0
Pituitary Gland						
Pars distalis hyperplasia	1	3	1	3	1	0
Pars distalis adenoma	1	0	2	3	3	1
Pars distalis carcinoma	0	0	0	1	0	0
Pars intermedia adenoma	0	0	0	1	0	0

(a) Ten animals in each group were examined.

TABLE 13. HEMATOLOGIC AND CLINICAL CHEMICAL ANALYSES FOR RATS IN THE FIFTEEN-MONTH GAVAGE STUDIES OF HYDROQUINONE (a)

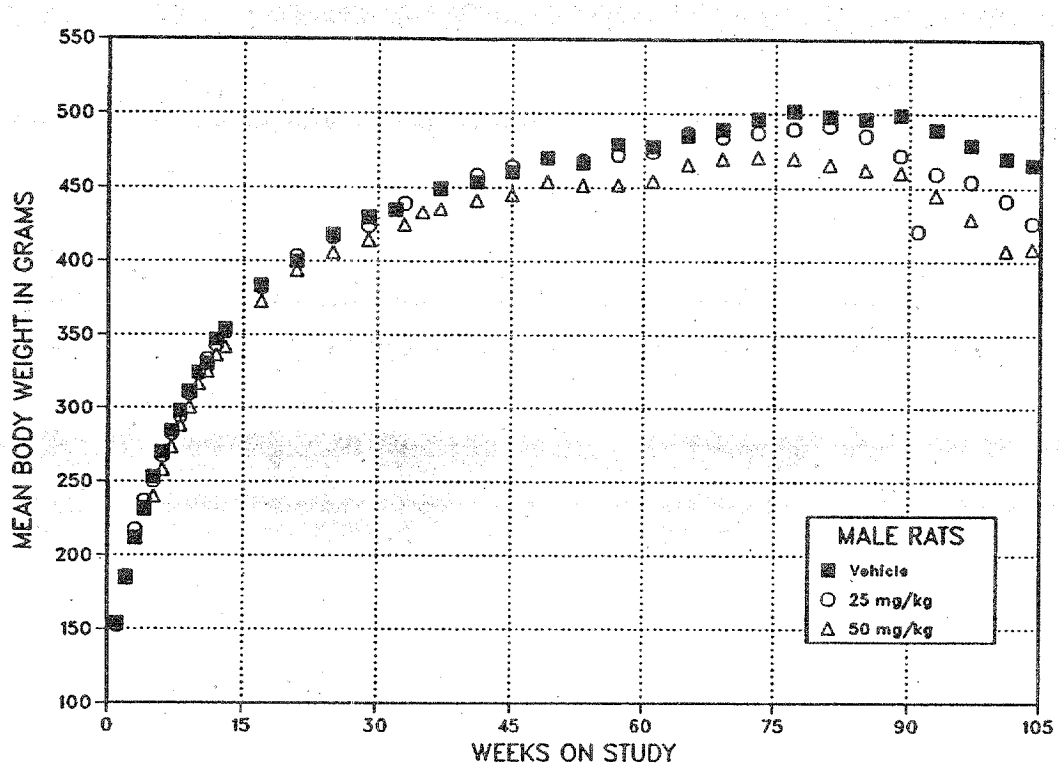
Analysis	Vehicle Control	25 mg/kg	50 mg/kg
MALE			
Leukocytes (1,000/µl)	2.6 ± 0.23	(b) 3.2 ± 0.26	4.4 ± 1.58
Lymphocytes (1,000/µl)	1.2 ± 0.14	*(b) 1.8 ± 0.15	1.9 ± 0.37
Segmented neutrophils (1,000/µl)	0.70 ± 0.077	(b) 0.88 ± 0.114	0.74 ± 0.093
Monocytes (1,000/µl)	0.02 ± 0.012	(b) 0.04 ± 0.012	0.03 ± 0.012
Eosinophils (1,000/µl)	0.06 ± 0.016	(b) 0.08 ± 0.011	0.06 ± 0.019
Atypical lymphocytes (1,000/µl)	0.07 ± 0.025	(b) 0.10 ± 0.021	0.15 ± 0.059
Atypical mononuclear cells (1,000/µl)	0.35 ± 0.056	(b) 0.21 ± 0.036	1.39 ± 1.176
Bands (1,000/µl)	0.29 ± 0.048	(b) 0.19 ± 0.026	0.29 ± 0.078
Hematocrit (percent)	37.2 ± 1.15	35.3 ± 2.31	35.0 ± 2.92
Hemoglobin (g/dl)	13.4 ± 0.41	12.8 ± 0.83	12.6 ± 1.01
Mean corpuscular hemoglobin (pg)	18.0 ± 0.21	17.8 ± 0.24	18.6 ± 0.46
Mean corpuscular hemoglobin concentration (g/dl)	36.2 ± 0.45	36.2 ± 0.16	36.4 ± 0.44
Mean cell volume (μ^3)	50.1 ± 0.23	49.5 ± 0.62	50.8 ± 0.83
Erythrocytes (10^6/µl)	7.5 ± 0.21	7.1 ± 0.40	6.9 ± 0.60
Reticulocytes (10^6/µl)	0.39 ± 0.029	0.50 ± 0.113	0.34 ± 0.033
Albumin (g/dl)	4.6 ± 0.05	4.5 ± 0.04	4.4 ± 0.07
Alkaline phosphatase (IU/liter)	139 ± 4.7	133 ± 5.1	*132 ± 17.6
Alanine aminotransferase (IU/liter)	81.6 ± 7.19	80.8 ± 6.65	125.1 ± 34.54
Blood urea nitrogen (mg/dl)	20.3 ± 1.32	18.2 ± 1.00	19.6 ± 1.20
Creatinine (mg/dl)	0.44 ± 0.027	0.41 ± 0.018	0.42 ± 0.013
Sorbitol dehydrogenase (SU/ml)	20.0 ± 1.22	19.9 ± 1.10	23.4 ± 2.92
Total protein (g/dl)	6.7 ± 0.05	6.6 ± 0.07	6.7 ± 0.13
FEMALE			
Leukocytes (1,000/µl)	1.9 ± 0.15	2.1 ± 0.23	2.0 ± 0.14
Lymphocytes (1,000/µl)	1.3 ± 0.08	1.3 ± 0.16	1.2 ± 0.07
Segmented neutrophils (1,000/µl)	0.34 ± 0.051	0.45 ± 0.072	0.44 ± 0.067
Monocytes (1,000/µl)	0.003 ± 0.003	0.007 ± 0.006	0.006 ± 0.004
Eosinophils (1,000/µl)	0.02 ± 0.006	0.02 ± 0.006	0.02 ± 0.004
Atypical lymphocytes (1,000/µl)	0.08 ± 0.011	0.07 ± 0.013	0.08 ± 0.010
Atypical mononuclear cells (1,000/µl)	0.07 ± 0.013	0.11 ± 0.017	0.14 ± 0.053
Bands (10,000/µl)	0.15 ± 0.036	0.15 ± 0.023	0.14 ± 0.030
Hematocrit (percent)	40.2 ± 0.69	38.9 ± 0.89	*36.0 ± 1.70
Hemoglobin (g/dl)	14.8 ± 0.27	14.3 ± 0.26	*13.4 ± 0.62
Mean corpuscular hemoglobin (pg)	20.2 ± 0.11	20.2 ± 0.20	21.1 ± 0.71
Mean corpuscular hemoglobin concentration (g/dl)	36.9 ± 0.27	36.8 ± 0.46	37.4 ± 0.27
Mean cell volume (μ^3)	54.9 ± 0.38	55.1 ± 0.43	56.7 ± 1.83
Erythrocytes (10^6/µl)	7.3 ± 0.13	7.1 ± 0.15	*6.5 ± 0.40
Reticulocytes (10^6/µl)	0.10 ± 0.019	0.13 ± 0.019	0.21 ± 0.117
Albumin (g/dl)	5.1 ± 0.11	4.9 ± 0.07	5.1 ± 0.20
Alkaline phosphatase (IU/liter)	119 ± 11.2	*150 ± 4.6	142 ± 5.9
Alanine aminotransferase (IU/liter)	48.9 ± 4.42	57.3 ± 4.83	61.9 ± 8.51
Blood urea nitrogen (mg/dl)	19.0 ± 1.05	20.7 ± 1.02	19.4 ± 1.21
Creatinine (mg/dl)	0.41 ± 0.023	0.42 ± 0.013	0.41 ± 0.018
Sorbitol dehydrogenase (SU/ml)	15.6 ± 1.33	17.8 ± 1.65	21.7 ± 3.25
Total protein (g/dl)	7.3 ± 0.16	7.0 ± 0.07	7.0 ± 0.27

(a) Mean ± standard error for groups of 10 animals unless otherwise specified; P values vs. the vehicle controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977). IU = international units; SU = Sigma units.
(b) Nine animals were examined.
*$P<0.05$

TABLE 14. MEAN BODY WEIGHTS OF RATS IN THE TWO-YEAR GAVAGE STUDIES OF HYDROQUINONE

Weeks on Study	Vehicle Control Av. Wt. (grams)	Vehicle Control Number Weighed	25 mg/kg Av. Wt. (grams)	25 mg/kg Wt. (percent of veh. controls)	25 mg/kg Number Weighed	50 mg/kg Av. Wt. (grams)	50 mg/kg Wt. (percent of veh. controls)	50 mg/kg Number Weighed
MALE								
1	154	65	152	99	65	154	100	65
2	186	65	186	100	65	185	99	65
3	212	65	217	102	65	212	100	65
4	232	65	237	102	65	231	100	65
5	253	65	250	99	65	240	95	65
6	270	65	268	99	65	258	96	65
7	285	65	283	99	65	274	96	65
8	298	65	295	99	65	288	97	65
9	311	65	310	100	65	301	97	65
10	325	65	323	99	65	317	98	65
11	331	65	333	101	65	325	98	65
12	347	65	343	99	65	336	97	65
13	354	65	352	99	65	342	97	65
17	384	65	382	99	64	373	97	65
21	400	65	403	101	64	394	99	65
25	418	65	416	100	64	406	97	65
29	430	65	424	99	64	414	96	65
37	449	64	450	100	63	436	97	65
41	454	64	458	101	62	441	97	65
45	460	64	464	101	62	445	97	65
49	470	64	470	100	62	454	97	64
53	467	64	468	100	62	452	97	64
57	479	62	472	99	62	452	94	64
61	478	62	474	99	61	455	95	64
65	485	62	487	100	59	466	96	64
69	490	(a) 51	484	99	(a) 48	470	96	(a) 53
73	497	50	487	98	47	471	95	52
77	502	49	490	98	47	470	94	51
81	499	47	492	99	45	466	93	49
85	497	46	485	98	43	462	93	47
89	499	41	474	95	39	461	92	41
93	489	40	460	94	36	445	91	38
97	479	37	454	95	29	429	90	31
101	470	34	441	94	26	408	87	25
104	466	30	426	91	22	409	88	18
FEMALE								
1	133	65	131	98	65	130	98	65
2	142	65	143	101	65	140	99	65
3	148	65	153	103	65	150	101	65
4	161	65	160	99	65	157	98	65
5	167	65	166	99	65	165	99	65
6	172	65	170	99	65	169	98	65
7	177	65	176	99	65	176	99	65
8	183	65	177	97	65	178	97	65
9	189	65	185	98	65	185	98	65
10	192	65	190	99	65	188	98	65
11	194	65	191	98	65	192	99	65
12	198	65	197	99	65	194	98	65
13	199	65	200	101	65	203	102	65
17	213	65	211	99	65	207	97	65
21	219	65	221	101	65	218	100	65
25	229	65	225	98	65	223	97	65
29	237	65	232	98	65	230	97	65
33	240	65	239	100	65	235	98	65
37	245	65	243	99	65	241	98	65
41	250	65	250	100	65	248	99	65
45	260	65	257	99	65	255	98	65
49	263	65	266	101	65	265	101	65
53	270	65	272	101	65	265	98	64
57	280	64	281	100	64	274	98	64
61	291	64	294	101	64	288	99	64
65	301	64	304	101	63	294	98	64
69	315	(a) 53	316	100	(a) 53	308	98	(a) 53
73	322	51	327	102	51	317	98	52
77	330	51	332	101	51	324	98	52
81	334	50	338	101	50	329	99	52
85	341	49	345	101	49	333	98	51
89	351	49	349	99	48	342	97	48
93	348	49	346	99	44	336	97	46
97	344	46	348	101	36	334	97	41
101	347	42	341	98	33	333	96	(b) 36
104	344	40	348	101	28	335	97	32

(a) Interim kill occurred.
(b) The number of animals weighed was lower than the number of animals surviving.



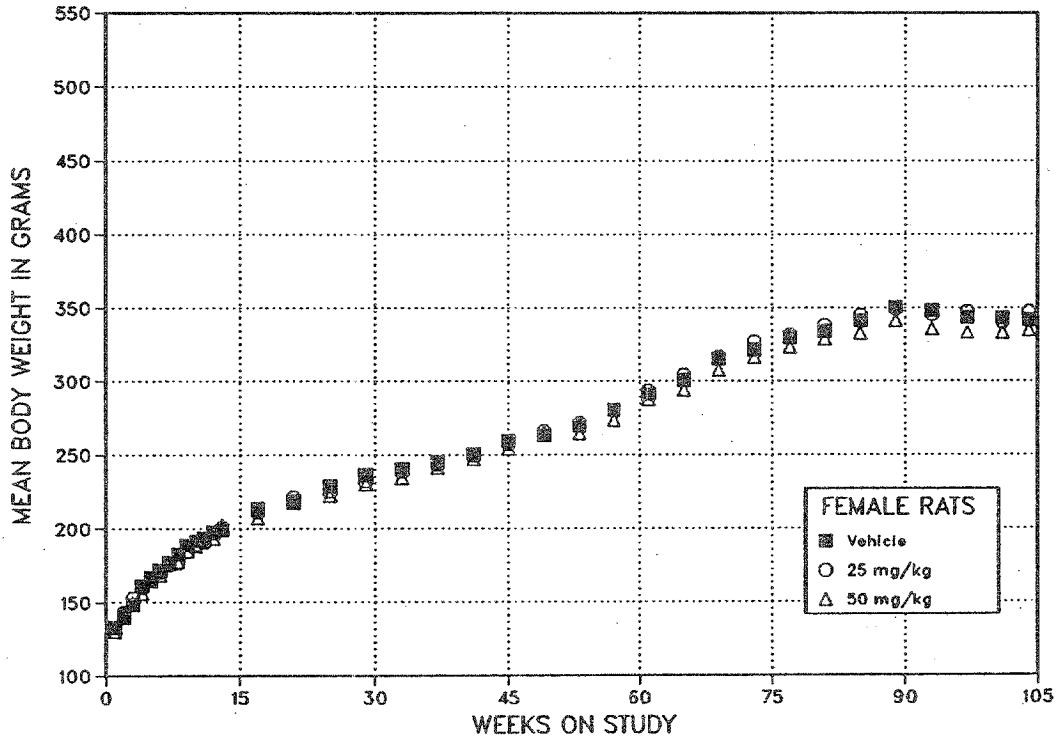


FIGURE 4. GROWTH CURVES FOR RATS ADMINISTERED HYDROQUINONE IN WATER BY GAVAGE FOR TWO YEARS

TABLE 15. RELATIVE ORGAN WEIGHTS FOR RATS IN THE TWO-YEAR GAVAGE STUDIES OF HYDROQUINONE (a)

Organ	Vehicle Control	25 mg/kg	50 mg/kg
MALE			
Number weighed	27	18	18
Body weight (grams)	464 ± 9.2	*434 ± 8.5	**402 ± 11.9
Brain	4.9 ± 0.11	*5.2 ± 0.12	**5.6 ± 0.18
Kidney	4.5 ± 0.12	4.7 ± 0.14	**(b) 6.6 ± 0.59
Liver	46.6 ± 2.09	47.6 ± 2.76	*53.9 ± 3.04
FEMALE			
Number weighed	39	27	31
Body weight (grams)	337 ± 7.1	360 ± 13.1	330 ± 7.4
Brain	5.9 ± 0.16	5.6 ± 0.17	6.0 ± 0.15
Kidney	3.9 ± 0.11	3.7 ± 0.12	4.0 ± 0.10
Liver	37.2 ± 0.96	38.1 ± 1.25	41.2 ± 1.71

(a) Mean ± standard error in milligrams of organ per gram body weight; P values vs. the vehicle controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
(b) Seventeen were weighed.
*P<0.05
**P<0.01

Survival

Estimates of the probabilities of survival for male and female rats administered hydroquinone at the doses used in these studies and for vehicle controls are shown in Table 16 and in the Kaplan and Meier curves in Figure 5. No significant differences in survival were observed between any groups of either sex.

Pathology and Statistical Analyses of Results

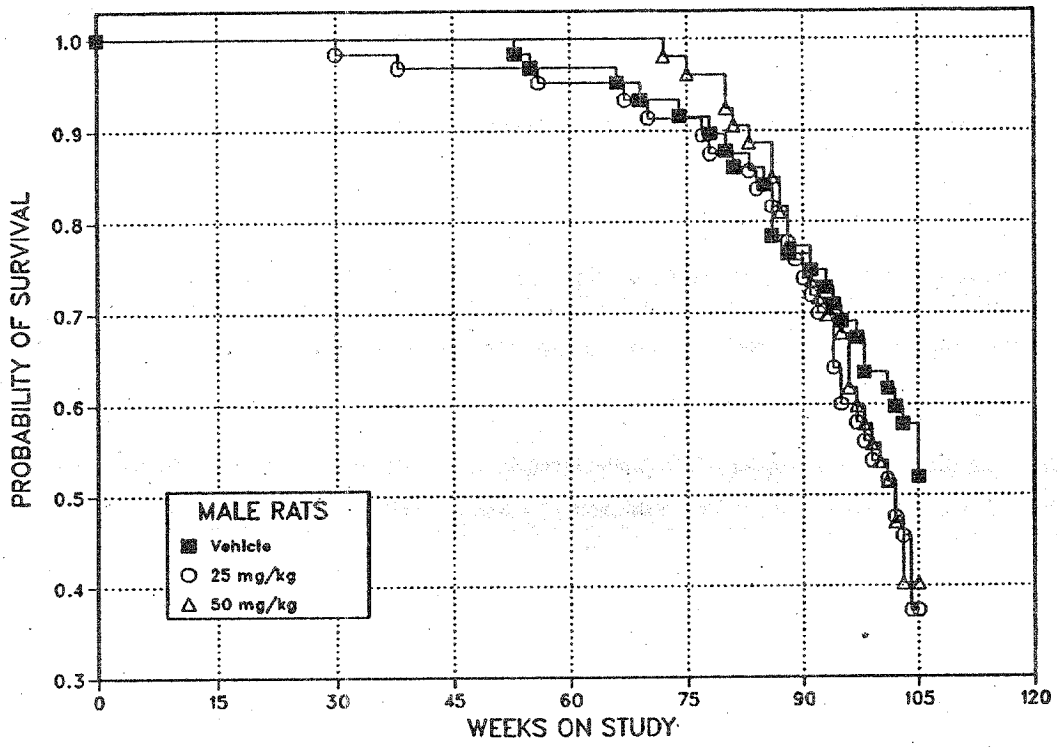
This section describes the statistically significant or biologically noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions of the kidney, hematopoietic system, adrenal gland, thyroid gland, and anterior pituitary gland.

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary tumors that occurred with an incidence of at least 5% in at least one animal group, and historical control incidences for the neoplasms mentioned in this section are presented in Appendixes A and B for male and female rats, respectively.

TABLE 16. SURVIVAL OF RATS IN THE TWO-YEAR GAVAGE STUDIES OF HYDROQUINONE

	Vehicle Control	25 mg/kg	50 mg/kg
MALE (a)			
Animals initially in study	55	55	55
Natural deaths	13	7	8
Moribund kills	13	25	22
Animals surviving until study termination	27	18	18
Accidentally killed	2	5	7
Survival P values (b)	0.371	0.217	0.427
FEMALE (a)			
Animals initially in study	55	55	55
Natural deaths	2	6	6
Moribund kills	14	19	14
Animals surviving until study termination	(c) 40	27	32
Accidentally killed	0	3	(d) 4
Survival P values (b)	0.350	0.067	0.380

(a) First day of termination period: male--731; female--729
(b) The result of the life table trend test is in the vehicle control column, and those of the life table pairwise comparisons with the vehicle controls are in the dosed columns.
(c) One animal died or was killed in a moribund condition and was combined, for statistical purposes, with those killed at termination.
(d) One animal was killed accidentally during the termination period and was combined, for statistical purposes, with those killed at termination.



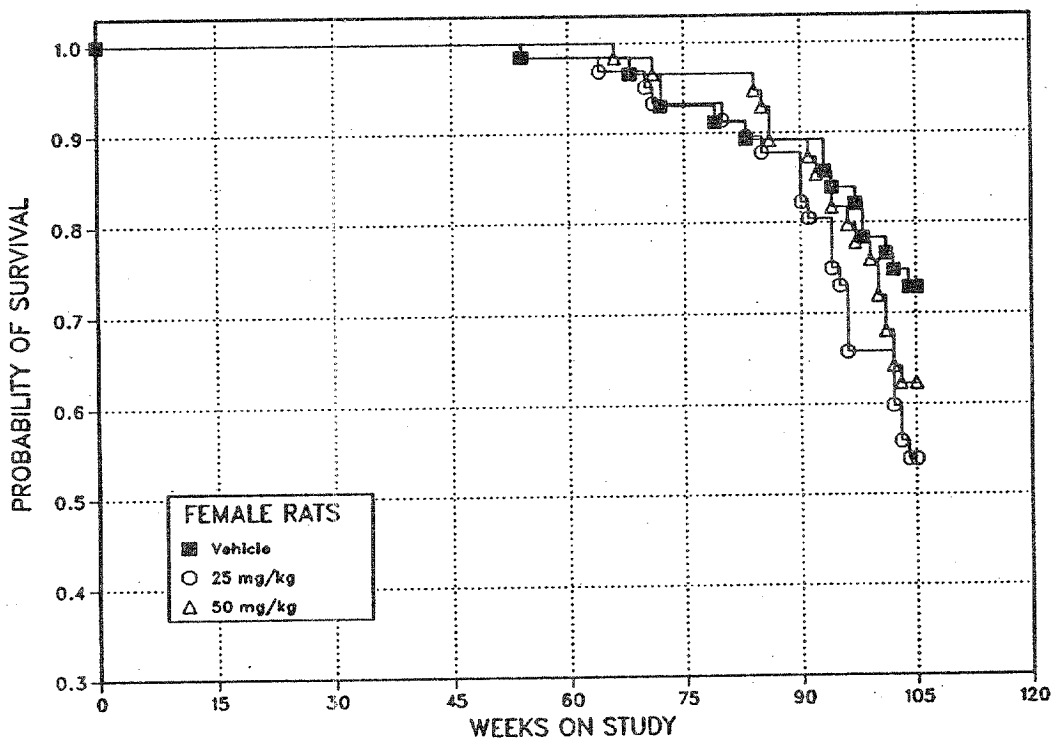


FIGURE 5. KAPLAN-MEIER SURVIVAL CURVES FOR RATS ADMINISTERED HYDROQUINONE IN WATER BY GAVAGE FOR TWO YEARS

Kidney: Spontaneous nephropathy occurred in nearly all male and most female rats of all dosed groups and vehicle controls; however, this age-related renal disease was judged to be more severe in high dose male rats relative to vehicle controls (Table 17). Nephropathy was characterized by varied degrees of degeneration and regeneration of tubular epithelium, atrophy and dilatation of some tubules, hyaline casts in the tubular lumina, glomerulosclerosis, interstitial fibrosis, and chronic inflammation. Papillary hyperplasia of the transitional epithelium overlying the renal papillae and cysts (dilated tubules in the renal cortex) were increased in dosed male rats. These changes are a component of severe nephropathy and reflect the increased number of male rats with advanced renal disease.

Renal tubular adenomas occurred in low and high dose male rats but not in vehicle controls; the incidence in the high dose group was statistically significant (Table 18). All tubular adenomas were identified during the examination of the routine kidney sections; none was observed macroscopically at necropsy. The tubular adenomas were discrete masses of epithelial cells arranged in solid clusters or nests separated by a scant stroma (Figures 6 to 9). In a few tumors, some of the cells exhibited poorly defined tubular formation that blended with the solid areas. The epithelial cells were relatively uniform with pale basophilic cytoplasm and round nuclei with prominent nucleoli. Tubular hyperplasia, consisting of tubules with stratified epithelial cells that partially filled the tubular lumina, was seen in two high dose male rats.

TABLE 17. NUMBER OF MALE RATS WITH INDICATED SEVERITY OF NEPHROPATHY IN THE TWO-YEAR GAVAGE STUDY OF HYDROQUINONE

Severity	Vehicle Control	25 mg/kg	50 mg/kg
Number of rats examined	55	55	55
No nephropathy	2	3	0
Minimal	3	1	3
Mild	12	12	5
Moderate	26	31	15
Marked	12	8	32

TABLE 18. RENAL TUBULE LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF HYDROQUINONE (a)

	Vehicle Control	25 mg/kg	50 mg/kg
Hyperplasia			
Overall Rates	0/55 (0%)	0/55 (0%)	2/55 (4%)
Adenoma (b)			
Overall Rates	0/55 (0%)	4/55 (7%)	8/55 (15%)
Terminal Rates	0/27 (0%)	2/18 (11%)	5/18 (28%)
Day of First Observation		392	598
Logistic Regression Tests	P=0.003	P=0.069	P=0.003

(a) The statistical analyses used are discussed in Section II (Statistical Methods) and Table A3 (footnotes).
(b) Historical incidence of renal tubular cell neoplasms in water gavage vehicle controls (mean ± SD): 1/298 (0.3% ± 0.8%); historical incidence in untreated controls: 9/1,928 (0.5% ± 1%)

Hematopoietic System: The incidences of mononuclear cell leukemia in dosed female rats were significantly greater than in vehicle controls (Table 19). The extent of organ involvement with the leukemia was staged to determine if this disease was the probable cause of death of the affected rats. Stage 1 leukemia was limited to the spleen, with increased numbers of mononuclear cells in the red pulp but with limited distortion of normal splenic architecture (Table 20). Leukemia was not considered the cause of death in these rats. Stage 2 leukemia caused effacement of splenic architecture, with large numbers of mononuclear cells in the red pulp and few neoplastic cells in the sinusoids of the liver or other organs. Stage 2 leukemia may have contributed to the deaths of rats with this disease.

Stage 3 leukemia consisted of marked effacement of the splenic architecture and advanced infiltration of the liver or other organs with neoplastic cells. Stage 3 leukemia was considered the most probable cause of death in the animals affected.

Adrenal Gland: Pheochromocytomas or malignant pheochromocytomas (combined) of the adrenal gland were observed at marginally increased incidences in dosed male rats (vehicle control, 14/55; low dose, 19/48; high dose, 21/55). These lesions were not considered to be related to the administration of hydroquinone because the increased incidences were marginally significant; the finding was not supported by observations from the 15-month interim kill, and the incidence of pheochromocytomas is rather variable in historical controls. The historical incidence of pheochromocytomas or malignant pheochromocytomas (combined) in male water gavage vehicle control F344/N rats is 40% ± 16% and in untreated controls is 26% ± 14%.

TABLE 19. HEMATOPOIETIC SYSTEM TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF HYDROQUINONE

	Vehicle Control	25 mg/kg	50 mg/kg
Mononuclear Leukemia (a)			
Overall Rates	9/55 (16%)	15/55 (27%)	22/55 (40%)
Adjusted Rates	19.4%	37.9%	49.6%
Terminal Rates	4/40 (10%)	6/27 (22%)	11/32 (34%)
Day of First Observation	553	576	492
Life Table Tests	P=0.003	P=0.048	P=0.003
Logistic Regression Tests	P=0.004	P=0.129	P=0.006

(a) Historical incidence of leukemia in water gavage vehicle controls (mean ± SD): 75/299 (25% ± 15%); historical incidence of leukemia in untreated controls: 383/1,983 (19% ± 7%)

TABLE 20. NUMBER OF FEMALE RATS WITH VARIOUS STAGES OF MONONUCLEAR CELL LEUKEMIA IN THE TWO-YEAR GAVAGE STUDY OF HYDROQUINONE

Stage (a)	Vehicle Control	25 mg/kg	50 mg/kg
1	0	2	1
2	4	5	7
3	5	8	14
Total	9	15	22

(a) Stage 1 leukemia probably was not a contributory cause of death; stage 2 is more severe and was probably a contributory cause of death; stage 3 was considered to be the probable cause of death for most animals in this category.

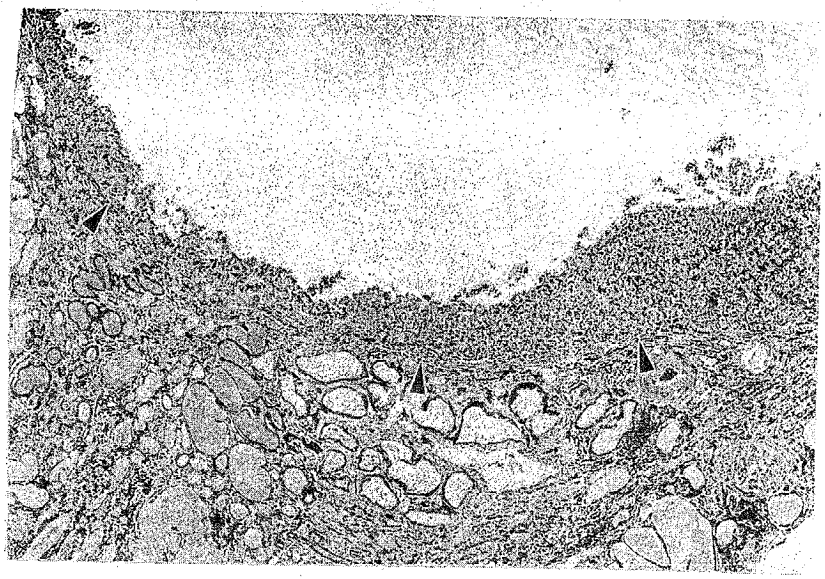


Figure 6. Large cystic tubular cell adenoma in the kidney of high dose male rat CID #271 (arrows).

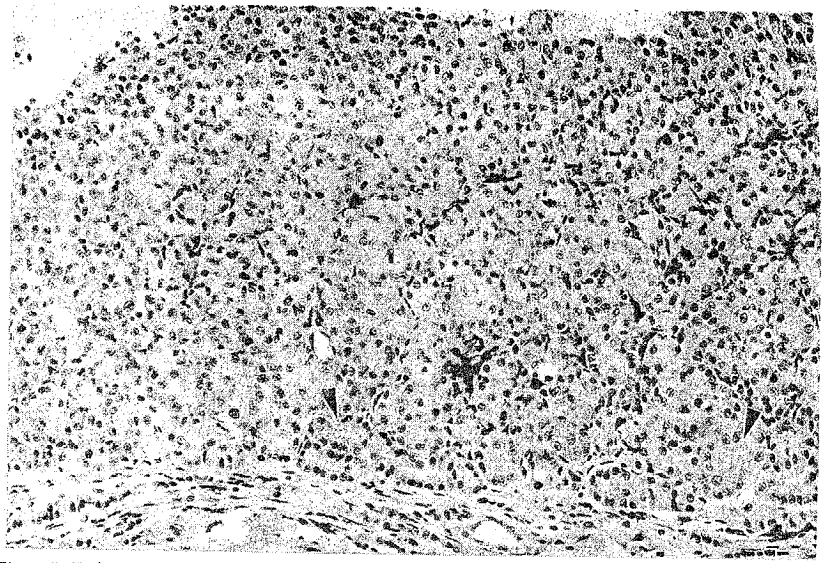


Figure 7. Higher magnification of tubular cell adenoma in Figure 6. Note the uniform cells arranged in tubular structures (arrows).

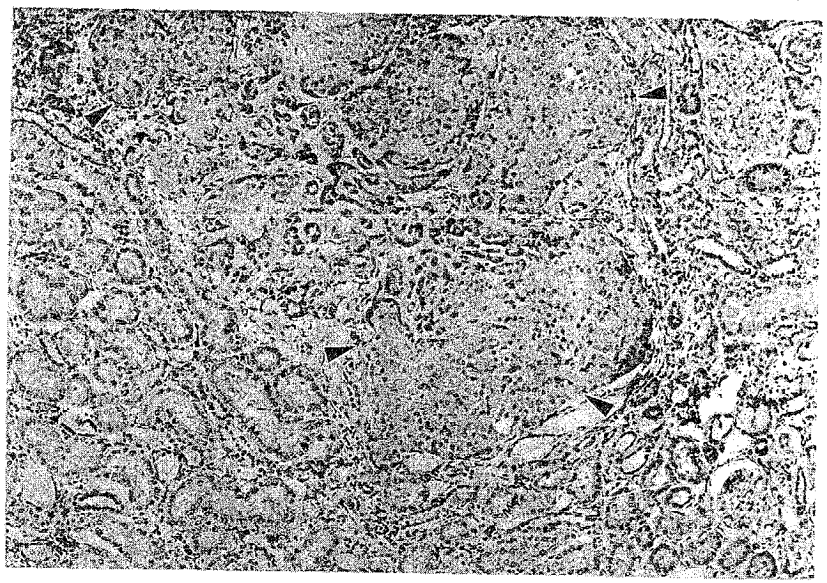


Figure 8. Tubular cell adenoma in the kidney of low dose male rat CID #152 (arrows).

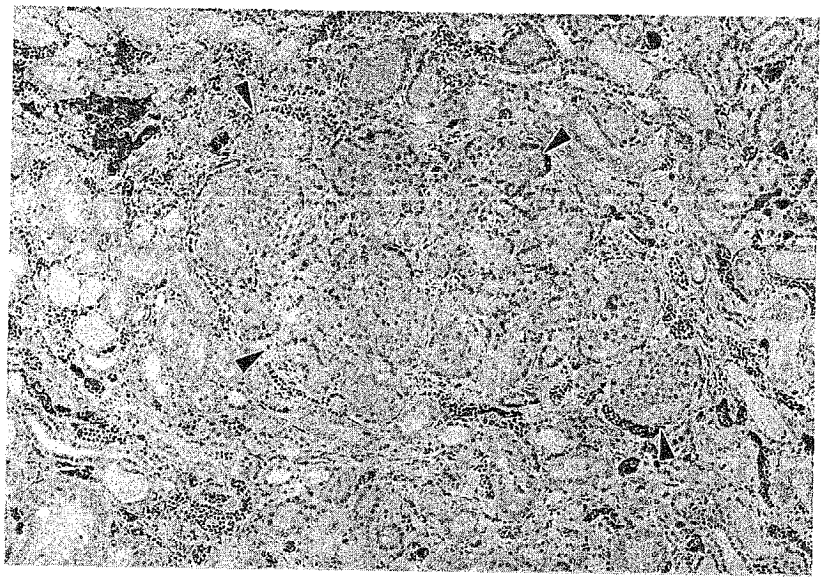


Figure 9. Tubular cell adenoma in the kidney of high dose male rat CID #312 (arrows).

Thyroid Gland: The incidence of C-cell adenomas or carcinomas (combined) in low dose female rats was significantly lower than that in vehicle controls (vehicle control, 13/55; low dose, 4/54; high dose, 8/55) (Table B3).

Anterior Pituitary Gland: Adenomas in male rats occurred with a significant negative trend; the incidence in the high dose group was significantly lower than in the vehicle controls (Table 21). Results of the pairwise comparison between the high dose group and the vehicle controls were only marginally significant, and the incidence of adenomas and carcinomas (combined) (9%) in the high dose group is within the historical range (5%-54%) for untreated controls (Table A4c). Therefore, this negative trend is not considered to be related to the administration of hydroquinone.

TABLE 21. ANTERIOR PITUITARY GLAND LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF HYDROQUINONE

	Vehicle Control	25 mg/kg	50 mg/kg
Hyperplasia			
Overall Rates	12/54 (22%)	14/54 (26%)	11/54 (20%)
Adenoma			
Overall Rates	13/54 (24%)	9/54 (17%)	5/54 (9%)
Terminal Rates	6/27 (22%)	3/17 (18%)	1/18 (6%)
Day of First Observation	459	466	598
Logistic Regression Tests	P=0.031N	P=0.303N	P=0.038N
Carcinoma			
Overall Rates	0/54 (0%)	1/54 (2%)	0/54 (0%)
Adenoma or Carcinoma (a)			
Overall Rates	13/54 (24%)	10/54 (19%)	5/54 (9%)
Terminal Rates	6/27 (22%)	3/17 (18%)	1/18 (6%)
Day of First Observation	459	466	598
Logistic Regression Tests	P=0.033N	P=0.392N	P=0.038N

(a) Historical incidence in water gavage vehicle controls (mean ± SD): 126/295 (43% ± 12%); historical incidence in untreated controls: 459/1,830 (25% ± 10%)

PRELIMINARY QUALITATIVE DERMAL ABSORPTION STUDY

Hydroquinone was qualitatively detected in the urine of male mice by thin-layer chromatography at both doses (4 or 40 mg per animal) as soon as 2 hours and as long as 72 hours after dosing. The intensity of the spot increased after enzymatic hydrolysis by β-glucuronidase and aryl sulfatase. Crystals of hydroquinone were observed on skin after application of the high dose. Contamination of the urine by crystals of hydroquinone from the skin of dosed animals could not be ruled out, especially at the higher dose.

FOURTEEN-DAY STUDIES

Dermal

All mice survived to the end of the studies (Table 22). Final mean body weights of all groups of mice were lower than the initial weights. Crystals were seen on the skin and fur of animals at 4,800 mg/kg. No compound-related clinical signs were observed.

TABLE 22. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE FOURTEEN-DAY DERMAL STUDIES OF HYDROQUINONE

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	25.6 ± 0.2	24.4 ± 0.7	−1.2 ± 0.6	
300	5/5	25.8 ± 0.7	24.2 ± 0.4	−1.6 ± 0.5	99.2
600	5/5	26.4 ± 1.3	24.4 ± 1.0	−2.0 ± 0.3	100.0
1,200	5/5	25.8 ± 1.2	24.8 ± 1.4	−1.0 ± 0.9	101.6
2,400	5/5	24.4 ± 0.5	23.0 ± 0.5	−1.4 ± 0.8	94.3
4,800	5/5	26.6 ± 0.7	25.0 ± 0.3	−1.6 ± 0.7	102.5
FEMALE					
0	5/5	19.6 ± 0.2	18.8 ± 0.4	−0.8 ± 0.2	
300	5/5	20.6 ± 0.5	20.0 ± 0.4	−0.6 ± 0.2	106.4
600	5/5	19.6 ± 0.5	18.8 ± 0.4	−0.8 ± 0.2	100.0
1,200	5/5	20.4 ± 0.5	19.4 ± 0.7	−1.0 ± 0.6	103.2
2,400	5/5	20.0 ± 0.3	19.0 ± 0.3	−1.0 ± 0.3	101.1
4,800	5/5	20.2 ± 0.6	19.4 ± 0.6	−0.8 ± 0.2	103.2

(a) Number surviving/number initially in 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

Gavage

Four of five male mice and 5/5 female mice receiving 500 mg/kg and 3/5 males receiving 250 mg/kg died within 3 days (Table 23). Other deaths were not compound related. The final mean body weights of male mice that received 250 or 500 mg/kg were 8% or 4% lower than that of the vehicle controls. Final mean body weights of dosed and vehicle control female mice were similar. Tremors followed by recovery or convulsions and death were seen in males and females receiving 500 mg/kg and males receiving 250 mg/kg. Tremors followed by recovery were seen in females receiving 250 mg/kg.

THIRTEEN-WEEK STUDIES

Eight of 10 male and 8/10 female mice receiving 400 mg/kg and 2/10 males receiving 200 mg/kg died before the end of the studies (Table 24). One death at 200 mg/kg was attributed to gavage error. Mean body weights of dosed and vehicle control mice were similar at necropsy. However, an unexplained drop in the mean body weight of the male vehicle control group was noted during weeks 12 and 13.

The most common clinical sign was lethargy, seen in all dosed males and the top three dosed groups of females. Tremors after dosing were seen in the top dose group of each sex and in the 200 mg/kg group of males. These tremors were often followed by convulsions in the top dose group only.

Liver weight to body weight ratios for dosed male mice were significantly greater than for vehicle controls (Table 25). Ulceration, inflammation, or epithelial hyperplasia of the forestomach was found in 3/10 male and 2/10 female mice receiving 400 mg/kg and in 1/10 females receiving 200 mg/kg.

TABLE 23. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE FOURTEEN-DAY GAVAGE STUDIES OF HYDROQUINONE

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	25.2 ± 1.2	28.2 ± 0.9	+3.0 ± 0.3	
31	(d) 4/5	25.2 ± 0.7	28.0 ± 1.1	+2.3 ± 0.6	99.3
63	5/5	26.2 ± 0.8	28.0 ± 0.8	+1.8 ± 0.2	99.3
125	5/5	25.2 ± 0.7	28.6 ± 0.9	+3.4 ± 1.0	101.4
250	(e) 2/5	24.4 ± 1.2	26.0 ± 3.0	+0.5 ± 0.5	92.2
500	(f) 1/5	25.6 ± 1.1	27.0	0.0	95.7
FEMALE					
0	5/5	21.0 ± 0.3	22.2 ± 0.5	+1.2 ± 0.4	
31	(g) 4/5	20.8 ± 0.4	21.8 ± 0.6	+1.0 ± 0.4	98.2
63	(h) 4/5	18.8 ± 0.4	21.3 ± 0.5	+2.3 ± 0.5	95.9
125	5/5	21.0 ± 0.5	21.4 ± 0.7	+0.4 ± 0.8	96.4
250	5/5	21.0 ± 0.6	22.4 ± 0.2	+1.4 ± 0.4	100.9
500	(i) 0/5	19.6 ± 0.6	(j)	(j)	(j)

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Death due to gavage error
(e) Day of death: 3,3; one death accidental.
(f) Day of death: all 1
(g) Day of death: 8
(h) Day of death: 3
(i) Day of death: 1,1,1,1,2
(j) No data are reported due to 100% mortality in this group.

TABLE 24. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE THIRTEEN-WEEK GAVAGE STUDIES OF HYDROQUINONE

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Necropsy	Change (c)	Necropsy Weight Relative to Vehicle Controls (percent)
MALE					
0	10/10	26.4 ± 0.3	30.5 ± 1.3	+4.1 ± 1.2	
25	10/10	26.6 ± 0.3	37.6 ± 1.3	+11.0 ± 1.3	123.3
50	10/10	26.3 ± 0.3	35.7 ± 0.5	+9.4 ± 0.6	117.0
100	10/10	26.7 ± 0.4	(d) 37.5 ± 0.8	+10.7 ± 0.8	123.0
200	(e) 8/10	26.7 ± 0.4	35.1 ± 0.7	+8.6 ± 0.7	115.1
400	(f) 2/10	26.2 ± 0.4	31.8 ± 1.3	+6.6 ± 0.7	104.3
FEMALE					
0	10/10	19.1 ± 0.2	25.0 ± 0.4	+5.9 ± 0.4	
25	10/10	19.1 ± 0.2	26.5 ± 0.4	+7.4 ± 0.4	106.0
50	10/10	19.4 ± 0.3	26.9 ± 0.5	+7.5 ± 0.5	107.6
100	10/10	19.4 ± 0.3	26.4 ± 0.6	+7.0 ± 0.4	105.6
200	10/10	18.9 ± 0.2	25.3 ± 0.4	+6.4 ± 0.4	101.2
400	(g) 2/10	19.3 ± 0.1	26.1 ± 0.3	+7.2 ± 0.1	104.4

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) One necropsy body weight was not recorded; body weight change is based on nine animals.
(e) Week of death: 1,9
(f) Week of death: 1,1,1,1,1,1,2,13
(g) Week of death: 1,1,1,1,8,9,10,12

TABLE 25. LIVER WEIGHTS FOR MICE IN THE THIRTEEN-WEEK GAVAGE STUDIES OF HYDROQUINONE (a)

Dose (mg/kg)	Number Weighed	Necropsy Body Weight (grams)	Liver Weight (mg)	Liver Weight/ Necropsy Body Weight (mg/g)
MALE				
0	10	30.5 ± 1.33	1,253 ± 56	41.2 ± 1.40
25	10	**37.6 ± 1.30	**2,016 ± 79	**54.0 ± 2.38
50	10	35.7 ± 0.53	**1,826 ± 46	**51.2 ± 1.05
100	(b) 9	**37.5 ± 0.75	**1,818 ± 37	**48.9 ± 1.35
200	8	35.1 ± 0.67	**2,000 ± 112	**57.1 ± 3.37
400	2	31.8 ± 1.30	*1,750 ± 120	**55.0 ± 1.53
FEMALE				
0	10	25.0 ± 0.41	1,230 ± 31	49.1 ± 1.08
25	10	26.5 ± 0.42	1,331 ± 62	50.4 ± 2.47
50	10	*26.9 ± 0.51	1,309 ± 33	48.8 ± 1.02
100	10	26.4 ± 0.55	*1,396 ± 60	52.8 ± 1.39
200	10	25.3 ± 0.38	1,338 ± 47	*52.9 ± 1.25
400	2	26.1 ± 0.35	*1,505 ± 85	*57.8 ± 4.04

(a) Mean ± standard error; P values vs. the vehicle controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
(b) All 10 livers were weighed, but one body weight was not recorded at necropsy; ratio is based on remaining nine animals.
*P<0.05
**P<0.01

Dose Selection Rationale: Because of deaths and forestomach lesions at higher doses in the 13-week studies, doses selected for mice for the 2-year studies were 50 and 100 mg/kg hydroquinone, administered in water by gavage 5 days per week.

FIFTEEN-MONTH STUDIES

Significant increases were observed for the hematocrit value, erythrocyte count, serum albumin concentration, total protein concentration, and serum alkaline phosphatase and sorbitol dehydrogenase activity for high dose male mice and for the serum albumin and total protein concentration for high dose female mice; significantly lower activity was observed for alanine aminotransferase and sorbitol dehydrogenase activity for high dose female mice (Table 26).

The relative liver weights for high dose male and female mice, the relative kidney weights for dosed female mice, and the relative brain weight for high dose female mice were significantly higher than those for vehicle controls (Table 27). Compound-related lesions were seen in the liver of male mice (Table 28). In dosed male mice, hepatocytes in the centrilobular areas contained multiple small, clear cytoplasmic vacuoles characteristic of lipid. Hepatocytes in the periportal areas were large and had dense, finely granular eosinophilic cytoplasm. These changes were diagnosed as centrilobular fatty changes and cytomegaly, respectively. Occasional hepatocytes had multiple nuclei (syncytial cells). These lesions were not observed in female mice. Several hepatocellular neoplasms were observed in male and female mice but were too few for any conclusion to be made regarding their relationship to administration of hydroquinone.

TWO-YEAR STUDIES

Body Weights, Organ Weights, and Clinical Signs

Mean body weights of high dose male mice were 5%-8% lower than those of vehicle controls from week 93 to the end of the study (Table 29 and Figure 10). Mean body weights of high dose female mice were 5%-8% lower than those of vehicle controls from week 20 to week 44 and 10%-14% lower thereafter. The relative liver weights were increased for dosed male and high dose female mice (Table 30). No compound-related clinical signs were observed.

TABLE 26. HEMATOLOGIC AND CLINICAL CHEMICAL ANALYSES FOR MICE IN THE FIFTEEN-MONTH GAVAGE STUDIES OF HYDROQUINONE (a)

Analysis	Vehicle Control	50 mg/kg	100 mg/kg
MALE			
Leukocytes (1,000/µl)	3.5 ± 0.63	4.7 ± 0.71	3.8 ± 0.55
Lymphocytes (1,000/µl)	1.7 ± 0.34	2.6 ± 0.49	1.7 ± 0.23
Segmented neutrophils (1,000/µl)	1.6 ± 0.36	1.6 ± 0.41	1.8 ± 0.55
Monocytes (1,000/µl)	0.06 ± 0.011	0.05 ± 0.021	0.06 ± 0.029
Eosinophils (1,000/µl)	0.01 ± 0.006	0.01 ± 0.007	0.02 ± 0.008
Atypical lymphocytes (1,000/µl)	0.08 ± 0.031	0.22 ± 0.065	0.11 ± 0.030
Bands (1,000/µl)	0.09 ± 0.029	0.41 ± 0.231	0.17 ± 0.050
Hematocrit (percent)	36.5 ± 2.38	39.5 ± 1.27	*41.3 ± 1.17
Hemoglobin (g/dl)	12.2 ± 0.75	13.0 ± 0.38	13.6 ± 0.32
Mean corpuscular hemoglobin (pg)	17.0 ± 0.47	16.4 ± 0.17	16.4 ± 0.24
Mean corpuscular hemoglobin concentration (g/dl)	33.6 ± 0.40	33.0 ± 0.24	32.9 ± 0.31
Mean cell volume (μ^3)	51.1 ± 0.92	49.7 ± 0.30	49.7 ± 0.60
Erythrocytes (10^6/µl)	7.3 ± 0.52	8.0 ± 0.29	*8.3 ± 0.28
Reticulocytes (10^6/µl)	0.35 ± 0.149	0.20 ± 0.031	0.23 ± 0.035
Albumin (g/dl)	3.3 ± 0.05	(b) 3.5 ± 0.08	**3.8 ± 0.15
Alkaline phosphatase (IU/liter)	38.4 ± 2.34	(c) 38.8 ± 1.41	*50.0 ± 3.92
Alanine aminotransferase (IU/liter)	39.6 ± 3.97	(b) 39.6 ± 4.49	56.4 ± 9.34
Blood urea nitrogen (mg/dl)	24.8 ± 1.41	26.2 ± 1.88	28.1 ± 1.00
Creatinine (mg/dl)	0.2 ± 0.01	(b) 0.2 ± 0.02	0.2 ± 0.01
Sorbitol dehydrogenase (SU/ml)	35.8 ± 1.24	(b) 35.6 ± 2.05	**43.0 ± 1.79
Total protein (g/dl)	5.2 ± 0.04	(b) 5.4 ± 0.15	**5.9 ± 0.23
FEMALE			
Leukocytes (1,000/µl)	5.7 ± 1.25	3.7 ± 0.73	5.2 ± 0.89
Hematocrit (percent)	43.8 ± 0.75	44.1 ± 0.50	43.6 ± 1.86
Hemoglobin (g/dl)	14.7 ± 0.18	14.8 ± 0.13	14.2 ± 0.21
Mean corpuscular hemoglobin (pg)	16.7 ± 0.12	16.7 ± 0.22	16.4 ± 0.11
Mean corpuscular hemoglobin concentration (g/dl)	33.6 ± 0.33	33.7 ± 0.28	32.9 ± 0.79
Mean cell volume (μ^3)	50.0 ± 0.60	49.7 ± 0.47	50.1 ± 1.24
Erythrocytes (10^6/µl)	8.8 ± 0.12	8.9 ± 0.13	8.7 ± 0.17
Albumin (g/dl)	3.5 ± 0.06	3.6 ± 0.10	**3.9 ± 0.04
Alkaline phosphatase (IU/liter)	104 ± 9.7	101 ± 9.9	102 ± 6.3
Alanine aminotransferase (IU/liter)	38.9 ± 6.62	31.6 ± 6.19	**23.7 ± 1.33
Blood urea nitrogen (mg/dl)	23.5 ± 1.92	22.8 ± 1.27	26.0 ± 2.70
Creatinine (mg/dl)	0.2 ± 0.03	0.2 ± 0.02	0.2 ± 0.01
Sorbitol dehydrogenase (SU/ml)	35.6 ± 1.09	33.7 ± 1.37	*32.4 ± 0.73
Total protein (g/dl)	5.3 ± 0.07	5.5 ± 0.12	**5.7 ± 0.05

(a) Mean ± standard error for groups of 10 animals unless otherwise specified; P values vs. the vehicle controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977). IU = international units; SU = Sigma units.
(b) Nine animals were examined.
(c) Eight animals were examined.
*P<0.05
**P<0.01

TABLE 27. RELATIVE ORGAN WEIGHTS FOR MICE IN THE FIFTEEN-MONTH GAVAGE STUDIES OF HYDROQUINONE (a)

Organ	Vehicle Control	50 mg/kg	100 mg/kg
MALE			
Body weight (grams)	45.6 ± 1.26	44.9 ± 1.34	46.9 ± 1.01
Brain	10.6 ± 0.41	11.0 ± 0.33	10.2 ± 0.21
Kidney	18.0 ± 0.63	19.6 ± 0.41	19.2 ± 0.57
Liver	44.6 ± 2.32	52.5 ± 5.35	**54.8 ± 3.88
FEMALE			
Body weight (grams)	46.7 ± 2.51	42.4 ± 2.54	40.6 ± 1.17
Brain	10.7 ± 0.48	12.2 ± 0.74	*12.6 ± 0.35
Kidney	11.2 ± 0.30	**13.1 ± 0.44	**13.3 ± 0.26
Liver	40.5 ± 0.95	40.9 ± 1.15	**45.2 ± 1.25

(a) Mean ± standard error in milligrams of organ per gram body weight for groups of 10 animals; P values vs. the vehicle controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
*P<0.05
**P<0.01

TABLE 28. NUMBERS OF MICE WITH SELECTED LESIONS IN THE FIFTEEN-MONTH GAVAGE STUDIES OF HYDROQUINONE (a)

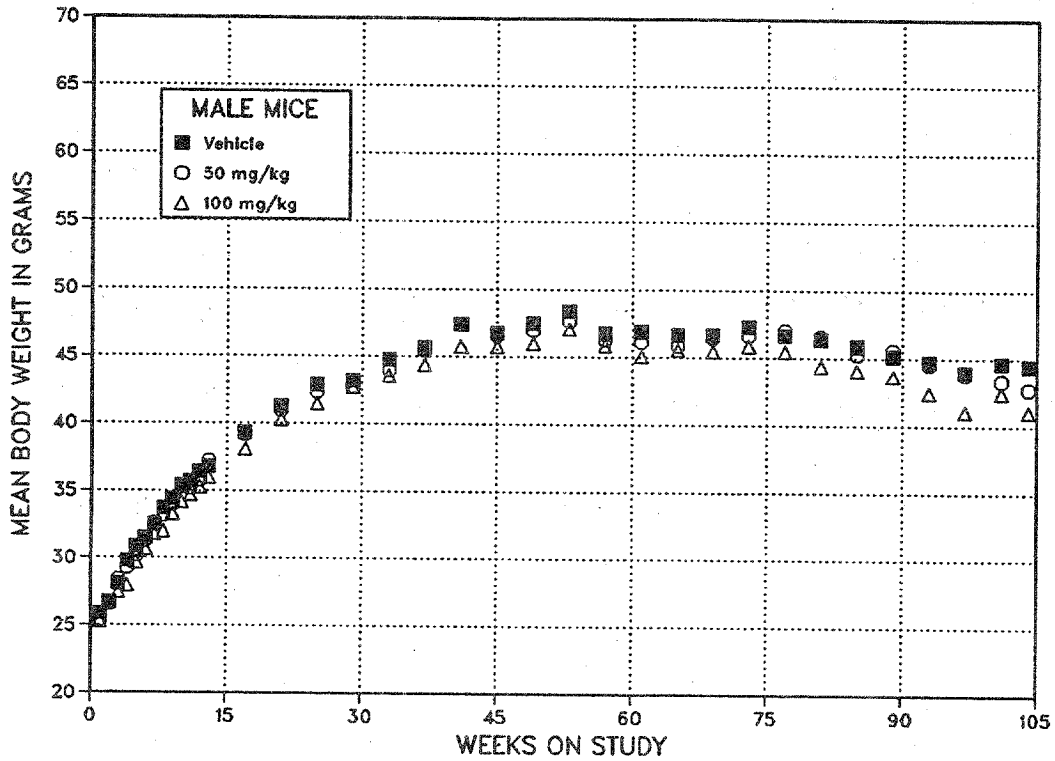
Site/Lesion	Male			Female		
	0 mg/kg	50 mg/kg	100 mg/kg	0 mg/kg	50 mg/kg	100 mg/kg
Liver						
Diffuse centrilobular fatty change	1	0	7	0	0	0
Diffuse fatty change	0	0	0	1	3	0
Diffuse cytomegaly	0	8	10	0	0	0
Syncytial cells	1	1	4	0	0	0
Basophilic focus	0	0	1	0	0	0
Clear cell focus	0	0	0	0	0	1
Hepatocellular adenoma	1	1	4	0	1	0
Hepatocellular carcinoma	2	1	1	0	0	0
Hepatocellular adenoma or carcinoma	3	2	4	0	1	0
Thyroid Gland						
Follicular cell hyperplasia	0	0	0	0	0	2

(a) Ten animals in each group were examined.

TABLE 29. MEAN BODY WEIGHTS OF MICE IN THE TWO-YEAR GAVAGE STUDIES OF HYDROQUINONE

Weeks on Study	Vehicle Control Av. Wt. (grams)	Vehicle Control Number Weighed	50 mg/kg Av. Wt. (grams)	50 mg/kg Wt. (percent of veh. controls)	50 mg/kg Number Weighed	100 mg/kg Av. Wt. (grams)	100 mg/kg Wt. (percent of veh. controls)	100 mg/kg Number Weighed
MALE								
1	25.9	65	25.4	98.1	65	25.3	97.7	65
2	26.7	65	26.6	99.6	65	26.7	100.0	65
3	28.1	65	28.3	100.7	64	27.5	97.9	65
4	29.8	65	29.3	98.3	64	28.0	94.0	65
5	30.9	65	30.2	97.7	64	29.7	96.1	65
6	31.5	65	31.3	99.4	64	30.7	97.5	65
7	32.5	65	32.6	100.3	64	31.8	97.8	65
8	33.7	65	33.2	98.5	64	32.0	95.0	65
9	34.4	65	34.0	98.8	64	33.3	96.8	65
10	35.4	65	35.3	99.7	64	34.2	96.6	65
11	35.7	65	35.2	98.6	64	34.7	97.2	65
12	36.4	65	35.8	98.4	64	35.3	97.0	65
13	36.8	65	37.2	101.1	64	36.0	97.8	65
17	39.3	65	39.2	99.7	64	38.1	96.9	65
21	41.3	65	41.0	99.3	64	40.3	97.6	65
25	42.9	65	42.4	98.8	64	41.5	96.7	65
29	43.2	65	42.8	99.1	64	42.7	98.8	65
33	44.8	65	44.0	98.2	63	43.6	97.3	65
37	45.7	65	45.5	99.6	63	44.4	97.2	65
41	47.4	65	47.4	100.0	63	45.8	96.6	65
45	46.8	65	46.6	99.6	63	45.8	97.9	65
49	47.5	64	47.0	98.9	63	46.0	96.8	64
53	48.4	63	47.7	98.6	63	47.1	97.3	64
57	46.8	63	46.3	98.9	62	45.9	98.1	64
61	46.9	61	46.2	98.5	61	45.1	96.2	64
65	46.7	61	45.9	98.3	60	45.6	97.6	64
69	46.7	(a) 51	46.5	99.6	(a) 49	45.5	97.4	(a) 54
73	47.3	51	46.7	98.7	49	45.9	97.0	53
77	46.7	51	47.0	100.6	49	45.5	97.4	52
81	46.4	49	46.6	100.4	48	44.4	95.7	52
85	45.9	49	45.3	98.7	48	44.1	96.1	50
89	45.2	47	45.6	100.9	47	43.7	96.7	50
93	44.8	45	44.6	99.6	47	42.5	94.9	46
97	44.0	42	43.9	99.8	41	41.1	93.4	41
101	44.8	(b) 33	43.6	97.3	37	42.5	94.9	36
104	44.5	33	42.8	96.2	37	41.1	92.4	36
FEMALE								
1	21.5	65	22.1	102.8	65	21.7	100.9	65
2	22.9	65	22.4	97.8	65	22.3	97.4	(b) 60
3	23.4	65	23.7	101.3	65	23.3	99.6	61
4	23.8	65	24.4	102.5	65	23.9	100.4	61
6	25.0	65	25.4	101.6	65	24.9	99.6	61
7	26.4	65	26.2	99.2	65	25.8	97.7	61
8	27.2	65	27.0	99.3	(b) 64	26.4	97.1	61
9	26.8	65	27.2	101.5	65	26.8	100.0	61
10	27.6	65	27.2	98.6	65	26.8	97.1	61
11	27.9	65	28.1	100.7	65	27.2	97.5	61
12	28.4	65	28.8	101.4	65	27.6	97.2	61
16	30.6	65	30.4	99.3	65	29.5	96.4	61
20	33.0	65	31.9	96.7	65	31.0	93.9	60
25	33.4	65	33.4	100.0	65	31.8	95.2	60
28	35.0	65	34.6	98.9	65	32.9	94.0	60
32	36.3	65	34.9	96.1	(b) 64	33.8	93.1	60
36	37.7	65	37.5	99.5	65	35.7	94.7	60
40	40.4	65	40.0	99.0	65	38.0	94.1	60
44	41.8	64	40.9	97.8	65	38.6	92.3	60
48	43.9	64	43.2	98.4	65	39.4	89.7	60
53	46.6	64	44.4	95.3	65	41.3	88.6	60
56	45.6	(b) 62	45.1	98.9	65	40.5	88.8	59
60	45.7	62	44.3	96.9	64	40.8	89.3	59
64	46.3	61	45.0	97.2	63	41.1	88.8	59
69	46.6	(a) 50	46.1	98.9	53	41.7	89.5	49
72	47.5	(b) 48	46.4	97.7	53	42.2	88.8	49
77	49.0	49	48.4	98.8	(a) 53	43.8	89.4	(a) 49
80	49.8	47	49.7	99.8	52	43.4	87.1	48
84	50.7	46	50.1	98.8	51	44.8	88.4	47
88	51.3	45	51.0	99.4	49	44.5	86.7	47
92	52.0	43	52.6	101.2	47	46.3	89.0	44
96	51.4	41	49.2	95.7	44	44.3	86.2	41
100	53.4	37	51.5	96.4	42	45.9	86.0	39
103	52.6	37	51.1	97.1	40	45.8	87.1	37

(a) Interim kill occurred.
(b) The number of animals weighed was lower than the aumber of animals surviving.



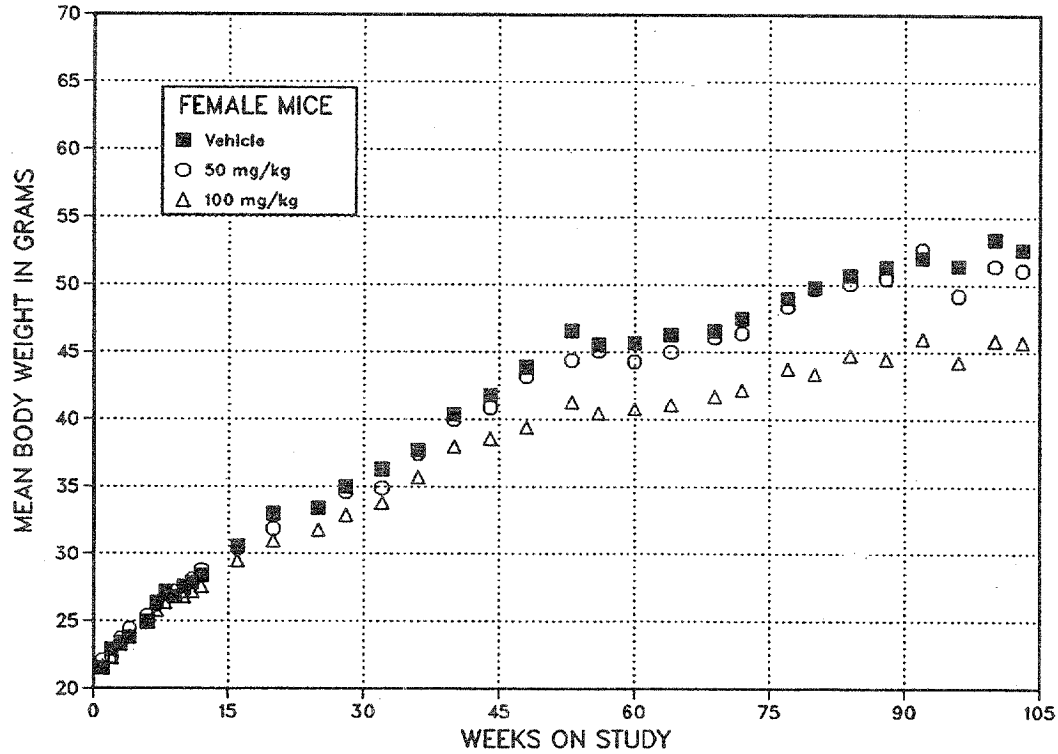


FIGURE 10. GROWTH CURVES FOR MICE ADMINISTERED HYDROQUINONE IN WATER BY GAVAGE FOR TWO YEARS

TABLE 30. RELATIVE ORGAN WEIGHTS FOR MICE IN THE TWO-YEAR GAVAGE STUDIES OF HYDROQUINONE (a)

Organ	Vehicle Control	50 mg/kg	100 mg/kg
MALE			
Number weighed	33	36	36
Body weight (grams)	44.0 ± 0.76	43.0 ± 0.72	42.0 ± 0.95
Brain	11.7 ± 0.21	11.9 ± 0.22	12.1 ± 0.28
Kidney	11.8 ± 0.38	(b) 11.7 ± 0.23	12.4 ± 0.32
Liver	67.2 ± 4.80	*(b) 76.4 ± 4.82	*70.0 ± 3.17
FEMALE			
Number weighed	37	39	36
Body weight (grams)	50.7 ± 1.65	51.5 ± 1.46	47.8 ± 1.24
Brain	10.7 ± 0.40	10.5 ± 0.38	11.0 ± 0.29
Kidney	(c) 7.5 ± 0.44	7.4 ± 0.31	7.3 ± 0.22
Liver	(c) 52.0 ± 3.32	52.0 ± 2.65	*55.1 ± 2.68

(a) Mean ± standard error in milligrams of organ per gram body weight; P values vs. the vehicle controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
(b) Thirty-five were weighed.
(c) Thirty-six were weighed.
*$P<0.05$

Survival

Estimates of the probabilities of survival for male and female mice administered hydroquinone at the doses used in these studies and for vehicle controls are shown in Table 31 and in the Kaplan and Meier curves in Figure 11. No significant differences in survival were observed between any groups of either sex.

Pathology and Statistical Analyses of Results

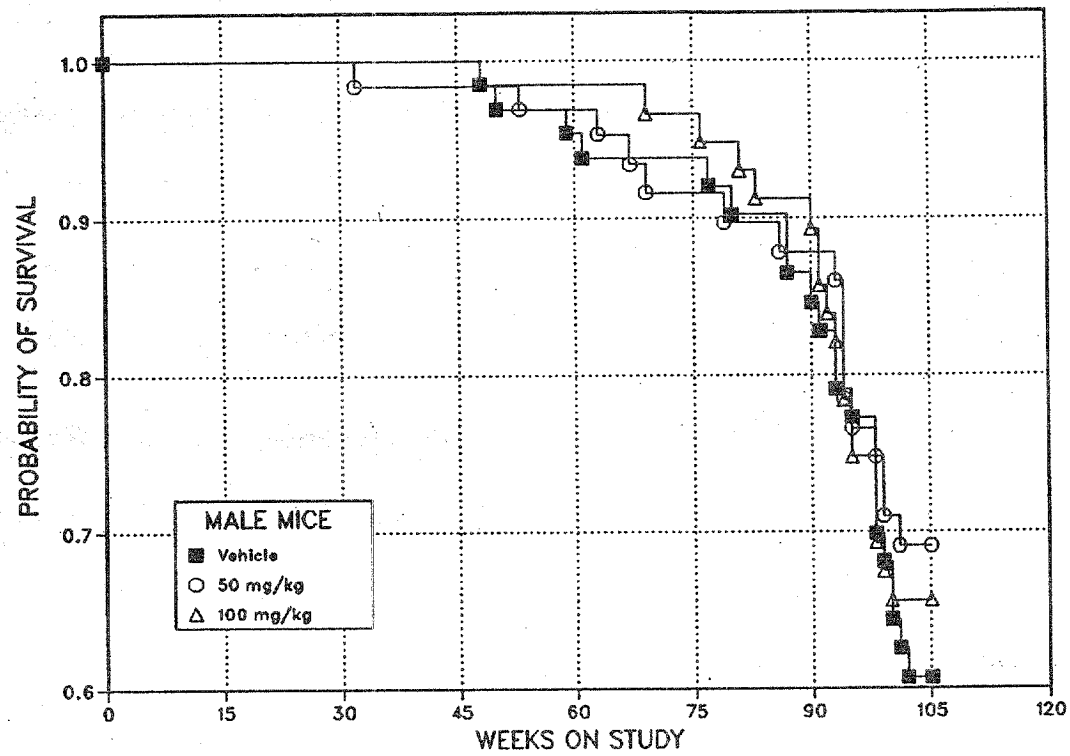
This section describes the statistically significant or biologically noteworthy changes in the incidences of mice with neoplastic or nonneoplastic lesions of the liver and thyroid gland.

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary tumors that occurred with an incidence of at least 5% in at least one animal group, and historical control incidences for the neoplasms mentioned in this section are presented in Appendixes C and D for male and female mice, respectively.

TABLE 31. SURVIVAL OF MICE IN THE TWO-YEAR GAVAGE STUDIES OF HYDROQUINONE

	Vehicle Control	50 mg/kg	100 mg/kg
MALE (a)			
Animals initially in study	55	55	55
Natural deaths	10	7	14
Moribund kills	12	10	5
Animals surviving until study termination	33	37	36
Animals missexed	0	1	0
Survival P values (b)	0.649	0.494	0.719
FEMALE (a)			
Animals initially in study	55	55	55
Natural deaths	11	5	9
Moribund kills	7	11	6
Animals surviving until study termination	37	39	36
Accidentally killed	0	0	4
Survival P values (b)	0.638	0.690	0.714

(a) First day of termination period: male--729; female--735
(b) The result of the life table trend test is in the vehicle control column, and those of the life table pairwise comparisons with the vehicle controls are in the dosed columns.



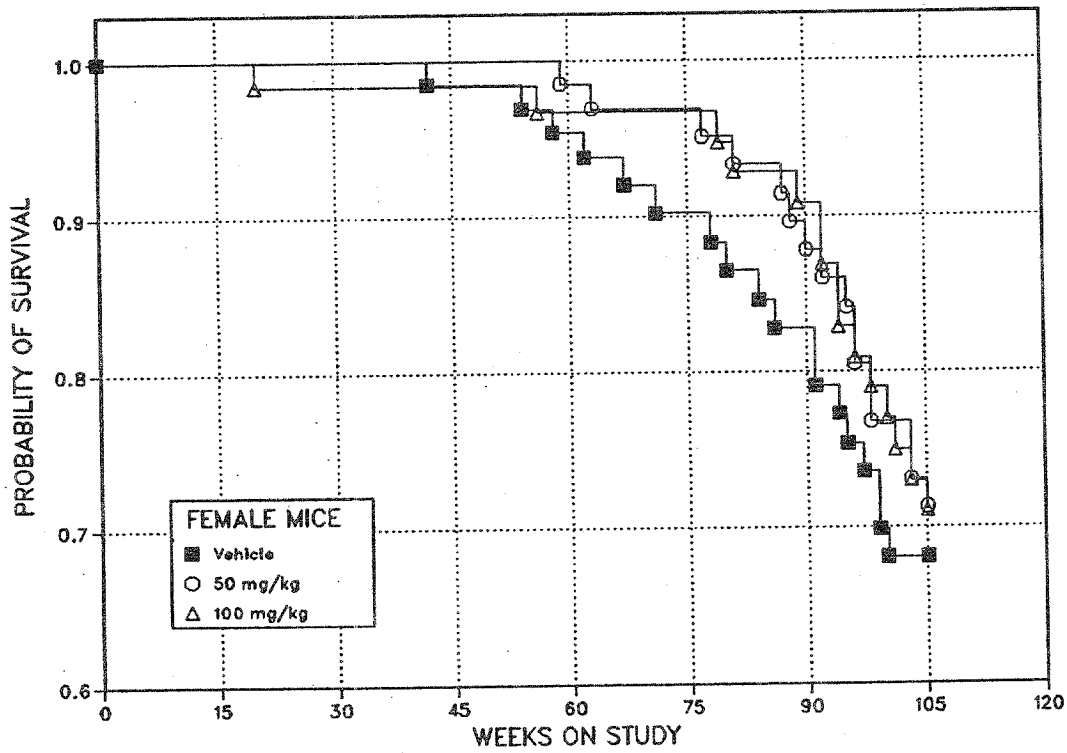


FIGURE 11. KAPLAN-MEIER SURVIVAL CURVES FOR MICE ADMINISTERED HYDROQUINONE IN WATER BY GAVAGE FOR TWO YEARS

Liver: Nonneoplastic lesions attributable to the administration of hydroquinone occurred in dosed male mice (Table 32). These included anisokaryosis, syncytial alteration, and basophilic focus. Anisokaryosis is a variation in the size of hepatocyte nuclei. Mice normally have a slight variation in nuclear size, but dosed mice were considered to show excessive variation. Syncytial alteration consisted of hepatocytes with more than five nuclei per cell. This lesion, occasionally seen in vehicle control animals, occurred more frequently in high dose male mice and appeared to contain more nuclei. Basophilic foci consisted of well-defined areas of hepatocytes with altered staining qualities of the cytoplasm (increased basophilia), hypertrophy of the affected cells, and slight distortion in the arrangement of the hepatic plates.

Foci of cellular alteration, such as the basophilic focus and hepatocellular adenoma, form a morphologic continuum. The adenomas are larger than foci (e.g., involve several or more hepatocellular lobules) and exhibit loss of lobular architecture, with alteration in growth pattern of the hepatic plates, and greater cellular atypia. The incidences of hepatocellular adenomas were increased in dosed male mice, but these increases were offset by decreases in hepatocellular carcinomas. The incidences of adenomas or carcinomas (combined) in dosed male mice were not significantly different from that in vehicle controls.

The incidences of hepatocellular adenomas were significantly increased in dosed female mice (Table 32). Hepatocellular carcinomas also occurred in one vehicle control, two low dose, and two high dose female mice.

Thyroid Gland: Follicular cell hyperplasia was observed at increased incidences in dosed mice of each sex (Table 33). Follicular cell adenomas were seen in 2/55 vehicle control, 1/53 low dose, and 2/54 high dose male mice. Follicular cell adenomas were seen in 3/55 vehicle control, 5/55 low dose, and 6/55 high dose female mice; a follicular cell carcinoma was seen in a seventh high dose female mouse. The highest observed historical incidence of thyroid gland follicular cell adenomas or carcinomas (combined) in female water gavage vehicle control $B6C3F_1$ mice is 3/48 (6%).

Thyroid follicular cell hyperplasia varied in extent and severity among animals. In some mice, the lesion was focal and involved one or more adjacent follicles, whereas in others, multiple, sometimes coalescing, foci involved much of the gland (Figure 12). The affected follicles had cuboidal to columnar epithelial cells with papillary infoldings into the lumen. The cells were hypertrophied with basophilic and occasionally vacuolated cytoplasm, and the nuclei were enlarged and contained abundant heterochromatin.

The follicular cell adenomas were discrete masses displacing normal or hyperplastic follicles. The neoplastic epithelium was arranged in poorly defined, irregular tubular, follicular, or papillary structures (Figure 13). The cells were enlarged with abundant basophilic cytoplasm and round hyperchromatic nuclei. Slight cellular pleomorphism and atypia were seen. The follicular cell carcinoma was distinguished from the adenomas primarily on the basis of cytologic features, including cellular anaplasia and atypia (Figures 14 and 15).

TABLE 32. HEPATOCELLULAR LESIONS IN MICE IN THE TWO-YEAR GAVAGE STUDIES OF HYDROQUINONE (a)

	Vehicle Control	50 mg/kg	100 mg/kg
MALE			
Anisokaryosis			
Overall Rates	0/55 (0%)	2/54 (4%)	12/55 (22%)
Syncytial Alteration			
Overall Rates	5/55 (9%)	3/54 (6%)	25/55 (45%)
Basophilic Focus			
Overall Rates	2/55 (4%)	5/54 (9%)	11/55 (20%)
Adenoma			
Overall Rates	9/55 (16%)	21/54 (39%)	20/55 (36%)
Terminal Rates	7/33 (21%)	16/37 (43%)	17/36 (47%)
Day of First Observation	694	441	661
Logistic Regression Tests	P=0.018	P=0.008	P=0.015
Carcinoma			
Overall Rates	13/55 (24%)	11/54 (20%)	7/55 (13%)
Adenoma or Carcinoma (b)			
Overall Rates	20/55 (36%)	29/54 (54%)	25/55 (45%)
Terminal Rates	11/33 (33%)	21/37 (57%)	19/36 (53%)
Day of First Observation	537	441	526
Logistic Regression Tests	P=0.223	P=0.053	P=0.250
FEMALE			
Basophilic Focus			
Overall Rates	2/55 (4%)	6/55 (11%)	3/55 (5%)
Adenoma			
Overall Rates	2/55 (4%)	15/55 (27%)	12/55 (22%)
Terminal Rates	2/37 (5%)	13/39 (33%)	9/36 (25%)
Day of First Observation	735	534	656
Logistic Regression Tests	P=0.007	P=0.001	P=0.005
Carcinoma			
Overall Rates	1/55 (2%)	2/55 (4%)	2/55 (4%)
Adenoma or Carcinoma (c)			
Overall Rates	3/55 (5%)	16/55 (29%)	13/55 (24%)
Terminal Rates	3/37 (8%)	13/39 (33%)	10/36 (28%)
Day of First Observation	735	534	656
Logistic Regression Tests	P=0.009	P=0.002	P=0.007

(a) The statistical analyses used are discussed in Section II (Statistical Methods) and Table C3 (footnotes).
(b) Historical incidence in water gavage vehicle controls (mean ± SD): 106/347 (31% ± 6%); historical incidence in untreated controls: 609/2,032 (30% ± 8%)
(c) Historical incidence in water gavage vehicle controls (mean ± SD): 29/348 (8% ± 5%); historical incidence in untreated controls: 184/2,032 (9% ± 5%)

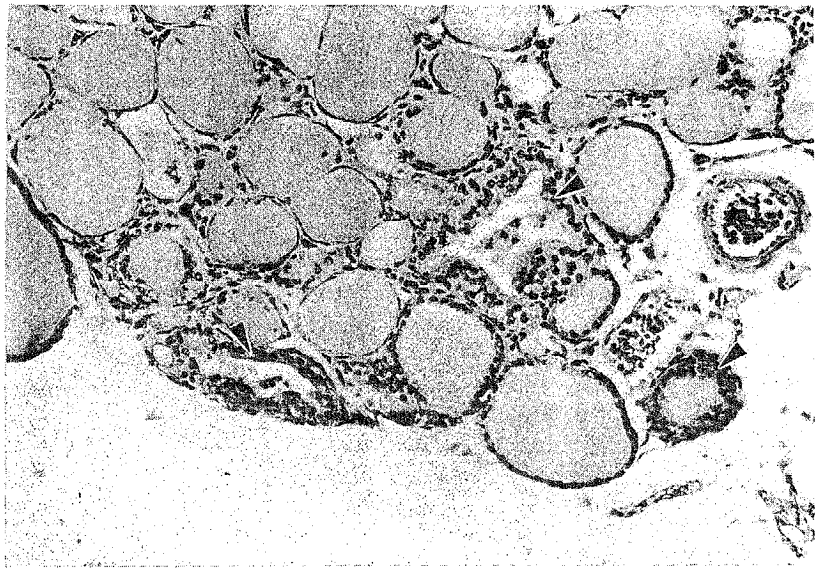


Figure 12. Follicular cell hyperplasia in thyroid of low dose female mouse CID #615 (arrows). The hyperplastic follicles are lined by cuboidal or columnar cells. Note the flattened follicular cells lining the normal follicles.

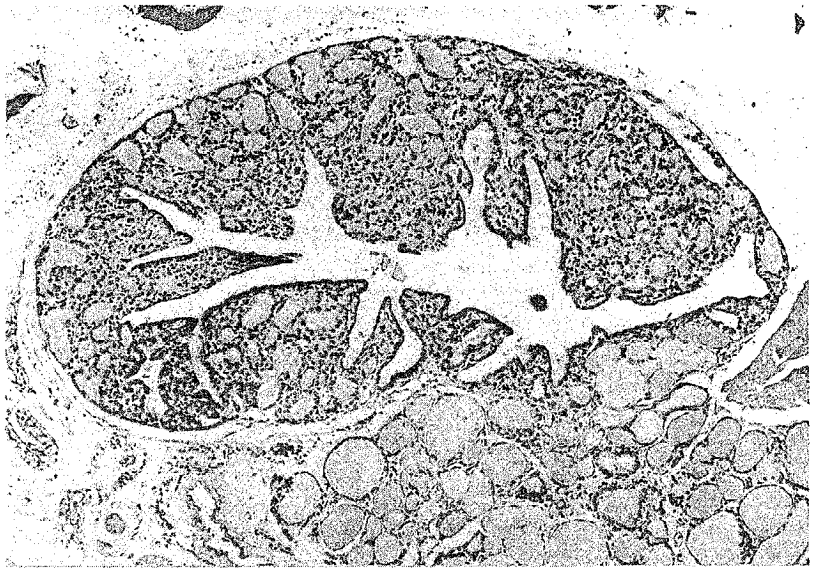


Figure 13. Thyroid follicular cell adenoma in low dose female mouse CID #615. The adenoma is a well-delineated mass composed of neoplastic follicular epithelium arranged in papillary and microfollicular patterns.

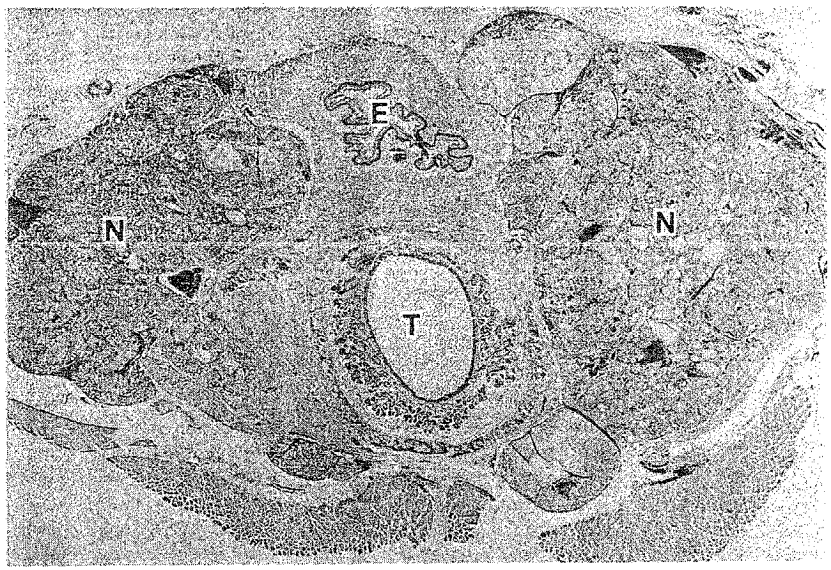


Figure 14. Thyroid follicular cell carcinoma in high dose female mouse CID #671 with trachea (T) and esophagus (E). Note the neoplasm (N) that has obliterated all normal follicular architecture.

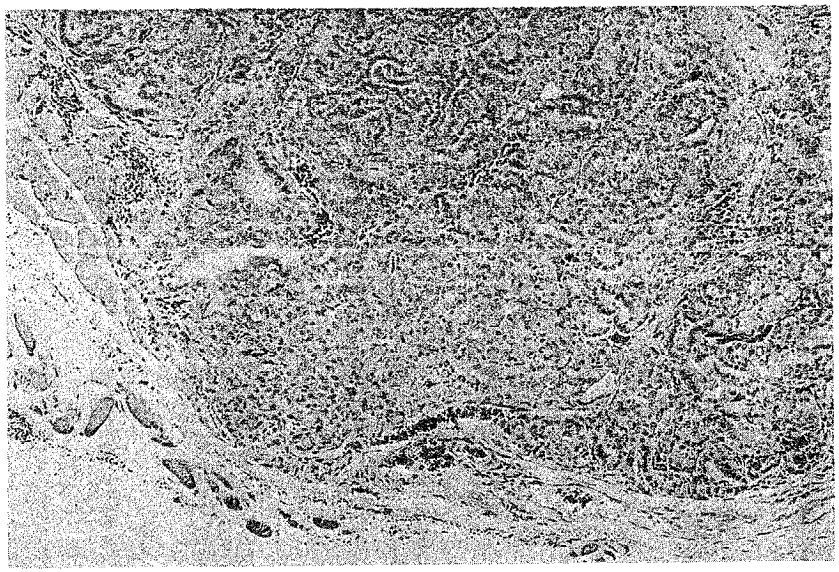


Figure 15. Higher magnification of follicular cell carcinoma in Figure 14. Note the papillary and tubular arrangement of the neoplastic epithelial cells.

TABLE 33. THYROID FOLLICULAR CELL LESIONS IN MICE IN THE TWO-YEAR GAVAGE STUDIES OF HYDROQUINONE

	Vehicle Control	50 mg/kg	100 mg/kg
MALE			
Hyperplasia			
Overall Rates	5/55 (9%)	15/53 (28%)	19/54 (35%)
Adenoma			
Overall Rates	2/55 (4%)	1/53 (2%)	2/54 (4%)
Carcinoma			
Overall Rates	0/55 (0%)	0/53 (0%)	0/54 (0%)
Adenoma or Carcinoma			
Overall Rates	2/55 (4%)	1/53 (2%)	2/54 (4%)
FEMALE			
Hyperplasia			
Overall Rates	13/55 (24%)	47/55 (85%)	45/55 (82%)
Adenoma			
Overall Rates	3/55 (5%)	5/55 (9%)	6/55 (11%)
Terminal Rates	2/37 (5%)	4/39 (10%)	4/36 (11%)
Day of First Observation	664	668	548
Logistic Regression Tests	P=0.186	P=0.397	P=0.233
Carcinoma			
Overall Rates	0/55 (0%)	0/55 (0%)	1/55 (2%)
Adenoma or Carcinoma (a)			
Overall Rates	3/55 (5%)	5/55 (9%)	7/55 (13%)
Terminal Rates	2/37 (5%)	4/39 (10%)	5/36 (14%)
Day of First Observation	664	668	548
Logistic Regression Tests	P=0.115	P=0.397	P=0.152

(a) Historical incidence in water gavage vehicle controls (mean ± SD): 10/337 (3% ± 2%); historical incidence in untreated controls: 49/1,937 (3% ± 3%)

Hydroquinone was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with a preincubation protocol at doses up to 666 µg/plate in the presence or absence of Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 (Haworth et al., 1983; Table 34). In the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y/TK cells, hydroquinone was positive at doses of 1.25 µg/ml and higher in the absence of S9 and at 2.5 µg/ml and higher in the presence of Aroclor 1254-induced male F344 rat liver S9 (McGregor et al., 1988; Table 35). In tests for cytogenetic effects in cultured Chinese hamster ovary (CHO) cells, hydroquinone induced sister chromatid exchanges (SCEs) with and without Aroclor 1254-induced male Sprague Dawley rat liver S9; doses that elicited a positive response without any indication of cell cycle delay ranged from 0.50 to 5.0 µg/ml in the absence of S9 and from 50 to 800 µg/ml in the presence of S9 (Galloway et al., 1987; Table 36). Although SCE induction by hydroquinone was stronger in the absence of S9, in the chromosomal aberration test with CHO cells, hydroquinone was positive only in the presence of S9 at doses of 450 and 600 µg/ml; without S9, an increase in aberrations was observed at the highest dose tested (20 µg/ml), but this was not statistically significant (Galloway et al., 1987; Table 37). Hydroquinone, dissolved in saline and administered by feeding at 26,400 and 30,000 ppm, produced an equivocal increase in sex-linked recessive lethal mutations in male Drosophila; administration of hydroquinone by injection produced no increase (above control levels) in the number of sex-linked recessive lethal mutations (Table 38).

TABLE 34. MUTAGENICITY OF HYDROQUINONE IN *SALMONELLA TYPHIMURIUM* (a)

Strain	Dose (µg/plate)	Revertants/Plate (b) −S9 Trial 1	−S9 Trial 2	+S9 (hamster) Trial 1	+S9 (hamster) Trial 2	+S9 (rat) Trial 1	+S9 (rat) Trial 2
TA100	0	100 ± 3.0	95 ± 6.6	116 ± 11.2	90 ± 0.0	124 ± 16.0	121 ± 9.7
	10	97 ± 1.5	91 ± 0.3	105 ± 8.2	122 ± 16.7	117 ± 5.7	122 ± 4.0
	33	107 ± 6.7	115 ± 5.1	114 ± 6.3	108 ± 15.7	102 ± 9.2	127 ± 7.8
	100	108 ± 3.9	119 ± 5.9	122 ± 4.3	107 ± 10.1	121 ± 8.0	92 ± 2.6
	333	Toxic	Toxic	109 ± 7.0	112 ± 13.7	103 ± 8.0	80 ± 5.5
	666	Toxic	Toxic	118 ± 11.0	123 ± 16.0	116 ± 4.8	115 ± 13.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		1,447 ± 9.7	1,877 ± 37.6	1,499 ± 64.7	1,394 ± 40.8	1,048 ± 95.9	1,093 ± 48.8
TA1535	0	18 ± 3.2	15 ± 1.2	8 ± 3.5	7 ± 0.3	14 ± 4.3	7 ± 2.0
	10	21 ± 1.0	15 ± 2.7	12 ± 4.6	9 ± 1.8	13 ± 4.2	11 ± 2.5
	33	15 ± 2.3	9 ± 1.2	10 ± 1.5	8 ± 0.9	6 ± 1.5	11 ± 1.3
	100	13 ± 1.2	8 ± 1.2	9 ± 0.9	11 ± 2.3	9 ± 2.7	7 ± 0.6
	333	(d) 11 ± 1.7	Toxic	10 ± 2.3	11 ± 0.3	8 ± 0.9	8 ± 1.3
	666	Toxic	Toxic	10 ± 2.2	8 ± 2.1	10 ± 1.2	9 ± 1.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		1,110 ± 45.4	1,185 ± 20.7	107 ± 9.5	120 ± 10.9	76 ± 6.8	60 ± 1.7
TA1537	0	9 ± 0.3	8 ± 0.3	11 ± 0.9	8 ± 2.2	9 ± 1.2	7 ± 1.5
	10	9 ± 0.0	7 ± 0.7	6 ± 1.2	7 ± 2.0	6 ± 0.9	6 ± 2.0
	33	9 ± 1.8	6 ± 0.9	11 ± 1.7	8 ± 0.7	9 ± 2.5	7 ± 1.2
	100	(d) 6 ± 1.2	(d) 5 ± 1.8	10 ± 2.3	10 ± 2.7	7 ± 2.1	7 ± 1.2
	333	Toxic	Toxic	8 ± 1.7	6 ± 1.5	8 ± 1.2	6 ± 1.2
	666	Toxic	Toxic	9 ± 1.7	8 ± 0.9	7 ± 1.0	8 ± 0.7
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		192 ± 20	461 ± 105.2	219 ± 19.2	150 ± 11.9	95 ± 0.9	129 ± 4.0
TA98	0	20 ± 1.7	20 ± 1.5	28 ± 4.0	30 ± 0.9	25 ± 2.6	23 ± 1.7
	10	18 ± 2.5	19 ± 1.7	24 ± 3.3	30 ± 1.0	25 ± 3.3	25 ± 5.5
	33	21 ± 0.9	22 ± 3.7	25 ± 1.2	29 ± 0.6	23 ± 3.5	26 ± 3.0
	100	(d) 20 ± 6.7	19 ± 0.5	28 ± 2.8	25 ± 0.9	22 ± 1.2	21 ± 5.9
	333	Toxic	Toxic	24 ± 3.8	26 ± 4.1	24 ± 0.3	25 ± 2.2
	666	Toxic	Toxic	22 ± 2.0	29 ± 1.2	24 ± 2.9	17 ± 2.6
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		1,549 ± 24.6	1,762 ± 8.3	2,009 ± 23.1	1,415 ± 77.7	1,194 ± 30.1	950 ± 298.9

(a) Study performed at EG&G Mason Research Institute. The detailed protocol is presented by 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) 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 TA1537.
(d) Slight toxicity

TABLE 35. INDUCTION OF TRIFLUOROTHYMIDINE RESISTANCE BY HYDROQUINONE IN MOUSE L5178Y LYMPHOMA CELLS (a,b)

Compound	Concentration (µg/ml)	Cloning Efficiency (percent)	Relative Total Growth (percent)	Tft-Resistant Cells	Mutant Fraction (c)
−S9					
Trial 1					
Methanol (d)		78.8 ± 6.9	100.0 ± 2.2	50.8 ± 3.5	22.3 ± 2.3
Hydroquinone	3.125	35.5 ±22.5	17.5 ±12.5	349.0 ±123.0	(e) 419.5 ±145.5
	6.25	42.5 ± 5.5	11.0 ± 2.0	688.0 ± 90.0	(e) 541.5 ± 0.5
	12.5	26.0 ± 1.0	7.0 ± 0.0	660.5 ± 36.5	(e) 857.0 ± 14.0
	25	29.0 ± 1.0	6.5 ± 0.5	663.5 ± 52.5	(e) 773.0 ± 87.0
	50	Lethal	--	--	--
Ethyl methanesulfonate (f)	250	120	114	390	108
Trial 2					
Dimethyl sulfoxide (d)		74.3 ±10.2	100.0 ± 6.7	82.5 ± 15.2	38.3 ± 6.5
Hydroquinone	0.625	91.5 ±10.5	69.5 ± 1.5	135.5 ± 20.5	49.0 ± 2.0
	1.25	32.0 ± 4.0	19.0 ± 3.0	168.0 ± 7.0	(e) 179.5 ± 15.5
	2.5	13.5 ± 1.5	5.5 ± 1.5	250.5 ± 19.5	(e) 624.5 ± 17.5
	(g) 5	13	1	718	1,915
	10	Lethal	--	--	--
Ethyl methanesulfonate	250	81.5 ±11.5	62.0 ± 4.0	748.0 ±125.0	(e) 306.0 ± 7.0
+S9 (h)					
Dimethyl sulfoxide (d)		77.0 ± 5.2	99.8 ± 0.9	161.3 ± 10.6	69.8 ± 2.6
Hydroquinone	0.625	92.0 ± 2.0	124.5 ± 8.5	198.5 ± 11.5	72.5 ± 5.5
	1.25	77.0 ± 4.0	102.0 ± 3.0	178.5 ± 9.5	77.5 ± 8.5
	2.5	86.5 ±11.5	87.0 ± 6.0	345.0 ± 35.0	(e) 134.0 ± 4.0
	5	65.5 ± 4.5	19.0 ± 1.0	644.5 ± 25.5	(e) 328.0 ± 10.0
	10	43.5 ± 0.5	7.0 ± 0.0	606.0 ± 24.0	(e) 464.0 ± 21.0
Methylcholanthrene	2.5	50.0 ± 5.0	52.0 ± 5.0	673.0 ± 20.0	(e) 451.5 ± 28.5

(a) Study performed at Inveresk Research International. The experimental protocol is presented in detail by McGregor et al. (1988) and follows the basic format of Clive et al. (1979). The highest dose of study compound is determined by solubility or toxicity and may not exceed 5 mg/ml. All doses are tested in duplicate except as noted; the average for the two tests is presented in the table. Cells (6×10^5/ml) were treated for 4 hours at 37° C in medium, washed, resuspended in medium, and incubated for 48 hours at 37° C. After expression, 3×10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine (Tft) for selection of Tft-resistant cells, and 600 cells were plated in nonselective medium and soft agar to determine the cloning efficiency.
(b) Mean ± standard error from replicate trials of approximately 1×10^6 cells each. All data are evaluated statistically for both trend and peak response ($P<0.05$ for at least one of the three highest dose sets). Both responses must be significantly ($P<0.05$) positive for a chemical to be considered capable of inducing Tft resistance. If only one of these responses is significant, the call is "equivocal"; the absence of both trend and peak response results in a "negative" call.
(c) Mutant fraction (frequency) is a ratio of the Tft-resistant cells to the cloning efficiency, divided by 3 (to arrive at MF per 1×10^6 cells treated); MF = mutant fraction.
(d) Data presented are for four tests.
(e) Significant positive response; occurs when the relative mutant fraction (average MF of treated culture/average MF of solvent control) is greater than or equal to 1.6.
(f) Data presented are for one test.
(g) Data presented are for one test; the dose in one test was lethal.
(h) Tests conducted with metabolic activation were performed as described in (a) except that S9, prepared from the liver of Aroclor 1254-induced F344 rats, was added at the same time as the study chemical and/or solvent.

TABLE 36. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY HYDROQUINONE (a)

Compound	Dose (µg/ml)	No. of Total Cells	Chromosomes	SCEs/ No. of SCEs	Chromosome	SCEs/ Cell	Relative Hours in BrdU	SCEs/Cell (percent) (b)
−S9 (c)--Summary: Positive								
Dimethyl sulfoxide		50	1,019	374	0.37	7.5	25.5	
Hydroquinone	0.5	50	1,022	545	0.53	10.9	25.5	145.3
	1.67	50	1,025	866	0.84	17.3	25.5	230.7
	5	50	1,024	1,013	0.99	20.3	25.5	270.7
Mitomycin C	0.005	25	515	817	1.59	32.7	25.5	436.0
+S9 (d)								
Trial 1--Summary: Positive								
Dimethyl sulfoxide		50	1,045	461	0.44	9.2	25.8	
Hydroquinone	50	50	1,036	559	0.54	11.2	25.8	121.7
	167	50	1,040	593	0.57	11.9	25.8	129.3
	500	50	1,026	671	0.65	13.4	25.8	145.7
Cyclophosphamide	1.5	25	524	1,032	1.97	41.3	25.8	448.9
Trial 2--Summary: Positive								
Dimethyl sulfoxide		50	1,041	461	0.44	9.2	25.5	
Hydroquinone	600	50	1,043	765	0.73	15.3	25.5	166.3
	700	50	1,018	899	0.88	18.0	25.5	195.7
	800	50	1,044	876	0.84	17.5	25.5	190.2
Cyclophosphamide	1.5	25	530	634	1.20	25.4	25.5	276.1

(a) Study performed at Litton Bionetics, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway et al. (1985, 1987), and the data are included in Galloway et al. (1987). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethyl sulfoxide) as described in (c) and (d) below and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air dried, and stained.
(b) SCEs/cell of culture exposed to study chemical relative to those of culture exposed to solvent
(c) In the absence of S9, Chinese hamster ovary cells were incubated with study compound or solvent for 2 hours at 37° C. Then BrdU was added, and incubation was continued for 24 hours. Cells were washed, fresh medium containing BrdU and colcemid was added, and incubation was continued for 2-3 hours.
(d) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 26 hours, with colcemid present for the final 2-3 hours. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.

TABLE 37. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY HYDROQUINONE (a)

−S9 (b)					+S9 (c)				
Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Harvest time: 10.5 hours					Harvest time: 10.5 hours				
Dimethyl sulfoxide					Dimethyl sulfoxide				
	100	3	0.03	3.0		100	1	0.01	1.0
	100	3	0.03	3.0					
Hydroquinone									
5	100	2	0.02	2.0	150	100	5	0.05	4.0
7.5	100	2	0.02	2.0	450	100	22	0.22	17.0
10	100	4	0.04	4.0	600	100	29	0.29	19.0
20	50	5	0.10	8.0					
Summary: Negative					Summary: Positive				
Mitomycin C					Cyclophosphamide				
1	50	10	0.20	20.0	25	50	10	0.20	18.0

(a) Study performed at Litton Bionetics, Inc.; Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway et al. (1985, 1987), and the data are included in Galloway et al. (1987). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethyl sulfoxide) as indicated in (b) and (c). Cells were arrested in first metaphase by addition of colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.
(b) In the absence of S9, cells were incubated with study compound or solvent for 8-10 hours at 37° C. Cells were then washed, and fresh medium containing colcemid was added for an additional 2-3 hours followed by harvest.
(c) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8-10 hours. Colcemid was added for the last 2-3 hours of incubation before harvest. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.

TABLE 38. INDUCTION OF SEX-LINKED RECESSIVE LETHAL MUTATIONS IN DROSOPHILA BY HYDROQUINONE (a)

Route of Exposure	Dose (ppm)	Induced Incidence of Deaths (percent)	Induced Incidence of Sterility (percent)	No. of Lethals/No. of X Chromosomes Tested			Overall Total (b)
				Mating 1	Mating 2	Mating 3	
Injection	1,500	3	0	3/2,211	1/1,782	2/1,504	6/5,497 (0.11%)
	0			3/2,211	0/1,892	4/1,087	7/5,190 (0.13%)
Feeding	26,400	25	43	0/1,111	1/874	2/742	3/2,727 (0.11%)
	0			2/1,502	0/1,466	0/1,212	2/4,180 (0.05%)
Feeding	30,000	4	16	1/1,665	3/783	1/955	5/3,403 (0.15%)
	0			0/785	0/625	0/748	0/2,158 (0.00%)

(a) Study performed at The University of Wisconsin--Madison. A detailed protocol of the sex-linked recessive lethal assay is presented in Zimmering et al. (1985). Exposure by feeding was done by allowing 24-hour-old Canton-S males to feed for 3 days on a solution of the study chemical dissolved in 5% sucrose. In the injection experiments, 24-hour-old Canton-S males were treated with a solution of the chemical dissolved in 0.7% saline and allowed 24 hours to recover. Exposed males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three broods of 3, 2, and 2 days; sample sperm from successive matings were treated as spermatozoa (mating 1), spermatids (mating 2), and spermatocytes (mating 3). F_1 heterozygous females were crossed to their siblings and placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters; no clusters were found. After 17 days, presumptive lethal mutations were identified as vials containing no wild-type males; these were retested. Results were considered to be equivocal (Margolin et al., 1983).
(b) Combined total of number of lethal mutations/number of X chromosomes tested for three mating trials

IV. DISCUSSION AND CONCLUSIONS

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Hydroquinone has a high production volume and is either used directly as an antioxidant or processed into derivatives that are used as antioxidants. It is an effective antioxidant for nonfood industrial fats and oils. Hydroquinone is also an important commercial developing agent for photographic film. Minor uses are as a polymerization inhibitor for vinyl monomers and as an ingredient in dermatologic preparations to bleach hyperpigmented skin.

Fourteen-day, 90-day, 15-month, and 2-year studies of the toxicity and carcinogenicity of hydroquinone were conducted in F344/N rats and $B6C3F_1$ mice of each sex. For the 14-day studies, hydroquinone was administered in corn oil by gavage or in 95% ethanol by percutaneous application. All subsequent studies used the gavage route of administration.

Two routes of administration were used in the 14-day studies in rats and mice to assess the relative toxicity of hydroquinone by dermal application and gavage. Mortality and body weight increases at 14 days were used as criteria of toxicity. In rats of each sex gavaged with hydroquinone at doses ranging from 63 to 1,000 mg/kg body weight, chemical-related deaths were observed at the top two doses, but no substantial changes in weight gain occurred at lower doses. No deaths or notable differences in body weight gain of rats were observed after dermal application of hydroquinone at doses ranging from 24 to 384 mg per animal.

Similarly, with gavage administration of the study chemical to mice at doses of 31-500 mg/kg, most male mice in the top two dosed groups and all females in the top dosed group died before the end of the 14-day studies. Again, no substantial changes in weight gain occurred. When hydroquinone was applied dermally to mice at doses of up to 96 mg per animal, no deaths occurred, and no toxic symptoms were observed.

The preliminary qualitative dermal absorption studies indicated that applied doses of hydroquinone, 4 or 40 mg per animal for rats and mice, resulted in the appearance of conjugated (glucuronide and/or sulfate) metabolites as soon as 2 hours after dosing. Thus, dermal application of hydroquinone, at the doses bracketed in the toxicity studies, resulted in systemic availability of the parent compound, as evidenced by excretion of urinary metabolites. However, dermal application was accompanied by crystallization of hydroquinone on the surface of the skin, and since no toxic effects were seen, it was apparent that the dermal route was inappropriate for evaluation of the systemic toxicity of this compound. Accordingly, gavage administration was employed in further evaluations.

In the 13-week gavage studies, doses for rats ranged from 25 to 400 mg/kg body weight. All male and female rats died in the groups exposed at 400 mg/kg, and 3/10 females died at 200 mg/kg. Tremors and convulsions before death were common in the 14-day and 13-week studies with both species and confirm findings from other studies (Angel and Rogers, 1972). Hydroquinone has been shown to alter neuromuscular activity by central nervous system-mediated stimulation of presynaptic acetylcholine release (Otsuka and Monomura, 1963).

In the 13-week studies, some male rats at 200 mg/kg hydroquinone were noted to be lethargic after 10 weeks of dosing, and females at this dose exhibited tremors and convulsions. However, no remarkable clinical signs were seen in the lower dosed groups. Absolute and relative liver weights were decreased in all groups of dosed male rats and were significantly increased in the three top dosed groups of female rats. The reason for this apparent dichotomy is not known. Grossly discernible lesions were limited to the 100, 200, and 400 mg/kg groups and included perioral staining, reddened mucosa in the stomach, and intra-abdominal bleeding. Similarly, microscopically diagnosed lesions were also limited to the top three dosed groups and included inflammation and epithelial hyperplasia of the forestomach and toxic nephropathy. At those doses showing some indication of toxicity, the central nervous system, liver, kidney, and forestomach were identified as target organs. Thus, for rats of each sex, hydroquinone at doses of 50 mg/kg and below had negligible effects on body weight gain, clinical signs, and gross and histopathologic findings. Doses of 0, 25, and 50 mg/kg were therefore chosen for the 2-year evaluation of hydroquinone in rats of each sex.

The 13-week studies with mice employed five doses of hydroquinone ranging from 25 to 400 mg/kg body weight. Eight of 10 male and 8/10 female mice at 400 mg/kg and 2/10 males at 200 mg/kg died before the end of the studies (see Table 24). Final mean body weights of dosed and vehicle control mice were similar. The most common clinical sign was lethargy, which was seen in all dosed males and in the top three dosed groups of females. Tremors after dosing were seen in the top dosed group of each sex and in the 200 mg/kg group of males.

Relative liver weights for dosed male mice in the 13-week studies were higher than that for vehicle controls (see Table 25). Ulceration, inflammation, or epithelial hyperplasia of the forestomach was observed in the top two dosed groups. These studies identified the liver, central nervous system, and forestomach as target organs for hydroquinone-induced toxicity. With the exception of a moderate relative increase in liver weight for male mice, hydroquinone doses of 100 mg/kg and below resulted in no discernible indices of toxicity which would preclude long-term growth and survival. Accordingly, doses of 0, 50, and 100 mg/kg were chosen for the 2-year studies of hydroquinone in mice of each sex.

Results of the 15-month interim kill confirmed that the kidney of male rats was a target organ for the chemical-related toxicity. Although the kidney of both male and female rats was affected at the higher doses used in the 13-week studies, the lesions were less severe in females than in males at the same doses. This may explain the lack of chemical-related kidney toxicity in female rats at the 15-month observation.

Mild regenerative anemia was also observed in female rats at the 15-month kill. This was evident from the slightly decreased hematocrit, hemoglobin concentration, and erythrocyte count (see Table 13) and is consistent with the documented toxicity of hydroquinone toward bone marrow (Carlson and Brewer, 1953; Greenlee et al., 1981).

Centrilobular fatty change and cytomegaly were observed in the liver of male mice at 15 months but not in the animals killed at 2 years. This may be explained by the fact that at 15 months, the necropsy occurred within 24 hours of the last dose, whereas in the 2-year studies, hydroquinone dosing was stopped 2 weeks before necropsy. The centrilobular fatty change and cytomegaly were relatively subtle microscopic lesions that likely regressed after cessation of chemical administration.

In the 2-year studies, the survival of male and female rats exposed to hydroquinone was similar to that of vehicle controls for the first 90 weeks, after which survival was somewhat decreased (see Figure 5). The number of animals killed in a moribund condition was greater for dosed male rats after 90 weeks, suggesting chemical-induced morbidity in these groups. No statistically significant differences in the number of rats surviving to the terminal kill were observed between dosed rats and vehicle controls. Thus, although survival of dosed male rats was lower than typical, sufficient numbers of animals were at risk to permit adequate evaluation of long-term toxicity and carcinogenicity. Body weights of dosed male rats were similar to those of vehicle controls for the first 73 weeks, and body weights of dosed and vehicle control female rats were similar throughout the study. The inflammation and hyperplasia observed in the forestomach of rats and mice in the 13-week studies were not observed at 15 months or at 2 years. However, other chemical-related nonneoplastic and neoplastic lesions were observed.

Results from these 2-year studies provide substantial evidence that long-term administration of hydroquinone induced a variety of nonneoplastic and neoplastic lesions in both rats and mice. Dose-related incidences of renal tubular cell adenomas were observed in dosed male rats, whereas none was observed in the vehicle controls (see Table 18). The absence of this neoplasm in vehicle controls is consistent with historical observations for this strain of male rats: the historical incidence of tubular cell adenomas of the kidney is less than 0.5% in both untreated (9/1,928) and water vehicle gavage (1/298) control male F344/N rats. The incidences in both dosed groups exceed the highest historical incidences of renal tubular cell neoplasms observed in either untreated (3/50, 6%) or water gavage vehicle (1/50, 2%) controls (Table A4a). The

appearance of renal tubular cell hyperplasia in high dose male rats, combined with evidence of chemical-influenced nephropathy at 15 months (see Table 12) and 2 years (see Table 17), provide supportive evidence that the neoplastic lesions were chemically induced. There does not appear to be a relationship between the chemical-related nephropathy observed in the 13-week studies and the hyperplasia and tubular adenomas observed in the 2-year studies. The neoplasms occurred in male rats, and the nephropathy was seen in both male and female rats.

No hyaline droplet formation was seen in the kidney in rats in the 13-week studies, although it has been suggested that the likelihood of observing this might have been lessened by the fact that some necropsies were conducted as late as 72 hours after dosing (see Table 6). Furthermore, the kidney in these studies revealed no evidence of granular cast formation in the loop of Henle or of linear mineralization. Considered together, this information indicates that hydroquinone administration was not associated with hyaline droplet formation or with other aspects of the $\alpha_{2\mu}$-globulin nephrotoxic syndrome (Short et al., 1987).

Results from these 2-year studies provide evidence that hydroquinone increased the incidences of mononuclear cell leukemia in female rats. The incidence in the concurrent vehicle controls (16%) is somewhat lower than historical mean incidences in either untreated controls (19%) or water gavage vehicle controls (25%). However, the incidences in both dosed groups exceed the historical means observed in untreated or water gavage vehicle control groups. Furthermore, the incidence in the high dose group (40%) exceeds the control incidences for this neoplasm in all but one of 46 studies that collectively include almost 2,300 untreated or water gavage control female F344/N rats (Table B4a). No histopathologic evidence of mononuclear cell leukemia was observed in the 15-month interim-kill animals.

These studies provide appreciable evidence for the induction of nonneoplastic and neoplastic lesions in the liver of dosed mice. Of particular significance are the increased incidences of hepatocellular adenomas in dosed female mice. The vehicle control incidence of 4% is within the historical range (2%-18%) observed in approximately 40 other untreated control groups (about 2,000 female mice) and in 7 water gavage vehicle control groups (0%-12%) containing nearly 350 mice (Table D4a). The incidences observed in both dosed groups (27% and 22%) are significantly above the concurrent vehicle control incidence, and values in both dosed groups exceed the highest incidence observed in control female mice in recent NTP experience.

In contrast, hydroquinone did not influence the incidences of hepatocellular adenomas and carcinomas in male mice, although anisokaryosis, multinucleated hepatocytes, and basophilic foci (possible precursors in the development of hepatocellular neoplasia) were all increased (see Table 32).

Follicular cell hyperplasia of the thyroid gland was increased in dosed male mice and particularly in dosed female mice compared with vehicle controls. Hyperplasia was also seen in two high dose females at 15 months. Although not statistically significant, a dose-related marginal increase in the incidence of neoplasms of the thyroid gland occurred in female mice, but the incidence in the high dose group (13%) approximates the maximum observed incidence for untreated controls (15%) in the historical data base. A relationship between goitrogen-induced hyperplasia and a resultant increase in follicular cell neoplasia is well documented in rats and mice (Paynter et al., 1988). Since the higher doses employed in the 13-week studies had no apparent effect on the thyroid gland and thyroid and pituitary hormones were not assessed, conclusions regarding goitrogenic activity of hydroquinone remain speculative.

The metabolic interrelationship between hydroquinone and benzene (see Figure 1) invites comparisons between these studies of hydroquinone and those of benzene (NTP, 1986; Huff et al., 1988). Both compounds were evaluated by the gavage route and were studied in the same strains of rats and mice under similar experimental protocols. All eight sex-species studies had at least one dose (50 mg/kg) in common. Although both chemicals caused neoplasms in

both species, little similarity was seen in the species-specific topography of the chemical-induced tumorigenesis. Benzene caused lesions at multiple sites in all four sex-species studies, whereas hydroquinone induced neoplasia in male rats (one site), female rats (one site), and female mice (one site). The administration of benzene to mice was associated with increased incidences of primary neoplasms in at least nine sites, including the forestomach, ovary, liver, lung, and preputial, mammary, harderian, and Zymbal glands. Increased incidences of lymphomas were also observed. Results of these hydroquinone studies in mice are similar to those from the benzene studies only in that both chemicals increased liver neoplasms in female mice. In rats, benzene was associated with increased neoplasms in the Zymbal gland, skin, and oral cavity; none of these sites was affected by hydroquinone. This suggests that hydroquinone contributes little to the observed carcinogenicity of benzene.

Hydroquinone is generally not mutagenic in bacteria, but there is extensive evidence demonstrating its clastogenicity with mammalian cells, both in vivo and in vitro. It induces chromosomal aberrations in Chinese hamster ovary cells (Galloway et al., 1987) and micronuclei in bone marrow cells of NMRI mice (Gocke et al., 1981; Tunek et al., 1982). The mutagenic responses seen with hydroquinone parallel those observed with benzene, which is known to be metabolized to phenol and hydroquinone and which generally requires the addition of exogenous metabolic activation to produce its genotoxic effects. Quinones and semiquinones, proposed as the ultimate binding species of metabolically activated phenol (Irons and Pfeifer, 1982; Smart and Zannoni, 1984), are probably involved in the DNA and protein binding properties of hydroquinone as well.

Reaction of semiquinone radicals with oxygen liberates superoxide anion radicals (Chignell, 1985). The mutagenicity associated with the one-electron reduction of various quinones tested in *Salmonella typhimurium* TA104 was attributed to generation of such oxygen radicals (Chesis et al., 1984). Benzene and hydroquinone might produce positive responses in *S. typhimurium* TA104. The mechanism for this genotoxic response, however, would be distinctly different from that involved with covalently binding nucleophilic macromolecules.

Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity** of hydroquinone for male F344/N rats, as shown by marked increases in tubular cell adenomas of the kidney. There was *some evidence of carcinogenic activity* of hydroquinone for female F344/N rats, as shown by increases in mononuclear cell leukemia. There was *no evidence of carcinogenic activity* of hydroquinone for male $B6C3F_1$ mice administered 50 or 100 mg/kg in water by gavage. There was *some evidence of carcinogenic activity* of hydroquinone for female $B6C3F_1$ mice, as shown by increases in hepatocellular neoplasms, mainly adenomas.

Administration of hydroquinone was associated with thyroid follicular cell hyperplasia in both male and female mice and anisokaryosis, multinucleated hepatocytes, and basophilic foci of the liver in male mice.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 7.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on pages 10-11.

V. REFERENCES

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APPENDIX A

SUMMARY OF LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF HYDROQUINONE

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TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF HYDROQUINONE

	Vehicle Control	Low Dose	High Dose
Animals initially in study	65	65	65
Animals removed	65	65	65
Animals examined histopathologically	55	55	55
ALIMENTARY SYSTEM			
Intestine large, cecum	(50)	(34)	(50)
Leukemia mononuclear	2 (4%)	2 (6%)	
Colon, serosa, rectum, mesothelioma malignant, metastatic		1 (3%)	
Intestine large, colon	(51)	(35)	(53)
Leukemia mononuclear	5 (10%)	4 (11%)	
Serosa, histiocytic sarcoma, metastatic	1 (2%)		
Intestine large, rectum	(53)	(32)	(52)
Leukemia mononuclear	5 (9%)	1 (3%)	
Intestine small, duodenum	(52)	(35)	(55)
Leukemia mononuclear	5 (10%)	2 (6%)	1 (2%)
Ileum, mesothelioma malignant, metastatic		1 (3%)	
Serosa, mesothelioma malignant, metastatic		1 (3%)	
Serosa, ileum, jejunum, histiocytic sarcoma, metastatic	1 (2%)		
Intestine small, ileum	(48)	(33)	(49)
Leukemia mononuclear	4 (8%)	2 (6%)	1 (2%)
Intestine small, jejunum	(44)	(30)	(48)
Leiomyosarcoma	1 (2%)	1 (3%)	
Leukemia mononuclear	1 (2%)	1 (3%)	
Liver	(55)	(55)	(55)
Hepatocellular adenoma	3 (5%)		1 (2%)
Histiocytic sarcoma			1 (2%)
Histiocytic sarcoma, metastatic	2 (4%)	1 (2%)	
Leiomyosarcoma, metastatic, intestine small	1 (2%)		
Leukemia mononuclear	27 (49%)	26 (47%)	30 (55%)
Mesothelioma malignant		1 (2%)	
Mesothelioma malignant, metastatic		2 (4%)	
Neoplastic nodule		2 (4%)	1 (2%)
Bile duct, leiomyosarcoma, extension, metastatic, intestine small		1 (2%)	
Mesentery	*(55)	*(55)	*(55)
Histiocytic sarcoma, metastatic	2 (4%)		
Leiomyosarcoma, extension, metastatic, intestine small	1 (2%)	1 (2%)	
Leukemia mononuclear	3 (5%)	4 (7%)	6 (11%)
Mesothelioma malignant		1 (2%)	1 (2%)
Mesothelioma malignant, metastatic	1 (2%)	2 (4%)	
Pancreas	(53)	(36)	(54)
Histiocytic sarcoma, metastatic	2 (4%)		
Leukemia mononuclear	5 (9%)	4 (11%)	5 (9%)
Mesothelioma malignant			1 (2%)
Mesothelioma malignant, metastatic		2 (6%)	
Pharynx	*(55)	*(55)	*(55)
Palate, carcinoma, extension, metastatic, Zymbal gland	(2%)		
Salivary glands	(54)	(37)	(55)
Leukemia mononuclear	5 (9%)	1 (3%)	5 (9%)
Stomach, forestomach	(55)	(36)	(55)
Leukemia mononuclear	4 (7%)	2 (6%)	
Mesothelioma malignant			1 (2%)
Serosa, glandular, histiocytic sarcoma, metastatic	1 (2%)		
Serosa, glandular, mesothelioma malignant, metastatic		2 (6%)	
Stomach, glandular	(54)	(34)	(55)
Leukemia mononuclear	3 (6%)	5 (15%)	1 (2%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF HYDROQUINONE (Continued)

	Vehicle Control	Low Dose	High Dose
ALIMENTARY SYSTEM (Continued)			
Tongue	*(55)	*(55)	*(55)
Papilloma squamous	1 (2%)		
Tooth	*(55)	*(55)	*(55)
Pulp, leukemia mononuclear	8 (15%)	9 (16%)	8 (15%)
CARDIOVASCULAR SYSTEM			
Heart	(55)	(38)	(55)
Leukemia mononuclear	13 (24%)	17 (45%)	20 (36%)
Schwannoma, NOS			1 (2%)
Atrium, histiocytic sarcoma, metastatic		1 (3%)	
Atrium right, liposarcoma, metastatic, skin		1 (3%)	
Endocardium, schwannoma, NOS	2 (4%)		
ENDOCRINE SYSTEM			
Adrenal gland, cortex	(54)	(37)	(54)
Histiocytic sarcoma, metastatic	1 (2%)		
Leukemia mononuclear	15 (28%)	14 (38%)	19 (35%)
Capsule, mesothelioma malignant, metastatic		1 (3%)	
Adrenal gland, medulla	(55)	(48)	(55)
Leukemia mononuclear	14 (25%)	13 (27%)	18 (33%)
Pheochromocytoma malignant	1 (2%)	2 (4%)	3 (5%)
Pheochromocytoma benign	9 (16%)	14 (29%)	13 (24%)
Bilateral, pheochromocytoma benign	4 (7%)	3 (6%)	6 (11%)
Islets, pancreatic	(54)	(36)	(54)
Adenoma	1 (2%)	1 (3%)	
Parathyroid gland	(54)	(36)	(54)
Leukemia mononuclear	1 (2%)		1 (2%)
Pituitary gland	(54)	(54)	(54)
Leukemia mononuclear	6 (11%)	9 (17%)	8 (15%)
Pars distalis, adenoma	13 (24%)	9 (17%)	5 (9%)
Pars distalis, carcinoma		1 (2%)	
Thyroid gland	(55)	(38)	(55)
Histiocytic sarcoma, metastatic	1 (2%)		
Leukemia mononuclear	4 (7%)	3 (8%)	3 (5%)
C-cell, adenoma	5 (9%)	2 (5%)	3 (5%)
C-cell, carcinoma	2 (4%)	2 (5%)	3 (5%)
Follicular cell, adenocarcinoma	2 (4%)		
Follicular cell, adenoma	1 (2%)		1 (2%)
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Coagulating gland	*(55)	*(55)	*(55)
Leukemia mononuclear			1 (2%)
Epididymis	(53)	(37)	(55)
Histiocytic sarcoma, metastatic	1 (2%)		
Leukemia mononuclear	2 (4%)	1 (3%)	3 (5%)
Mesothelioma malignant			1 (2%)
Mesothelioma malignant, metastatic	1 (2%)	1 (3%)	
Penis	*(55)	*(55)	*(55)
Leukemia mononuclear		1 (2%)	
Preputial gland	(53)	(34)	(54)
Adenoma	11 (21%)	8 (24%)	7 (13%)
Carcinoma	1 (2%)	1 (3%)	3 (6%)
Histiocytic sarcoma, metastatic	1 (2%)		
Leukemia mononuclear	5 (9%)	7 (21%)	7 (13%)
Mesothelioma malignant, metastatic	1 (2%)		

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF HYDROQUINONE (Continued)

	Vehicle Control	Low Dose	High Dose
GENITAL SYSTEM (Continued)			
Prostate	(53)	(41)	(55)
Adenoma		1 (2%)	
Leukemia mononuclear	4 (8%)	4 (10%)	5 (9%)
Serosa, histiocytic sarcoma, metastatic	1 (2%)		
Serosa, mesothelioma malignant, metastatic		1 (2%)	
Seminal vesicle	(53)	(36)	(55)
Leukemia mononuclear	5 (9%)	6 (17%)	6 (11%)
Serosa, histiocytic sarcoma, metastatic	1 (2%)		
Serosa, mesothelioma malignant, metastatic		1 (3%)	
Testes	(54)	(54)	(55)
Leukemia mononuclear	8 (15%)	9 (17%)	6 (11%)
Bilateral, interstitial cell, adenoma	37 (69%)	36 (67%)	43 (78%)
Interstitial cell, adenoma	9 (17%)	13 (24%)	6 (11%)
Tunic, histiocytic sarcoma, metastatic	1 (2%)		
Tunic, mesothelioma malignant	1 (2%)	3 (6%)	1 (2%)
HEMATOPOIETIC SYSTEM			
Blood	*(55)	*(55)	*(55)
Histiocytic sarcoma, metastatic	1 (2%)		
Leukemia mononuclear	17 (31%)	20 (36%)	21 (38%)
Bone marrow	(55)	(37)	(55)
Histiocytic sarcoma, metastatic	1 (2%)		
Leukemia mononuclear	11 (20%)	14 (38%)	18 (33%)
Lymph node	(55)	(41)	(55)
Axillary, leukemia mononuclear	1 (2%)		
Bronchial, leukemia mononuclear	1 (2%)	1 (2%)	
Deep cervical, leukemia mononuclear	1 (2%)	1 (2%)	
Iliac, leukemia mononuclear	1 (2%)	1 (2%)	
Inguinal, leukemia mononuclear	3 (5%)	1 (2%)	1 (2%)
Lumbar, leukemia mononuclear	4 (7%)	4 (10%)	2 (4%)
Mediastinal, histiocytic sarcoma, metastatic		1 (2%)	
Mediastinal, leukemia mononuclear	6 (11%)	7 (17%)	9 (16%)
Pancreatic, leukemia mononuclear	9 (16%)	3 (7%)	4 (7%)
Renal, leukemia mononuclear	4 (7%)		1 (2%)
Lymph node, mandibular	(52)	(38)	(54)
Leukemia mononuclear	15 (29%)	14 (37%)	16 (30%)
Lymph node, mesenteric	(53)	(38)	(54)
Leukemia mononuclear	18 (34%)	15 (39%)	20 (37%)
Mesothelioma malignant, metastatic		2 (5%)	
Inguinal, lumbar, mediastinal, histiocytic sarcoma, metastatic	1 (2%)		
Mediastinal, mandibular, histiocytic sarcoma, metastatic	1 (2%)		
Spleen	(55)	(52)	(55)
Histiocytic sarcoma, metastatic	1 (2%)		
Leiomyosarcoma, metastatic, intestine small		1 (2%)	
Leukemia mononuclear	28 (51%)	26 (50%)	31 (56%)
Capsule, histiocytic sarcoma, metastatic	1 (2%)		
Capsule, mesothelioma malignant		1 (2%)	
Capsule, mesothelioma malignant, metastatic		2 (4%)	
Thymus	(46)	(35)	(46)
Histiocytic sarcoma, metastatic	1 (2%)		
Leukemia mononuclear	10 (22%)	15 (43%)	10 (22%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF HYDROQUINONE (Continued)

	Vehicle Control	Low Dose	High Dose
INTEGUMENTARY SYSTEM			
Mammary gland	(49)	(29)	(53)
Adenocarcinoma	1 (2%)		
Adenoma	1 (2%)		
Fibroadenoma	3 (6%)		
Histiocytic sarcoma, metastatic	1 (2%)		
Leukemia mononuclear	2 (4%)		
Skin	(55)	(37)	(54)
Basal cell adenoma			2 (4%)
Basosquamous tumor malignant		1 (3%)	
Keratoacanthoma	2 (4%)		2 (4%)
Hair follicle, leukemia mononuclear	1 (2%)		
Posterior, leukemia mononuclear	1 (2%)		
Subcutaneous tissue, fibroma	1 (2%)	1 (3%)	
Subcutaneous tissue, fibrosarcoma	1 (2%)	1 (3%)	
Subcutaneous tissue, histiocytic sarcoma	2 (4%)	1 (3%)	
Subcutaneous tissue, leukemia mononuclear	6 (11%)	4 (11%)	2 (4%)
Subcutaneous tissue, lipoma		1 (3%)	
Subcutaneous tissue, liposarcoma		1 (3%)	
MUSCULOSKELETAL SYSTEM			
Bone	(55)	(37)	(55)
Rib, osteosarcoma			1 (2%)
Vertebra, leukemia mononuclear	1 (2%)		
Skeletal muscle	*(55)	*(55)	*(55)
Histiocytic sarcoma, metastatic	1 (2%)		
Leiomyosarcoma, metastatic, intestine small		1 (2%)	
Leukemia mononuclear	2 (4%)		2 (4%)
Osteosarcoma, extension, metastatic, bone			1 (2%)
NERVOUS SYSTEM			
Brain	(55)	(37)	(55)
Leukemia mononuclear	5 (9%)	5 (14%)	3 (5%)
Meninges, leukemia mononuclear	3 (5%)	1 (3%)	5 (9%)
Meninges, cerebrum, histiocytic sarcoma, metastatic		1 (3%)	
Spinal cord	*(55)	*(55)	*(55)
Leukemia mononuclear	2 (4%)	1 (2%)	
Meninges, leukemia mononuclear	6 (11%)	4 (7%)	7 (13%)
RESPIRATORY SYSTEM			
Lung	(55)	(38)	(55)
Alveolar/bronchiolar adenoma	1 (2%)		
Carcinoma, metastatic, Zymbal gland		1 (3%)	
Histiocytic sarcoma			1 (2%)
Histiocytic sarcoma, metastatic	2 (4%)	1 (3%)	
Leukemia mononuclear	21 (38%)	17 (45%)	26 (47%)
Liposarcoma, metastatic, skin		1 (3%)	
Osteosarcoma, metastatic, bone			1 (2%)
Pheochromocytoma malignant, metastatic, adrenal gland			1 (2%)
Nose	(55)	(37)	(55)
Leukemia mononuclear	5 (9%)	11 (30%)	12 (22%)
Trachea	(53)	(37)	(55)
Leukemia mononuclear	1 (2%)		

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF HYDROQUINONE (Continued)

	Vehicle Control	Low Dose	High Dose
SPECIAL SENSES SYSTEM			
Ear	*(55)	*(55)	*(55)
Canal, carcinoma, extension, metastatic, Zymbal gland	1 (2%)		
Eye	*(55)	*(55)	*(55)
Leukemia mononuclear	1 (2%)		
Harderian gland	*(55)	*(55)	*(55)
Leukemia mononuclear	1 (2%)		
Zymbal gland	*(55)	*(55)	*(55)
Adenoma		1 (2%)	
Carcinoma	1 (2%)	3 (5%)	1 (2%)
URINARY SYSTEM			
Kidney	(55)	(55)	(55)
Histiocytic sarcoma			1 (2%)
Leukemia mononuclear	16 (29%)	22 (40%)	25 (45%)
Capsule, histiocytic sarcoma, metastatic	1 (2%)		
Capsule, mesothelioma malignant, metastatic		1 (2%)	
Renal tubule, adenoma		4 (7%)	8 (15%)
Urinary bladder	(51)	(37)	(55)
Leukemia mononuclear	5 (10%)	4 (11%)	6 (11%)
Serosa, histiocytic sarcoma, metastatic	1 (2%)		
Serosa, mesothelioma malignant, metastatic	1 (2%)	2 (5%)	
Transitional epithelium, carcinoma, papillary		1 (3%)	
SYSTEMIC LESIONS			
Multiple organs	*(55)	*(55)	*(55)
Leukemia mononuclear	28 (51%)	26 (47%)	31 (56%)
Mesothelioma malignant	1 (2%)	3 (5%)	1 (2%)
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	65	65	65
Terminal sacrifice	27	18	18
Moribund	13	25	22
Interval sacrifice	10	10	10
Gavage death	2	5	7
Dead	13	7	8
TUMOR SUMMARY			
Total animals with primary neoplasms **	53	53	54
Total primary neoplasms	145	140	145
Total animals with benign neoplasms	50	50	52
Total benign neoplasms	102	96	98
Total animals with malignant neoplasms	35	37	38
Total malignant neoplasms	41	44	46
Total animals with secondary neoplasms ***	5	6	2
Total secondary neoplasms	37	34	3
Total animals with neoplasms uncertain Benign or malignant	2		1
Total uncertain neoplasms	2		1

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
*** 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 HYDROQUINONE: VEHICLE CONTROL

WEEKS ON STUDY	039	053	055	066	069	074	078	080	081	085	086	086	088	091	093	094	095	097	098	098	101	102	102	103
CARCASS ID	031	061	095	134	045	133	132	054	014	094	073	065	085	103	044	035	014	053	043	013	102	124	123	093	084
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	A	+	+	+	+	+	A
Leukemia mononuclear						X																X			
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	A
Leukemia mononuclear			X			X			X						X										
Serosa, histiocytic sarcoma, metastatic													X												
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X			X													X			
Intestine small	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+
Leukemia mononuclear			X			X									X							X			
Serosa, ileum, jejunum, histiocytic sarcoma, metastatic													X												
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	A		+	+	+	+	A	A	+	+	+	A	+	A
Leukemia mononuclear			X			X			X																
Intestine small, jejunum	A	+	A	+	+	+	+	+	+	+	M	A		+	+	+	+	A	A	+	+	+	A	+	A
Leiomyosarcoma					X																				
Leukemia mononuclear						X																			
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																							X		
Histiocytic sarcoma, metastatic				X									X												
Leiomyosarcoma, metastatic, intestine small					X																				
Leukemia mononuclear		X	X			X	X	X	X						X	X					X	X			
Mesentery		+		+	+	+							+			+						+			
Histiocytic sarcoma, metastatic				X									X												
Leiomyosarcoma, extension, metastatic, intestine small					X																				
Leukemia mononuclear						X																X			
Mesothelioma malignant, metastatic																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+
Histiocytic sarcoma, metastatic				X									X												
Leukemia mononuclear			X			X									X	X									
Pharynx																	+								
Palate, carcinoma, extension, metastatic, Zymbal gland																	X								
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Leukemia mononuclear						X		X							X										
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X									X										
Serosa, glandular, histiocytic sarcoma, metastatic													X												
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X																X			
Tongue										+	+														
Papilloma squamous										X															
Tooth			+			+									+	+					+				
Pulp, leukemia mononuclear			X			X									X	X					X				
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X			X		X							X	X					X	X			
Endocardium, schwannoma, NOS															X										
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma, metastatic													X												
Leukemia mononuclear			X			X	X	X	X						X	X					X	X			
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X			X	X	X							X	X					X	X			
Pheochromocytoma malignant																									
Pheochromocytoma benign														X			X						X		
Bilateral, pheochromocytoma benign																									X
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									X
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X			X															X	X			
Pars distalis, adenoma				X								X	X				X			X				X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma, metastatic				X																					
Leukemia mononuclear						X		X							X										
C-cell, adenoma															X										
C-cell, carcinoma																			X						
Follicular cell, adenocarcinoma																									X
Follicular cell, adenoma																									
GENERAL BODY SYSTEM																									
None																									

+: Tissue examined microscopically
: Not examined
−: Present but not examined microscopically
I: Insufficient tissue

M: Missing
A: Autolysis precludes examination
X: Incidence of listed morphology

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

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	105
CARCASS ID	083	042	122	011	012	013	024	033	034	052	063	064	071	072	081	082	101	121	021	022	023	032	041	051	062
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Intestine large, colon	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Serosa, histiocytic sarcoma, metastatic																									
Intestine large, rectum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							X																		
Intestine small	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								X	
Serosa, ileum, jejunum, histiocytic sarcoma, metastatic																									
Intestine small, ileum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																			X						
Intestine small, jejunum	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Leiomyosarcoma																									
Leukemia mononuclear																									
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																							X		
Histiocytic sarcoma, metastatic																									
Leiomyosarcoma, metastatic, intestine small																									
Leukemia mononuclear			X	X	X		X		X	X		X	X	X			X		X		X			X	X
Mesentery												+										+			
Histiocytic sarcoma, metastatic																									
Leiomyosarcoma, extension, metastatic, intestine small																									
Leukemia mononuclear																									
Mesothelioma malignant, metastatic																						X			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma, metastatic																									
Leukemia mononuclear																									
Pharynx																									
Palate, carcinoma, extension, metastatic, Zymbal gland																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear													X											X	
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								X	
Serosa, glandular, histiocytic sarcoma, metastatic																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								X	
Tongue																									
Papilloma squamous																									
Tooth												+							+						
Pulp, leukemia mononuclear												X							X						
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear												X	X				X		X					X	
Endocardium, schwannoma, NOS																			X						
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma, metastatic																									
Leukemia mononuclear												X	X				X		X					X	
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear												X	X				X		X					X	
Pheochromocytoma malignant																									
Pheochromocytoma benign		X							X		X	X									X				
Bilateral, pheochromocytoma benign																		X						X	
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								X	
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								X	
Pars distalis, adenoma		X		X							X			X											X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma, metastatic																									
Leukemia mononuclear																									
C-cell, adenoma										X			X										X		
C-cell, carcinoma																						X			
Follicular cell, adenocarcinoma																									X
Follicular cell, adenoma						X																			
GENERAL BODY SYSTEM																									
None																									

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

WEEKS ON STUDY	105	105	105	105	105	TOTAL: TISSUES TUMORS
CARCASS ID	091	092	111	112	113	
ALIMENTARY SYSTEM						
Esophagus	+	+	+	+	+	55
Intestine large	+	+	+	+	+	53
Intestine large, cecum	+	+	+	+	+	50
Leukemia mononuclear						2
Intestine large, colon	+	+	+	+	+	51
Leukemia mononuclear				X		5
Serosa, histiocytic sarcoma, metastatic						1
Intestine large, rectum	+	+	+	+	+	53
Leukemia mononuclear				X		5
Intestine small	+	+	+	+	+	52
Intestine small, duodenum	+	+	+	+	+	52
Leukemia mononuclear						5
Serosa, ileum, jejunum, histiocytic sarcoma, metastatic						1
Intestine small, ileum	+	+	+	+	+	48
Leukemia mononuclear						4
Intestine small, jejunum	+	+	+	+	+	44
Leiomyosarcoma						1
Leukemia mononuclear						1
Liver	+	+	+	+	+	55
Hepatocellular adenoma		X				3
Histiocytic sarcoma, metastatic						2
Leiomyosarcoma, metastatic, intestine small						1
Leukemia mononuclear		X		X	X	27
Mesentery			+	+	+	12
Histiocytic sarcoma, metastatic						2
Leiomyosarcoma, extension, metastatic, intestine small						1
Leukemia mononuclear				X		3
Mesothelioma malignant, metastatic						1
Pancreas	+	+	+	+	+	53
Histiocytic sarcoma, metastatic						2
Leukemia mononuclear				X		5
Pharynx						1
Palate, carcinoma, extension, metastatic, Zymbal gland						1
Salivary glands	+	+	+	+	+	54
Leukemia mononuclear						5
Stomach	+	+	+	+	+	55
Stomach, forestomach	+	+	+	+	+	55
Leukemia mononuclear				X		4
Serosa, glandular, histiocytic sarcoma, metastatic						1
Stomach, glandular	+	+	+	+	+	54
Leukemia mononuclear						3
Tongue						2
Papilloma squamous						1
Tooth				+		8
Pulp, leukemia mononuclear				X		8
CARDIOVASCULAR SYSTEM						
Heart	+	+	+	+	+	55
Leukemia mononuclear				X		13
Endocardium, schwannoma, NOS						2
ENDOCRINE SYSTEM						
Adrenal gland	+	+	+	+	+	55
Adrenal gland, cortex	+	+	+	+	+	54
Histiocytic sarcoma, metastatic						1
Leukemia mononuclear				X		15
Adrenal gland, medulla	+	+	+	+	+	55
Leukemia mononuclear				X		14
Pheochromocytoma malignant					X	1
Pheochromocytoma benign		X				9
Bilateral, pheochromocytoma benign				X		4
Islets, pancreatic	+	+	+	+	+	54
Adenoma						1
Parathyroid gland	+	+	+	+	+	54
Leukemia mononuclear						1
Pituitary gland	+	+	+	+	+	54
Leukemia mononuclear				X		6
Pars distalis, adenoma	X			X		13
Thyroid gland	+	+	+	+	+	55
Histiocytic sarcoma, metastatic						1
Leukemia mononuclear				X		4
C-cell, adenoma			X			5
C-cell, carcinoma						2
Follicular cell, adenocarcinoma						2
Follicular cell, adenoma						1
GENERAL BODY SYSTEM						
None						