NTP REPORT ON THE

TOXICITY STUDIES OF

D&C YELLOW NO. 11

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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

January 1991

NTP TOX 8
NIH Publication No. 91-3127

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

FOREWORD

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

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

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable Federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals. Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure.

Anyone who is aware of related ongoing or published studies not mentioned in this report, or of any errors in this report, is encouraged to make this information known to the NTP. Comments and questions should be directed to Dr. J.R. Bucher, NIEHS, P.O. Box 12333, Research Triangle Park, NC 27709 (919-541-4532).

These NTP Toxicity Study Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Toxicity Study Report are available without charge while supplies last from the NTP Public Information Office, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3991).

TOXICITY STUDIES OF

D&C YELLOW NO. 11

(CAS NO. 8003-22-3)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

William. Eastin, Ph.D., Study Scientist

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

January 1991

NTP TOX 8
NIH Publication No. 91-3127

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

CONTENTS

PAGE

ABSTRACT 3
CONTRIBUTORS 5
PEER REVIEW PANEL 6
SUMMARY OF PEER REVIEW COMMENTS 7

I. INTRODUCTION 8

II. MATERIALS AND METHODS 11

III. RESULTS 15

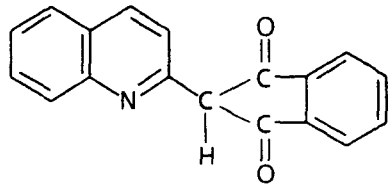
STUDIES IN RATS 15

STUDIES IN MICE 20

IV. DISCUSSION AND CONCLUSIONS 23

V. REFERENCES 25

APPENDIX: ORGAN WEIGHTS AND SERUM CHEMICAL DATA IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO. 11 28



D&C Yellow No. 11

CAS No. 8003-22-3

$C_{18}H_{11}NO_2$ Molecular weight 273.3

Synonym: 2-(2-quinolyl)-1,3-indandione

Names of Formulations: Arlosol Yellow S; Chinoline Yellow D soluble in spirits; Chinoline ZSS, CI 47000; C.I. Solvent Yellow 33; Nitro Fast Yellow SL; Oil Yellow SIS; Petrol Yellow C; Quinolin Yellow A Spirit Soluble; Quinoline Yellow Base; Quinoline Yellow Spirit Soluble; Quinoline Yellow SS; Solvent Yellow 33; Waxoline Yellow T

ABSTRACT

Toxicity studies were conducted by administering D&C Yellow No. 11 (approximately 99% pure) in feed at dietary concentrations of up to 50,000 ppm to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days or 13 weeks. A separate study was conducted to determine the effects of feeding diets containing D&C Yellow No. 11 to female rats during a reproductive cycle and to their offspring.

Although the estimated intake of D&C Yellow No. 11 by mice was more than twice that by rats, the results of the 14-day and 13-week studies were similar for both rats and mice. In both species, D&C Yellow No. 11 caused no deaths (5 animals per group in the 14-day studies and 10 per group in the 13-week studies) but did reduce body weight gain slightly in rats of each sex exposed to 17,000 or 50,000 ppm. Liver weights were increased in dosed rats and mice. There was minimal-to-mild degeneration of the periportal portion of the liver lobules of rats at dietary concentrations of 1,700 ppm and higher and of mice at 5,000 ppm and higher. A dose-related yellow-brown pigment was observed in hepatocytes, Kupffer cells, and biliary epithelium of the liver of each sex and species and in the tubular epithelium of the kidney of rats of each sex. Hepatocellular degeneration progressed slightly in severity with increased time of exposure (i.e., 14 days to 13 weeks) in rats but not in mice The number and size of hyaline droplets in the tubular epithelium of the cortex and outer medulla of the kidney were increased in all dosed groups of male rats.

In a perinatal toxicity study, body weight gain of rat dams given diets containing as much as 50,000 ppm D&C Yellow No. 11 for 4 weeks before mating to unexposed males was similar to that of controls at the time of mating but was lower at parturition and weaning. However, fertility, gestation length, litter size, and pup birth weights were unaffected by exposure. At weaning, body weights of pups from all dosed dams (5,000, 17,000, and 50,000 ppm) were lower than weights of pups from the controls. After exposure to D&C Yellow No. 11 for 4 weeks through the milk and to feed containing the same dietary concentrations that the dams received, weights of the 5,000-ppm pups were similar to those of the controls, but weights of the 17,000- and 50,000-ppm dose groups remained depressed. Microscopic evaluation showed lesions in the pups in all dosed groups; these lesions were similar to those described in the liver and kidney of rats in the 14-day and 13-week studies, including the male rat kidney cytoplasmic alterations.

The results of these studies indicate that compound-related effects occurred at all dietary concentrations of D&C Yellow No. 11; i.e., liver weights were increased in dosed rats and mice, and there was an increase in the number and size of hyaline droplets in all dosed groups of male rats.

CONTRIBUTORS

The NTP Report on the Two-Week and Thirteen-Week Toxicity Studies of D&C Yellow No. 11 is based on the 14-day and 13-week studies that began in October 1985 at EG&G Mason Research Institute (Worcester, MA).

National Toxicology Program
(Evaluated Experiment, Interpreted Results, and Reported Findings)

William Eastin, Ph D , Study Scientist

John R Bucher, Ph D
R E Chapin, Ph D
Michael Elwell, D V M , Ph D
Joel Leininger, D V M , Ph D
B A Schwetz, D V M , Ph D
James K Selkirk, Ph D
M B Thompson, D V M , Ph D

NTP Pathology Working Group
(Evaluated Slides and Prepared Pathology Report for Rats and Mice on 11/15/88)

Michael Elwell, D V M , Ph D (Chair) (NTP)
Sondra Grumbein, D V M , Ph D
Pathology Associates, Inc
Katharina Heider, D V M (NTP)
Joel Leininger, D V M , Ph D (NTP)
John Peckham, D V.M (Experimental
Pathology Laboratories, Inc)

Principal Contributors at EG&G Mason Research Institute
(Conducted Studies and Evaluated Tissues)

Herman S Lilja, Ph D
Carolyn Moyer, D V M

Principal Contributor at Experimental Pathology Laboratories, Inc.
(Provided Pathology Quality Assurance)

John Peckham, D V M

Principal Contributors at Analytical Sciences, Inc.
(Contractor for Statistical Analysis)

Steven Seilkop, M S
Janet Teague, M S

Principal Contributors at Carltech Associates, Inc.
(Contractor for Technical Report Preparation)

William D Theriault, Ph D
Abigail C Jacobs, Ph D
John Warner, M S
Naomi Levy, B A

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of D&C Yellow No. 11 on November 20, 1989, 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 four 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, and (d) to judge the significance of the experimental results by scientific criteria.

National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee

Robert A. Scala, Ph.D. (Chair)
Senior Scientific Advisor, Medicine and
Environmental Health Department
Research and Environmental Health Division
Exxon Biomedical Sciences, East Millstone, NJ

Daniel S. Longnecker, M.D.
Professor, Department of Pathology
Dartmouth Medical School
Hanover, NH

Ellen K. Silbergeld, Ph.D. (Principal Reviewer) Senior Scientist
Environmental Defense Fund
Washington, DC

Ad Hoc Subcommittee Panel of Experts

John Ashby, Ph.D.
Imperial Chemical Industries, PLC
Central Toxicology Laboratory
Alderley Park, England

Gary P. Carlson, Ph.D.
Professor of Toxicology, Department of Pharmacology and Toxicology
Purdue University, West Lafayette, IN

Harold Davis, D.V.M., Ph.D.
School of Aerospace Medicine
Brooks Air Force Base
San Antonio, TX

Robert H. Garman, D.V.M. (Principal Reviewer)
Consultants in Veterinary Pathology
Murrysville, PA

Lois Swirsky Gold, Ph.D.
University of California
Lawrence Berkeley Laboratory
Berkeley, CA

David W. Hayden, D.V.M., Ph.D.
Professor, Department of Veterinary Pathobiology
College of Veterinary Medicine
University of Minnesota, St. Paul, MN

Curtis D. Klaassen, Ph.D.
Professor, Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

Barbara McKnight, Ph.D.
Associate Professor
Department of Biostatistics
University of Washington
Seattle, WA

Lauren Zeise, Ph.D.
California Department of Health Services/RCHAS
Berkeley, CA

SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF D&C YELLOW NO. 11

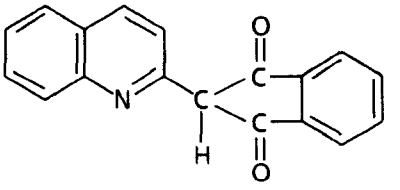
On November 20, 1989, the draft report on the toxicity studies of D&C Yellow No. 11 public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. W. Eastin, NIEHS, introduced the short-term toxicity studies by reviewing the rationale, experimental design, and results

Dr. Garman, a principal reviewer, said that the draft report was well written and thorough. In the concluding part of the Abstract, he thought that the special situation of hyaline droplet nephropathy in male rats could be discussed separately from other toxic effects.

Dr. Silbergeld, a second principal reviewer, said that the studies were acceptable within their design; her major criticism was with the study design itself, not with the conduct of the studies. Given that the major clinical observations for D&C Yellow No. 11 relate to its allergenic properties, she found it puzzling that the studies were not done by the dermal route and that immunotoxicology and dermal toxicity studies were not done. Dr. Eastin said that the nomination to study this color additive specifically requested the oral route because of the potential for ingestion. Dr. Silbergeld thought that the pathology for the nervous and female reproductive systems was inadequate.

The Panel recommended completion of the report with consideration of the points discussed.



D&C Yellow No. 11

CAS No. 8003-22-3

$C_{18}H_{11}NO_2$ Molecular weight 273.3

Synonym: 2-(2-quinolyl)-1,3-indandione

Names of Formulations: Arlosol Yellow S; Chinoline Yellow D soluble in spirits; Chinoline ZSS; CI 47000; C.I. Solvent Yellow 33; Nitro Fast Yellow SL; Oil Yellow SIS; Petrol Yellow C; Quinoline Yellow A Spirit Soluble; Quinoline Yellow Base; Quinoline Yellow Spirit Soluble; Quinoline Yellow SS; Solvent Yellow 33; Waxoline Yellow T

I. INTRODUCTION

Properties, Use, and Production

D&C Yellow No. 11 is the name given to 2-(2-quinolyl)-1,3-indandione when it has been certified for purity according to U.S. certification regulations (USCFR, 1988). The certified dye must conform to the following specifications and be free from impurities other than those named: volatile matter ≤ 1%; matter insoluble in ethyl alcohol ≤ 0.4%; phthalic acid ≤ 0.3%; quinaldine ≤ 0.2%; subsidiary colors ≤ 5%; lead ≤ 20 ppm; arsenic ≤ 3 ppm; and mercury ≤ 1 ppm. D&C Yellow No. 11 does not contain the methylated congener 6-methyl-2-(2-quinolyl)-1,3-indandione (FDA, personal communication). However, the noncertified dye, usually referred to as Solvent Yellow 33 (CTFA, 1982), is composed of two parts nonmethylated and one part methylated forms of the dye (Colour Index, 1982). In some toxicity studies, the D&C Yellow No. 11 used was reported to contain both 2-(2'-quinolyl)-1,3-indandione and 6'-methyl-2-(2'-quinolyl)-1,3-indandione (Bjorkner and Niklasson, 1983; Weaver, 1983; Sato et al., 1984).

D&C Yellow No. 11 is a yellow powder with a melting point of 240.9°-242.1° C. It is soluble in acetone, benzene, toluene, and xylene; slightly soluble in methanol, ethanol, ethyl acetate, linseed oil, mineral oil, and oleic acid; and insoluble in water (Colour Index, 1982). This dye is generally used in solvent form to color topical drug preparations and cosmetics (FDA, personal communication). In the United States, D&C Yellow No. 11 is approved only for external applications (Marmion, 1984).

Between 1979 and 1989, 29,968.5 lb of D&C Yellow No. 11 was certified and 173 cosmetic formulations were reported to include this dye (FDA, personal communication). It has been estimated that 14,314 workers were exposed to D&C Yellow No. 11 between 1981 and 1983 (NIOSH, 1989).

Metabolism and Disposition

In studies conducted for the National Toxicology Program using male F344/N rats, D&C Yellow No. 11 was rapidly distributed, metabolized, and excreted after intravenous or oral administration (El Dareer et al., 1988). After an intravenous dose of [^{14}C]D&C Yellow No. 11 (0.932 mg/kg), radioactivity distributed readily into most tissues. Fifty-four percent of the administered dose, all metabolites of D&C Yellow No. 11, was

recovered in the bile at 4 hours after dosing; 81.1% was excreted in the feces and 16% in the urine at 24 hours. In 11-day studies, D&C Yellow No. 11 (at dietary concentrations of 4.4, 37, 380, or 4,100 ppm) was given for 7 days; on day 8 only, [^{14}C]D&C Yellow No. 11 was included with the unlabeled dye and urine and feces collection was started. At the end of the studies, excreta and selected tissues were analyzed for radioactivity. Only trace amounts of radioactivity were present in tissue. On a microgram per gram basis, the liver and kidney contained the highest concentrations of radioactivity. In the 72 hours after [^{14}C]D&C Yellow No. 11 administration, total recovery of radioactivity was 89.1%-93.9% in the feces and 4.98%-6.25% in the urine. Assuming that the total amount excreted in urine was from absorbed compound, the excretion ratio of 5:1 (feces:urine), as determined in the intravenous studies, indicates that approximately 34% of the administered oral dose was absorbed. The percentage of the administered dose excreted in the urine was relatively constant in rats exposed to D&C Yellow No. 11 at up to 380 ppm in the diet but was slightly decreased at 4,100 ppm, the highest concentration studied. This decrease suggests that either the percentage of intestinal absorption was decreased or the mechanism for renal excretion was saturated at the 4,100-ppm concentration.

Short-Term Studies

D&C Yellow No. 11 has a low short-term oral toxicity in male Sprague Dawley rats (LD_{50} = 10,000 mg/kg) (Hazleton, 1962a) and male and female mongrel dogs (LD_{50} = 1,000 mg/kg) (Hazleton, 1962b). When the chemical was administered to Charles River rats in feed for 6 weeks at concentrations of 1,000, 2,300, 5,500, 12,900, or 30,000 ppm, no deaths occurred (Hazleton, 1962c). However, growth was suppressed at 30,000 ppm, and liver enlargement occurred at all concentrations. Microscopic evaluation indicated bile duct proliferation and excessive pigmentation of hepatocytes in rats that received 5,500, 12,900, or 30,000 ppm D&C Yellow No. 11. No deaths occurred in 13-week feed studies in albino rats (strain not given); however, the liver was enlarged at all concentrations studied (2,500-50,000 ppm) (Hansen et al., 1960).

F344/N rats were exposed to Solvent Yellow 33 by inhalation 6 hours per day, 5 days per week, for 4 days or 13 weeks (Sun et al., 1987). After 14 days of exposure (at concentrations of 10, 51, or 230 mg/m^3), rats at the highest concentration had body weights 8% lower than those of controls. After 13 weeks of exposure (at concentrations of 1, 10.8, or 100 mg/m^3), rats at the highest concentration had body weights 5% lower than those of controls and had an accumulation of vacuolated alveolar macrophages in the lung. However, tissue analysis by high-performance liquid chromatography showed very little Solvent Yellow 33 in the lung after exposure, indicating rapid clearance.

Long-Term Studies

In 1-year studies, D&C Yellow No. 11 given to Charles River rats at concentrations of 0.03%, 0.1%, 0.3%, or 1% by weight in feed (300, 1,000, 3,000, or 10,000 ppm) had no effect on survival; however, body weight gains by all dosed males and high dose females were depressed (Hazleton, 1967a). Liver weights progressively increased with increased concentrations. Morphologic alterations included bile duct proliferation and hepatocyte enlargement and pigment deposition in the renal proximal convoluted tubules and in the spleen of high dose females. When partial-hepatectomized male rats were fed 15,000 ppm D&C Yellow No. 11 in the diet for 10 days after surgery, liver regeneration was stimulated significantly compared with that in partial-hepatectomized controls (Gershbein, 1982).

As reported by Hazleton (1967b), beagle dogs were exposed orally to D&C Yellow No. 11 (7, 50, or 250 mg/kg per day) for up to 1 year. Three of six dogs in the mid dose group were dead by week 34. The deaths were attributed to severe weight loss during the initial phase of the studies because of nonacceptance of feed containing D&C Yellow No. 11 and coincidental disease. There were no other deaths during the study. Because of the refusal to eat the feed, the route of administration of the dye was changed to gelatin capsule in the mid dose groups after day 179 and in high dose groups after day 24. The most prominent pathologic changes from D&C Yellow No. 11 exposure were excessive pigment deposition in the cytoplasm of periportal hepatocytes (but not

Kupffer cells) of the liver and in the epithelium of the proximal convoluted tubules of the kidney.

D&C Yellow No. 11 (1% solution in benzene) applied at 1 mg/week to the back of male and female Swiss Webster mice for up to 95 weeks irritated the epidermis (Hazleton, 1967c). Survival was decreased in male mice, but extensive fighting among the group-housed male mice makes the interpretation of the effect of D&C Yellow No. 11 on survival unclear. D&C Yellow No. 11 at concentrations of 0.1% or 1% (1,000 or 10,000 ppm) applied once per day to intact (65 applications) or abraded (15 applications) skin of rabbits (0.5 g formulation/kg) for 5 days per week had no effect (Hazleton, 1965).

D&C Yellow No. 11 was shown to sensitize adult Hartley guinea pigs. Females induced with 40% D&C Yellow No. 11 in ethanol with an occluded patch, 1 day per week for 3 consecutive weeks, responded to a challenge concentration of 10% (Lamson et al., 1982). Hartley guinea pigs were also induced by injection of emulsified Freund's complete adjuvant into the nuchal region, followed by application of one of five test samples of D&C Yellow No. 11 to abraded skin for 2 days and topical application on days 8 and 9 (Sato et al., 1984). Challenge was carried out by topical application on day 21 to flank skin. In these studies, the threshold concentration for induction and challenge was 10 ppm. After 2 weeks, D&C Yellow No. 11 in Freund's adjuvant injected into the footpad produced a dose-response hypersensitivity in females exposed to intradermal challenges of the dye (Palazzolo and DiPasquale, 1983). Histopathologic examination of reaction sites indicated a cellular inflammatory response in guinea pigs consistent with delayed-type hypersensitivity.

Human Toxicity

D&C Yellow No. 11 has been shown to have a high allergenic potential in humans (Kita et al., 1984). Patients sensitized to D&C Yellow No. 11 in maximization tests exhibited an allergic contact dermatitis from the use of soaps (Jordan, 1981; Weaver, 1983) and facial cosmetics (Bjorkner and Magnusson, 1981; Calnan, 1981; Bjorkner and Niklasson, 1983; Rapaport, 1984) containing this dye. Positive reactions were seen in beauticians with hand dermatitis given Quinoline Yellow SS (0.5% in petrolatum) (Matsunaga et al., 1988).

Reproductive Effects and Teratology

No reproductive or teratology studies on D&C Yellow No. 11 were found in the literature.

Genetic Toxicology

D&C Yellow No. 11 was reported to be mutagenic in bacteria and mammalian cells treated in culture (Moore et al., 1985; Meyer et al., 1986; Zeiger et al., 1988). It produced chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells in vitro (NTP unpublished data) but was not clastogenic to mice exposed in vivo (Moore et al., 1985). Peripheral blood samples from mice in the 13-week studies were examined for the presence of micronucleated polychromatic and normochromic erythrocytes; no induction of micronuclei was observed in any of the dosed groups (NTP unpublished data).

Study Rationale

D&C Yellow No. 11 was nominated to the NTP for toxicity studies as a part of a larger regulatory effort mandated by Congress and undertaken by the Food and Drug Administration to determine the safety of a number of provisionally listed dyes. Since D&C Yellow No. 11 is currently regulated for external use, the recommendation to study D&C Yellow No. 11 by dietary exposure was based on the fact that it is a contaminant of D&C Yellow No. 10, a candidate for permanent listing as a chemical for which there is a potential for ingestion. Therefore, 14-day and 13-week feed studies were conducted in F344/N rats and B6C3F$_1$ mice of each sex to assess the toxicity of D&C Yellow No. 11. A study was also conducted in F344/N rats to determine the toxicity of perinatal dosing. Information from these studies will help to set doses should a long-term study be conducted using an in utero protocol.

II. MATERIALS AND METHODS

Procurement and Characterization of D&C Yellow No. 11

D&C Yellow No. 11 (2-(2-quinolyl)-1,3-indandione) was obtained in one lot from H. Kohnstamm and Company, Inc. (New York, NY) and certified by the Food and Drug Administration, Division of Color Technology. The material was identified as D&C Yellow No. 11 by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy and direct-inlet mass spectrometry.

The purity of the study material was determined to be approximately 99% by elemental analysis, Karl Fischer water analysis, thin-layer chromatography on silica gel plates, and high-performance liquid chromatography. The stability of the study material during the toxicity studies was monitored by gas chromatography. No deterioration of the D&C Yellow No. 11 was seen over the course of the studies.

Preparation and Characterization of Formulated Diets

Formulated diets were prepared by mixing the appropriate amounts of D&C Yellow No. 11/feed premix with feed in a twin-shell blender. The homogeneity and stability of D&C Yellow No. 11 in feed (500 ppm) was determined by high-performance liquid chromatographic analysis of acetone extracts of feed mixtures with an external standard of D&C Yellow No. 11. The chemical in feed was found to be homogeneously distributed and to be stable for at least 3 weeks in the dark at room temperature and for at least 7 days when stored open to air and light in a rodent cage. During the studies, formulated diets were stored for no longer than 3 weeks at 4° C.

Periodic analysis of formulated diets of D&C Yellow No. 11 was conducted at the study and analytical chemistry laboratories. The D&C Yellow No. 11 content of the administered diet was determined by spectrometric analysis of acetone extracts at 416 nm. Three complete sets of formulated diet mixtures were analyzed by the study laboratory during the 13-week studies; all samples were within specifications ($\pm$ 10% of the target concentration) (Table 1). The results of the analyses ranged from 97% to 103% of the target concentrations. Two referee analyses conducted by the analytical laboratory confirmed the results obtained by the study laboratory.

Fourteen-Day Study Design

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Frederick Cancer Research Facility and were held for 12-15 days before the studies began. The rats and mice were 6 weeks old when placed on study. Groups of five rats and five mice of each sex received diets containing 0, 500, 1,700, 5,000, 17,000, or 50,000 ppm D&C Yellow No. 11 for 14 days. Further details are given in Table 2.

Thirteen-Week Study Design

Groups of 10 rats and 10 mice of each sex were fed diets containing 0, 500, 1,700, 5,000, 17,000, or 50,000 ppm D&C Yellow No. 11 for 13 weeks.

TABLE 1. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO. 11

Target Concentration (ppm)	Determined Concentration (a) (ppm)	Coefficient of Variation
500	493 $\pm$ 7.6	1.5
1,700	1,683 $\pm$ 11.5	0.7
5,000	4,857 $\pm$ 5.8	0.1
17,000	17,133 $\pm$ 306	1.8
50,000	49,233 $\pm$ 58	0.1

(a) Mean $\pm$ standard deviation for three determinations; for each determination, all samples were analyzed in duplicate.

TABLE 2. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF D&C YELLOW NO. 11

Fourteen-Day Studies	Thirteen-Week Studies	Perinatal Toxicity Study
Strain and Species		
F344/N rats, B6C3F$_1$ mice	F344/N rats, B6C3F$_1$ mice	F344/N rats
Animal Source		
Frederick Cancer Research Facility (Frederick, MD)	Taconic Farms (Germantown, NY)	Same as 13-wk studies
Study Laboratory		
EG&G Mason Research Institute	EG&G Mason Research Institute	EG&G Mason Research Institute
Size of Study Groups		
5 males and 5 females of each species, rats housed 5 per cage, mice individually caged	10 males and 10 females of each species, rats housed 5 per cage, mice individually caged	F_0--12 males and 12 females, F_1--10 males and 10 females, F_0--1 male and 2 females housed together during breeding, females housed individually after mating, F_1--housed 5 per cage
Doses		
0, 500, 1,700, 5,000, 17,000, or 50,000 ppm D&C Yellow No 11 in feed	Same as 14-d studies	0, 5,000, 17,000, or 50,000 D&C Yellow No. 11 in feed to F_0 females and F_1 rats of each sex
Method of Animal Distribution		
Assigned to groups such that for a given sex and species all cage weights were approximately equal	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	Animals distributed to weight classes and then assigned to groups by a table of random numbers
Diet		
NIH 07 Rat and Mouse Ration (Zeigler Bros , Inc , Gardners, PA), available ad libitum	Same as 14 d studies	Same as 14-d studies
Animal Room Environment		
Temp--69 8°-73 4° F, hum--40% 55%, fluorescent light 12 h/d, >10 room air changes/h	Temp -70°-76° F; hum--44%-56%, fluorescent light 12 h/d, >10 room air changes/h	Temp--70°-78° F, hum--33%-70%, fluorescent light 12 h/d, >10 room air changes/h
Time Held Before Study		
Rats--12 13 d, mice--14 15 d	Rats--11 (male) or 13 (female) d, mice--13 (male) or 15 (female) d	F_0 females 22 d
Age When Placed on Study		
6 wk	6 wk	F_0--7 wk, F_1--in utero
Duration of Dosing		
14 consecutive d	13 wk	F_0 females--4 wk before breeding through weaning, F_1 males and females--in utero through 4 wk postweaning
Age When Killed		
8 wk	Rats and mice--19 wk	F_1--8 wk
Type and Frequency of Observation		
Observed 2 × d; weighed initially and 1 × wk thereafter	Observed 2 × d, weighed initially and 1 × wk thereafter	Observed 2 × d, female F_0 rats weighed initially, 1 × wk during the studies, at the end of the studies, on the day mating was observed (or on the last day of cohabitation), and at parturition; F_1 weighed at birth, 1 × wk thereafter, and at necropsy

TABLE 2. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF D&C YELLOW NO. 11 (Continued)

Fourteen-Day Studies	Thirteen-Week Studies	Perinatal Toxicity Study
Necropsy and Histologic Examinations		
Necropsy performed on all animals, tissues examined histologically for control and high dose groups Gross lesions and liver examined for all groups Organ weights obtained at necropsy	Necropsy performed on all animals; the following tissues examined histologically for control and high dose groups adrenal glands, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testes or ovaries/uterus, esophagus, femur or sternebrae or vertebrae including marrow, gallbladder (mice), gross lesions and tissue masses with regional lymph nodes, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal passage and turbinates, pancreas, parathyroid glands, pituitary gland, preputial/clitoral gland (rats), rectum, salivary glands, skin, spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder Tissues examined for all rats and mice gross lesions, liver, mandibular and mesenteric lymph nodes, and spleen Tissue examined for all rats. kidney Sperm morphology and vaginal cytology performed for control, 500-, 5,000-, and 50,000-ppm groups, serum chemical analyses performed and organ weights obtained at necropsy	Necropsy performed for all F_1 animals used in the studies, histologic exams of the same tissues examined in the 13-wk studies performed for all control and high dose animals Tissues examined for lower dose animals include gross lesions, kidneys, and liver

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 Taconic Farms. Animals were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms Animals were shipped to the study laboratory at 4 weeks of age The rats were quarantined at the study laboratory for 11-13 days and mice for 13-15 days. All animals were placed on study at 6 weeks of age.

All animals were observed two times per day Body weights were recorded once per week Animals found moribund and those surviving to the end of the studies were humanely killed Animals were anesthetized with Bio-Tal® barbiturate, and blood samples were drawn from the external jugular vein for serum chemical analyses Significant increases in serum activity of one or more marker (organ-specific) enzymes are usually indicative of cellular damage Because liver is a target organ of D&C Yellow No 11 exposure, serum activites of sorbitol dehydrogenase (SDH), alanine aminotransferase (ALAT), glutamic dehydrogenase (GDH), and ornithine carbamoyltransferase (OCT) were measured These four enzymes were selected because they are present in rodents in higher concentrations in liver than in other organs SDH is a cytoplasmic and mitochondrial enzyme that aids in the conversion of glucose to sorbitol (Asada and Galambos, 1963). ALAT is primarily a cytosolic enzyme that reversibly catalyzes conversion of alanine to α-ketoglutarate (Bergmeyer et al , 1978) GDH and OCT are mitochondrial enzymes that catalyze conversion of glutamate to α-ketoglutarate (Schmidt and Schmidt, 1983) and carbamoyl phosphate to ornithine (Ceriotti, 1983). A necropsy was performed on all animals

Sperm morphology and motility were evaluated for male rats and male mice that received 0, 500, 5,000, or 50,000 ppm D&C Yellow No. 11. The right epididymis was removed and quickly weighed; the cauda epididymis was removed at the junction of the vas deferens and the corpus epididymis and was weighed. Test yolk buffer (rats, 80 µl) or tyrodes buffer (mice, 80 µl) was applied to two prewarmed slides, and a small cut was made in the distal cauda epididymis. The sperm that effluxed from the epididymis were dispersed throughout the solution, coverslipped, and counted immediately on a warm microscope stage. In fields of 30 sperm or less, the number of moving and nonmoving sperm were counted in 5 fields on each slide.

After sperm sampling for motility estimation, the cauda was placed in phosphate-buffered saline (PBS), gently chopped with a razor blade, and allowed to sit for 15 minutes. The remaining clumps of tissue were removed, and the solution was mixed gently and heat-fixed at 65° C. Sperm density was then determined using a hemocytometer.

The right testis was frozen and stored. After thawing, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the testis in PBS containing 10% dimethyl sulfoxide. Homogenization-resistent spematid nuclei were enumerated using a hemocytometer; the data were expressed as spermatid heads per total testis and per gram of testis.

Vaginal smears were prepared during the 7 days before necropsy for females that received 0, 500, 5,000, or 50,000 ppm. For the 12 days prior to terminal kill, females were subject to vaginal lavage with saline. The aspirated cells were air-dried onto frosted slides, stained with Toluidine Blue O, and coverslipped. The relative preponderance of leukocytes, nucleated epithelial cells, and large squamous epithelial sheets were used to identify the stages of the estrual cycle.

Organs and tissues were examined for gross lesions. Tissues were preserved in 10% buffered formalin and routinely processed for preparation of histologic sections for microscopic examination. Tissues and groups examined are listed in Table 2. Additional sections of liver and kidney from rats in the 14-day studies were stained for bile (Hall's bile stain), lipofuscin (PAS, acid-fast oil red O), and hemosiderin (Perl's iron stain). Additional sections of kidney from control and high dose female and male rats (five each) from the 13-week studies were stained by the Mallory-Heidenhain method.

Upon completion of the histologic evaluation by the laboratory pathologist, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory and slide/block match. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed, and the results were reviewed and evaluated by the NTP Pathology Working Group (PWG). The final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman et al. (1985).

Perinatal Toxicity Study

Groups of twelve 7-week-old female F344/N rats were exposed to 0, 5,000, 17,000, or 50,000 ppm D&C Yellow No. 11 in feed for 4 weeks. For breeding, two female rats were housed with one control male (proven breeder) rat for 7 days. Females then were allowed to give birth and were continued on D&C Yellow No. 11 in feed until weaning (4 weeks), when they were killed. At birth, litter size was noted and litters were weighed and examined. On day 4, litters were culled by random selection to a maximum of eight pups. Randomly selected offspring were continued at the same exposure concentration as their dams for 4 weeks after weaning. A necropsy was performed on all F_1 animals at the end of the 4-week exposure period, and tissues were examined microscopically.

Statistical Methods

Organ weight to body weight ratios, serum enzyme activities, and reproductive function tests

were analyzed by the multiple comparison methods of Dunn (1964) and Shirley (1977). Jonckheere's test (Jonckheere, 1954) was used to evaluate the significance of dose-response trends for data on organ weight to body weight ratios, serum enzyme activities, and reproductive function tests. If the analysis indicated a significant trend, the nonparametric multiple comparison procedure of Shirley was used to assess the significance of pairwise comparisons between dosed and control groups; otherwise, Dunn's test was used for pairwise comparisons.

Quality Assurance

The studies of D&C Yellow No. 11 were performed in compliance with Good Laboratory Practices and regulations (21 CFR 58). The Quality Assurance Unit of EG&G Mason Research Institute performed audits and inspections of protocols, procedures, data, and reports throughout the conduct of the studies. The operations of the Quality Assurance Unit were monitored by the NTP, including site visits during the period of study performance.

III. RESULTS

STUDIES IN RATS

Fourteen-Day Studies

All rats lived to the end of the studies (Table 3). The final mean body weights of rats that received 50,000 ppm were 7% lower than that of controls for males and 4% lower for females. Feed consumption was unaffected by incorporation of D&C Yellow No. 11 into the diet. Compound-related clinical signs included yellow fur and feces for males that received 17,000 or 50,000 ppm and for females that received 1,700 ppm or more. Absolute liver weights and liver weight to body weight ratios were significantly increased for all groups of exposed rats.

TABLE 3. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE FOURTEEN-DAY FEED STUDIES OF D&C YELLOW NO. 11

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d)	Estimated Intake Dose (e)
MALE							
0	5/5	134	207	+73		18	
500	5/5	137	210	+73	101.4	18	52
1,700	5/5	135	204	+69	98.6	16	160
5,000	5/5	135	198	+63	95.7	16	480
17,000	5/5	134	200	+66	96.6	17	1,731
50,000	5/5	134	193	+59	93.2	17	5,199
FEMALE							
0	5/5	105	143	+38		13	
500	5/5	104	138	+34	96.5	13	54
1,700	5/5	105	140	+35	97.9	12	167
5,000	5/5	104	137	+33	95.8	12	498
17,000	5/5	104	139	+35	97.2	12	1,679
50,000	5/5	105	137	+32	95.8	12	4,959

(a) Number surviving/number initially in group
(b) Initial group mean body weight
(c) Mean body weight change of the group
(d) Grams per animal per day, averaged over both weeks of the studies; not corrected for scatter.
(e) Milligrams per kilogram per day, based on mean of initial and final body weights.

Minimal hepatocellular degeneration, characterized by cytoplasmic vacuolation in the periportal portion of the lobules, was present in the liver of all groups of dosed rats. In addition, a minimal amount of yellow-brown pigment granules or globules was present in the cytoplasm of Kupffer cells and hepatocytes; pigment was more prominent in females than in males. Special histologic stains of the liver pigment for hemosiderin, bile, and lipofuscin were negative. Cytoplasmic alteration was present in the kidney of all dosed male rats and consisted of an increase in the amount of hyaline droplets normally present in the cytoplasm of the tubular epithelium. The droplets were larger and the shape was much more irregular in dosed animals than in controls

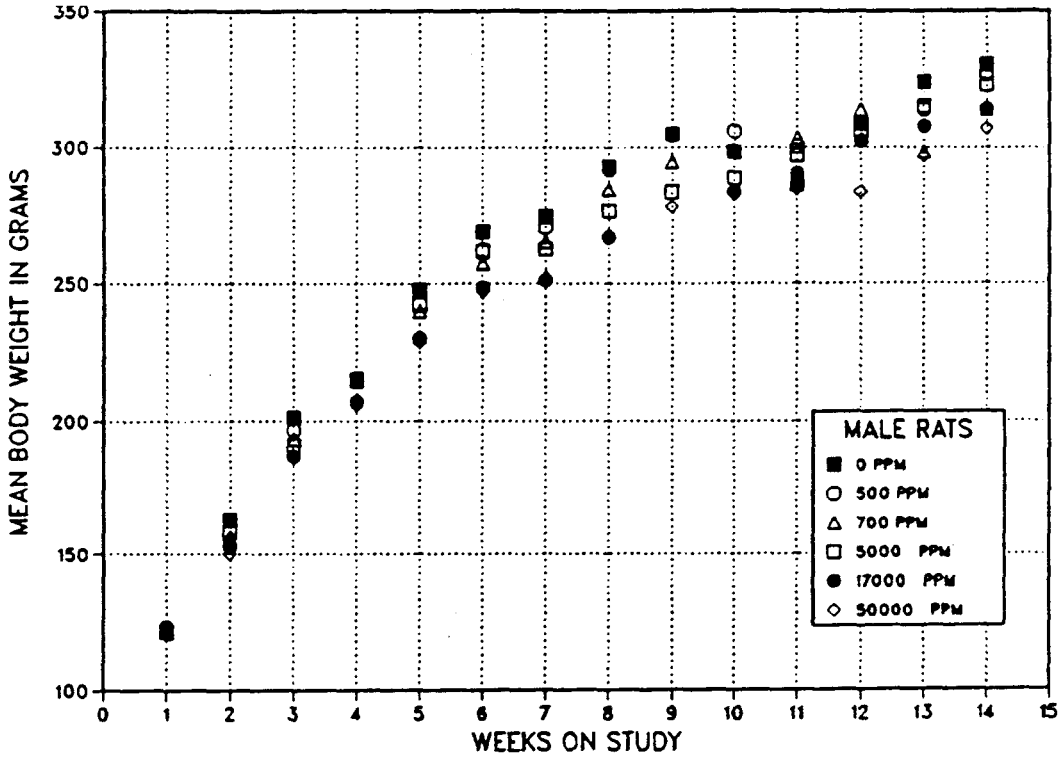
Thirteen-Week Studies

All rats lived to the end of the studies (Table 4). The final mean body weight of rats receiving 17,000 or 50,000 ppm was 5% or 7% lower than that of the controls (Figure 1). Compound-related clinical signs included yellow fur and skin for all exposed rats. Feed consumption was not affected by incorporation of D&C Yellow No. 11 in feed. The absolute liver weight and liver weight to body weight ratios were significantly increased for all exposed groups (concentrations as low as 500 ppm) (Table 5). Lung and thymus weights (Table A1), although significantly increased in some groups, were within the range of values seen in other recent National Toxicology Program studies. Effects on the liver were not clearly reflected by the serum enzymes analyzed (Table A2). Changes in alanine aminotransferase and glutamic dehydrogenase activities were not dose related for males or females; sorbitol dehydrogenase and ornithine carbamoyltransferase activities were increased in females, but not in males, that received 1,700 ppm or more, increases were not dose related Minimal-to-mild degeneration was present in the liver of males and females at concentrations of 1,700 ppm and higher (Table 6). Areas of degeneration were in the periportal portion of the hepatic lobules throughout the liver Hepatocytes in these areas were swollen and the cytoplasm contained irregularly shaped vacuolar spaces In some rats in the two highest dose groups, there was a confluence (bridging) between lobules of the periportal areas of degeneration, rarely, individual necrotic hepatocytes were present in these areas

TABLE 4. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO. 11

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Mean	Range	Estimated Intake Dose (e)
MALE								
0	10/10	121 ± 2	331 ± 3	+209 ± 3		16.7	13 9-18.5	
500	10/10	123 ± 2	327 ± 5	+204 ± 5	99	16.2	14 8-18 0	36
1,700	10/10	121 ± 2	314 ± 12	+193 ± 11	95	16.4	14 3-17.8	128
5,000	10/10	121 ± 2	323 ± 2	+202 ± 2	98	17.0	15 2-18.5	383
17,000	10/10	122 ± 2	314 ± 3	+192 ± 3	95	16 3	14 1-18.1	1,271
50,000	10/10	121 ± 2	307 ± 3	+186 ± 3	93	16.5	14.5-17.8	3,855
FEMALE								
0	10/10	105 ± 2	192 ± 3	+87 ± 3		11.1	10.3-11 6	
500	10/10	107 ± 2	193 ± 3	+86 ± 3	101	11.1	10.1-11.8	37
1,700	10/10	107 ± 2	191 ± 2	+84 ± 1	99	11.1	10.4-11.8	127
5,000	10/10	109 ± 2	190 ± 3	+81 ± 2	99	11.2	10.6-11 7	375
17,000	10/10	108 ± 1	183 ± 1	+75 ± 1	95	10.9	10.1-11.5	1,274
50,000	10/10	107 ± 1	179 ± 2	+72 ± 2	93	10.7	10.3-11.5	3,741

(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
(d) Grams per animal per day, averaged over the entire studies (range of weekly means); not corrected for scatter.
(e) Milligrams per kilogram per day, based on mean of initial and final body weights.



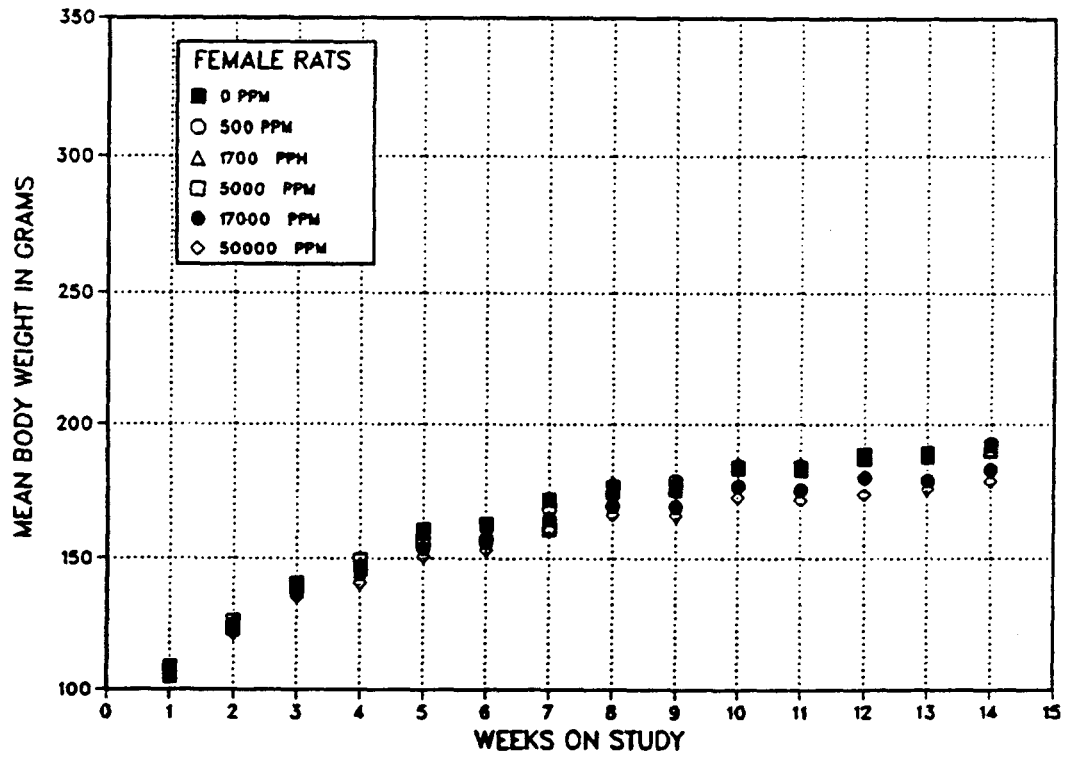


FIGURE 1. GROWTH CURVES FOR RATS FED DIETS CONTAINING D&C YELLOW NO. 11 FOR THIRTEEN WEEKS

TABLE 5. LIVER WEIGHTS OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO. 11 (a)

	Control	500 ppm	1,700 ppm	5,000 ppm	17,000 ppm	50,000 ppm
MALE						
Body weight (grams)	337 ± 3 6	332 ± 5 8	318 ± 10 8	326 ± 1 9	**316 ± 3 2	**309 ± 2.4
Liver						
Absolute	13,670 ± 270	*15,010 ± 360	**16,190 ± 730	**17,190 ± 340	**16,760 ± 380	**16,360 ± 270
Relative	40 6 ± 0 92	**45 2 ± 0 59	**50 9 ± 1 00	**52 7 ± 0 80	**52 9 ± 0 73	**52 9 ± 0 74
FEMALE						
Body weight (grams)	192 ± 2 6	191 ± 2 6	189 ± 2 3	188 ± 2 6	**180 ± 1 4	**176 ± 2 1
Liver						
Absolute	6,519 ± 149	*6,976 ± 129	**7,833 ± 158	**8,398 ± 145	**8,436 ± 152	**8,151 ± 248
Relative	34 0 ± 0 56	**36 5 ± 0 42	**41 5 ± 0 54	**44 6 ± 0 70	**46 8 ± 0 68	**46 2 ± 1 37

(a) Mean ± standard error (absolute in milligrams, relative in milligrams per gram unless otherwise specified) for groups of 10 animals, P values vs the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977)
*P<0 05
**P<0 01

TABLE 6. LIVER DEGENERATION IN RATS IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO. 11 (a)

Lesion	Control	500 ppm	1,700 ppm	5,000 ppm	17,000 ppm	50,000 ppm
MALE						
Periportal degeneration	0	0	4	10	10	10
Minimal (b)			4	9	3	0
Mild				1	7	10
FEMALE						
Periportal degeneration	0	0	2	7	9	10
Minimal			2	7	9	8
Mild						2

(a) Ten animals in each group were examined
(b) Number of rats with indicated severity

of degeneration, rarely, individual necrotic hepatocytes were present in these areas of degeneration Degeneration was less severe in females A finely granular-to-globular yellow-brown pigment was present in the hepatocytes, Kupffer cells, and biliary epithelium in the liver of dosed male and female rats This pigment was present in the areas of degeneration and was increased at higher doses It was more prominent in females than in males In the cortex and outer medulla of the kidney, a similar staining pigment was present in the cytoplasm of the tubular epithelium of males and females In males, cytoplasmic alteration was present in the tubular epithelium of the kidney in all dosed groups. This was characterized by the increased size and number of hyaline droplets in the cytoplasm of the tubular epithelium in the cortex and outer medulla These hyaline droplets often formed large globules or irregularly shaped crystalline

structures that stained similarly (Mallory-Heidenhain method) to the smaller granules of protein ($\alpha_{2\mu}$-globulin), typically seen in the renal tubular cell cytoplasm of male F344 rats.

The weights of the right cauda, epididymis, and testis of rats receiving the formulated diets did not differ from those of controls. Epididymal sperm density, sperm motility, and the percentage of abnormal sperm were not compound related (Table A2). Estrous stages and the length of the estrous cycle were not changed in a dose-related manner.

Perinatal Toxicity Study

No differences were found in the numbers of females that became pregnant in each group. Females that received 50,000 ppm weighed less than did controls when they gave birth and 4 weeks later at weaning (Table 7). The total litter size of exposed dams and the mean pup weights for all exposed groups were not affected by D&C Yellow No. 11. Dams were killed 4 weeks after weaning. Necropsies and microscopic evaluations were not performed.

TABLE 7. REPRODUCTIVE PERFORMANCE OF FEMALE RATS AND SURVIVAL AND BODY WEIGHTS OF F_1 PUPS IN THE PERINATAL TOXICITY FEED STUDY OF D&C YELLOW NO. 11 (a)

	Control	5,000 ppm	17,000 ppm	50,000 ppm
Number examined (b)	11	10	12	10
Body weight (grams)				
Initial	131 ± 4.4	132 ± 4.1	133 ± 4.0	132 ± 4.0
Day of mating	193 ± 3.4	188 ± 3.7	187 ± 4.2	187 ± 2.5
Parturition	225 ± 4.5	219 ± 2.7	214 ± 3.7	*210 ± 2.7
Weaning	235 ± 3.3	*225 ± 2.2	*224 ± 4.6	*222 ± 3.1
Length of gestation (days)	19.4 ± 0.61	20.2 ± 0.88	*21.3 ± 0.22	20.5 ± 0.65
Live pups per litter				
Number	12.5 ± 0.69	13.1 ± 0.31	11.6 ± 0.72	12.5 ± 0.45
Percent	97.8 ± 1.20	99.3 ± 0.70	99.4 ± 0.60	99.2 ± 0.80
Dams with litters/total number of dams	11/12	10/12	12/12	10/12
Mean litter size	12	14	12	11
Pup deaths during lactation/ total number of pups	0/140	2/132	0/130	0/126
Mean pup weight (grams) (c)				
At parturition (male and female)	5.3 ± 0.11	5.2 ± 0.06	5.2 ± 0.09	5.1 ± 0.06
Male (d)				
At 4 weeks	78.2 ± 2.5	*67.2 ± 2.6	**63.6 ± 1.4	**57.0 ± 1.8
At 8 weeks	220.0 ± 6.2	211.7 ± 4.4	**176.1 ± 7.1	**179.2 ± 2.5
Female (d)				
At 4 weeks	71.2 ± 2.4	*60.3 ± 2.3	**59.6 ± 1.7	**52.5 ± 2.2
At 8 weeks	151.7 ± 2.4	146.2 ± 2.0	**138.2 ± 2.2	**134.8 ± 1.8

(a) Mean ± standard error; P values vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
(b) Unless otherwise specified
(c) Ten litters from each group were averaged.
(d) Ten animals per group were weighed.
*$P<0.05$
**$P<0.01$

At week 4, weights of the F_1 generation dosed males and females were lower than those of controls. All dosed rats were yellow by day 7. In the liver, degeneration of hepatocytes was present in all dosed groups and was characterized by minimal cytoplasmic vacuolation similar to that seen in the 14-day and 13-week studies. All dosed rats had a minimal accumulation of a granular-to-globular yellow-brown pigment in the cytoplasm of cells in the liver and kidney. The pigment was morphologically similar to that seen in the 13-week studies and was more prominent in females than in males. In the liver, the pigment was primarily within periportal hepatocytes but was also in biliary epithelium and Kupffer cells. Pigment in the kidney was in the cytoplasm of the tubular epithelium in the cortex and outer medulla. Also present in the kidney of all dosed males was cytoplasmic alteration (hyaline droplets) morphologically similar to that observed in the 14-day and 13-week studies.

STUDIES IN MICE

Fourteen-Day Studies

All mice lived to the end of the studies (Table 8). The final mean body weights of dosed mice were higher than those of controls. Feed consumption was not clearly affected by the amount of D&C Yellow No. 11 incorporated into the diet. Compound-related clinical signs included yellow feces for female mice that received 17,000 or 50,000 ppm. Absolute liver weights and liver weight to body weight ratios were significantly increased for all groups of exposed mice. Minimal-to-mild degeneration in the liver characterized by cytoplasmic vacuolation of hepatocytes in the periportal region was present in groups of mice exposed to 1,700 ppm or more.

Thirteen-Week Studies

All mice lived to the end of the studies (Table 9). The final mean body weight of male mice that

TABLE 8. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE FOURTEEN-DAY FEED STUDIES OF D&C YELLOW NO. 11

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d)	Estimated Intake Dose (e)
MALE							
0	5/5	19.5	21.8	+2.3		4.7	
500	5/5	19.1	22.0	+2.9	100.9	4.5	109
1,700	5/5	19.4	22.8	+3.4	104.6	5.2	419
5,000	5/5	19.3	23.2	+3.9	106.4	3.8	894
17,000	5/5	20.0	23.0	+3.0	105.5	3.7	2,926
50,000	5/5	19.8	23.5	+3.7	107.8	4.4	10,162
FEMALE							
0	5/5	16.9	18.3	+1.4		4.2	
500	5/5	17.6	19.1	+1.5	104.4	4.9	134
1,700	5/5	17.3	19.1	+1.8	104.4	4.2	392
5,000	5/5	17.1	18.7	+1.6	102.2	4.0	1,117
17,000	5/5	16.7	18.9	+2.2	103.3	3.4	3,247
50,000	5/5	16.9	19.6	+2.7	107.1	4.1	11,233

(a) Number surviving/number initially in group
(b) Initial group mean body weight
(c) Mean body weight change of the group
(d) Grams per animal per day, averaged over both weeks of the studies; not corrected for scatter.
(e) Milligrams per kilogram per day, based on mean of initial and final body weights

TABLE 9. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO. 11

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Mean	Range	Estimated Intake Dose (e)
MALE								
0	10/10	22 4 ± 0 3	31.8 ± 0 9	+9 4 ± 0 8		3 9	3 2-5 1	
500	10/10	22 3 ± 0 3	30 7 ± 0 8	+8 5 ± 0.8	96 5	4 0	3 4-4 6	75
1,700	10/10	22 1 ± 0 3	30 6 ± 0 5	+8 5 ± 0 4	96 2	4 4	3.1-5 8	284
5,000	10/10	22 3 ± 0 3	31 3 ± 0 5	+9 0 ± 0 3	98 4	4 1	3 1-5 2	765
17,000	10/10	22 1 ± 0 3	30 9 ± 0 4	+8 8 ± 0 4	97 2	4 2	3 7 4 7	2,694
50,000	10/10	22 0 ± 0 3	29 9 ± 0 3	+7 9 ± 0 3	94 0	4 1	3 2 4 6	7,900
FEMALE								
0	10/10	18 2 ± 0 2	25 6 ± 0 3	+7 4 ± 0 3		5 0	4 2-6 7	
500	10/10	18 2 ± 0 2	25 9 ± 0 4	+7 7 ± 0 4	101 2	4 7	3 8-6 7	107
1,700	10/10	18 2 ± 0 2	27 2 ± 0 3	+9 0 ± 0 4	106 3	4 6	3 2 6 6	344
5,000	10/10	17 7 ± 0 3	25 4 ± 0 3	+7 7 ± 0 2	99 2	4 8	4 2-5 7	1,114
17,000	10/10	17 6 ± 0 3	25 4 ± 0 7	+7 8 ± 0 5	99 2	4 8	3 8-5 7	3,795
50,000	10/10	18 1 ± 0 2	26 6 ± 0 3	+8 5 ± 0 4	103 9	4 4	3 5-5 4	9,843

(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
(d) Grams per animal per day, averaged over both weeks of the studies, not corrected for scatter
(e) Milligrams per kilogram per day, based on mean of initial and final body weights

received 50,000 ppm was 6% lower than that of the controls (Figure 2) All exposed female groups gained more weight than did controls Feed consumption by males and females was not dose related Compound-related clinical signs included yellow fur for females Liver weight and liver weight to body weight ratios were significantly increased in all dosed groups of males and females (concentrations as low as 500 ppm) (Table 10) Absolute kidney weights, but not kidney weight to body weight ratios, were variably increased for all groups of exposed females Relative kidney weights were increased for males that received 5,000 ppm or more Absolute heart weights for all exposed groups and heart weight to body weight ratios for groups receiving 5,000 ppm or more were increased for exposed females (Table A3) However, these were within the range of normal heart weights seen in $B6C3F_1$ female mice Alanine aminotransferase activity was significantly increased for males receiving 50,000 ppm and was increased in all groups of dosed females (Table A4) Although sorbitol dehydrogenase activity was increased for females that received 50,000 ppm and ornithine carbamoyltransferase activity was increased at 17,000 and 50,000 ppm, values were not dose related and differences were not biologically meaningful Carcasses of most male and female mice receiving 5,000 ppm or more and of one male receiving 500 ppm were yellow Pigment was noted in the large and small intestines of males and females receiving 500 ppm or greater Periportal degeneration of minimal severity was found in the liver in 10/10 males and 5/10 females receiving 50,000 ppm, 6/10 males and 4/10 females receiving 17,000 ppm, 2/10 males receiving 5,000 ppm, and 0/10 male and 0/10 female controls This was characterized by a minimal cytoplasmic vacuolation of hepatocytes, which was similar to but less severe than that seen in the rats Mild-to-moderate yellow-brown pigmentation that increased with increasing concentration of D&C Yellow No 11 in the diet was found in periportal hepatocytes, Kupffer cells, and the biliary epithelium of all dosed animals Sperm motility was significantly decreased in males receiving 5,000 ($P<0.05$) or 50,000 ppm ($P<0.01$) (Table A4). Mice from the 1,700- and 17,000-ppm groups were not evaluated However, sperm motility in both dosed and control groups was substantially higher than that seen in historical control $B6C3F_1$ mice (Morrissey et al , 1988)

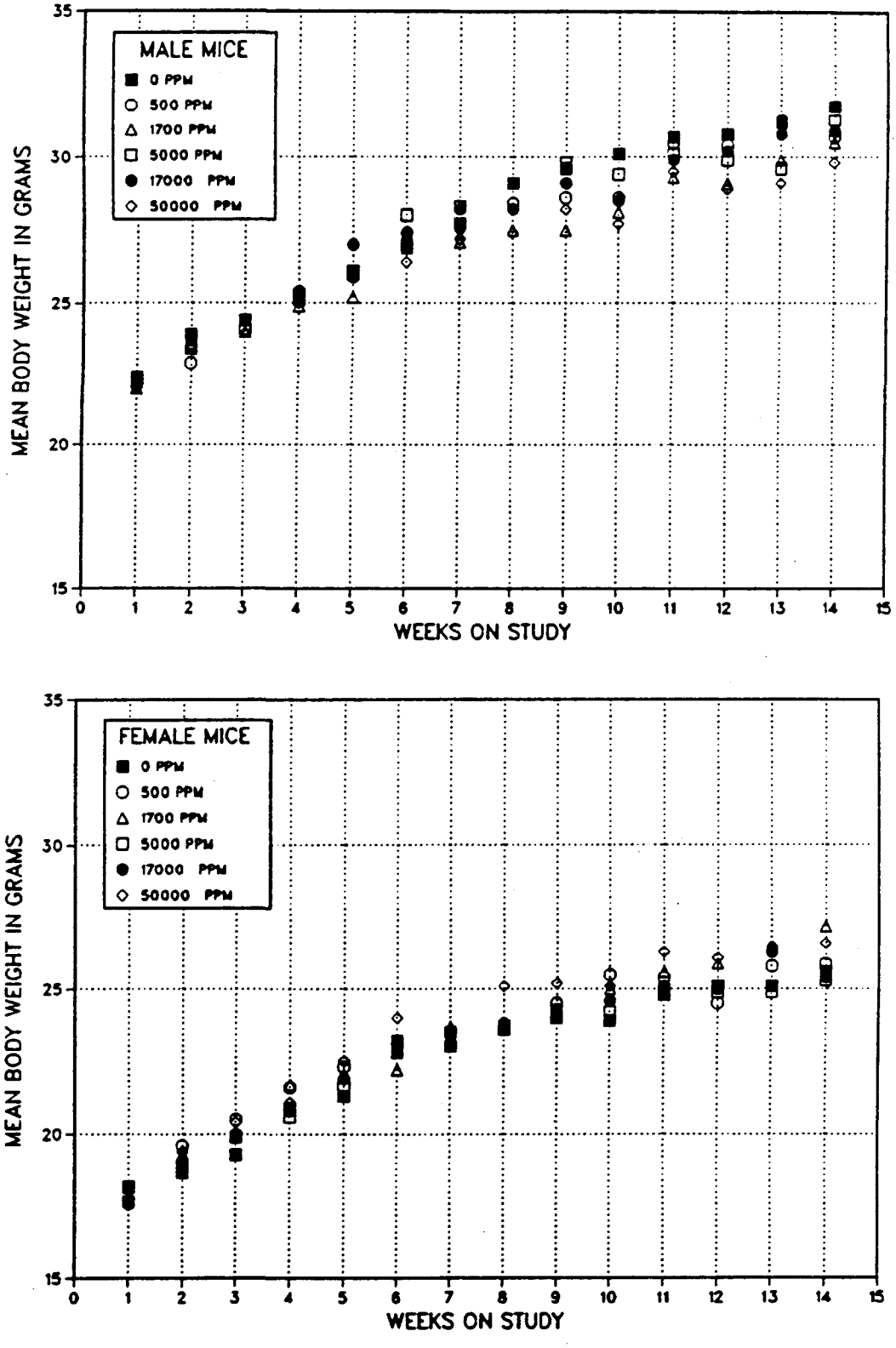


FIGURE 2. GROWTH CURVES FOR MICE FED DIETS CONTAINING D&C YELLOW NO. 11 FOR THIRTEEN WEEKS

TABLE 10. ORGAN WEIGHTS OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO. 11 (a)

Organ	Control	500 ppm	1,700 ppm	5,000 ppm	17,000 ppm	50,000 ppm
MALE						
Body weight (grams)	31 8 ± 0 88	30 9 ± 0 69	30 1 ± 0 49	30 7 ± 0 71	30 2 ± 0 45	*29 5 ± 0 35
Kidney						
Absolute	292 ± 7	298 ± 5	294 ± 6	*328 ± 9	304 ± 8	294 ± 7
Relative	9 3 ± 0 38	9 7 ± 0 24	9 8 ± 0 13	**10 7 ± 0 28	*10 1 ± 0 21	*10 0 ± 0 23
Liver						
Absolute	1,640 ± 31	**1,839 ± 37	**1,924 ± 33	**2,150 ± 48	**2,186 ± 52	**2,272 ± 62
Relative	51 9 ± 1 45	**59 8 ± 1 43	**64 0 ± 1 12	**70 0 ± 0 82	**72 4 ± 1 20	**76 9 ± 1 46
FEMALE						
Body weight (grams)	25 4 ± 0 42	26 0 ± 0 33	26 6 ± 0 32	25 7 ± 0 54	26 5 ± 0 48	26 6 ± 0 30
Kidney						
Absolute	187 ± 3	*201 ± 5	*202 ± 6	*205 ± 6	*198 ± 3	**206 ± 5
Relative	7 4 ± 0 21	7 8 ± 0 19	7 6 ± 0 24	8 0 ± 0 15	7 5 ± 0 12	7 7 ± 0 15
Liver						
Absolute	1,316 ± 42	**1,599 ± 44	**1,737 ± 33	**1,909 ± 74	**2,134 ± 95	**2,066 ± 56
Relative	51 8 ± 1 44	**61 6 ± 1 83	**65 3 ± 1 04	**74 2 ± 2 19	**80 7 ± 3 39	**77 5 ± 1 52

(a) Mean ± standard error (absolute in milligrams, relative in milligrams per gram unless otherwise specified) for groups of 10 animals. P values vs the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977)
*P<0 05
**P<0 01

IV. DISCUSSION AND CONCLUSIONS

The effects of dietary exposure to D&C Yellow No 11 on rats and mice were similar, but the effects on mice were not as severe, even though mice consumed twice as much compound as did rats on a gram per kilogram basis D&C Yellow No 11 did not reduce survival of F344/N rats or B6C3F$_1$ mice in the current studies at dietary concentrations up to 50,000 ppm (estimated daily intake, 4 g/kg in rats and 9 9 g/kg in mice) or of Charles River rats (10,000 ppm in feed) and beagle dogs (250 mg/kg) in short-term (Hazleton, 1962a,c,d) or 1-year studies (Hazleton, 1967a,b) There was no significant difference in feed consumption between groups in the current 13-week studies, and body weights were only slightly depressed (high dose rats, 7%, high dose male mice, 6%) Body weights were significantly (P<0 05) lower in high dose Charles River rats after 1 year on a 10,000-ppm D&C Yellow No 11 diet, but there was also a reduction in feed consumption recorded for at least the first 13 weeks, suggesting an unpalatable feed-dye mixture (Hazleton, 1967a) In other studies, beagles refused to eat feed containing D&C Yellow No 11 and the investigators had to complete the studies using encapsulated dye (Hazleton, 1967b)

The primary target organs of dietary D&C Yellow No 11 exposure were the liver in rats and mice and the kidney in rats Increased liver weights were observed for rats and mice of each sex at the end of the 13-week studies In F344/N rats of each sex, there was an apparent dose-related increase in liver weight up to the 5,000-ppm concentration, liver weights did not increase further at higher concentrations In disposition studies, the percentage of D&C Yellow No 11 absorbed by F344/N rats remained constant up to 380 ppm but was decreased at the next highest concentration (4,100 ppm) (El Dareer et al , 1988) Decreasing absorption of D&C Yellow No 11 at higher dietary concentrations

may explain, in part, the lack of further increase in liver weights seen in the current studies, but additional, higher dose disposition studies are needed to understand this effect more fully. In mice, D&C Yellow No. 11 produced a dose-related increase in liver weights at concentrations as high as 50,000 ppm. No absorption or disposition information for mice was found in the literature.

Microscopically, hepatocellular degeneration in rats progressed slightly in severity with the longer exposure in the 13-week studies, but the lesion was qualitatively the same as in the 14-day studies. A similar response was not observed in mice. Both rats and mice of each sex had yellow-brown pigment in hepatocytes, Kupffer cells, and the biliary epithelium of the liver. The yellow-brown pigment was most likely D&C Yellow No. 11, because special stains for bile, hemosiderin, and lipofuscin were negative. There was also no histologic evidence of increased red cell destruction. This conclusion concerning the identity of the pigment is also supported by rat disposition studies, which show that liver and kidney accumulate radioactivity after an intravenous dose of [^{14}C]D&C Yellow No. 11 and that parent compound and/or metabolites are eliminated in bile and urine (El Dareer et al., 1988). Increased liver weights were reported in rats from another 13-week feed study (Hansen et al., 1960). Microscopic evaluations showed that liver lesions from all exposed animals were similar to those described for the current studies (Hazleton, 1962c,d, 1967a,b).

In the 14-day and 13-week studies, rats of each sex, but not mice, also had yellow-brown pigment in the tubular epithelium of the kidney. Pigment deposition in the epithelium of the convoluted tubules of the kidney was consistently reported in Charles River rats and beagle dogs exposed to this dye in the feed (Hazleton, 1962c,d, 1967a,b). However, in the current studies, cytoplasmic alteration, characterized by increased size and number of irregularly shaped hyaline droplets in the renal tubular epithelium, was observed in the male F344/N rats but not seen in females; it was not reported in other studies with Charles River rats or beagle dogs. These droplets were morphologically similar to the abnormal accumulation of irregularly shaped hyaline droplets containing $\alpha_{2\mu}$-globulin, which is typically seen in chlorinated hydrocarbon nephropathy (e.g., tetrachlorobenzene [NTP, 1990a] and pentachlorobenzene [NTP, 1990b]), and they occurred only in male F344/N rats. However, it is noteworthy that, in contrast to the typical hydrocarbon nephropathy, renal tubular regeneration/necrosis, granular casts, and homogeneous protein casts in the tubules were not observed.

In F344/N rat perinatal toxicity studies, D&C Yellow No. 11 exposure decreased body weight gain of dams at birth and postnatally. Exposed dams gained weight more slowly than did controls during the 4 weeks preceding mating, through gestation, and lactation, and weights were significantly lower at weaning. In spite of the body weight effect, the dye did not affect fertility, litter size, pup survival, or mean pup birth weight. At 4 weeks of age, pups nursing from dams receiving feed containing D&C Yellow No. 11 had lower group mean body weights than those of controls. After weaning, pups were given the same dietary concentrations as their dams. At 8 weeks of age, body weights of low dose (5,000 ppm) rats were similar to those of control pups, but body weights of pups exposed at higher concentrations (17,000 or 50,000 ppm) remained lower than those of the controls. These rather marked weight effects indicate that rats are more sensitive to D&C Yellow No. 11 when dosing begins with perinatal exposure than when dosing begins at 6-7 weeks of age Microscopic evaluation showed that the lesions in the pups were similar to those described for liver and kidney of rats in the 14-day and 13-week studies, including the male rat kidney cytoplasmic alteration. It is not clear at what stage of development the effect on the F_1 occurs. D&C Yellow No. 11 is mutagenic in bacteria and mammalian cells in culture (Moore et al., 1985; Meyer et al., 1986; Zeiger et al., 1988). Thus, there could be an effect on germ cells or in utero development of the F_1, but pup birth weight and litter size of dosed dams were not different from those of control dams. Significant decreases in body weights were not detected until pups were 4 weeks of age, suggesting either an effect on pup growth rate of perinatal exposure or reduced

food consumption (e g , because of a decreased level of lactation or reduced milk/feed palatability) During the lactation period, there was potential for D&C Yellow No 11 exposure for litters initially through milk and later through a combination of milk and of feed provided to the dams

In summary, compound related effects were seen at all dietary concentrations of D&C Yellow No 11 in the current studies Liver weights were increased at all doses in both rats and mice Minimal hepatocellular degeneration was seen in rats receiving dietary concentrations of 1,700 ppm and above and in mice at 5,000 ppm and above In addition, hyaline droplets were seen in the tubular epithelial cells of the kidney in all dosed male rats Compound-related effects were also seen in all dosed rats in the perinatal toxicity studies Body weights of rat pups were lower than those of controls, and lesions in liver (both sexes) and kidney (males only) were similar to those seen in the 14-day and 13-week studies

V. REFERENCES

1 Asada, M , Galambos, J T (1963) Sorbitol dehydrogenase and hepatocellular injury An experimental and clinical study Gastroenterology 44 578

2 Bergmeyer, H U Scheibe, A W , Wahlefeld, A W (1978) Optimization of methods for aspartate aminotransferase and alanine aminotransferase Clin Chem 24 58-73

3 Bjorkner, B , Magnusson, B (1981) Patch test sensitization to D & C Yellow No 11 and simultaneous reaction to Quinoline Yellow Contact Dermatitis 7 1-4

4 Bjorkner, B , Niklasson, B (1983) Contact allergic reaction to D & C Yellow No 11 and Quinoline Yellow Contact Dermatitis 9 263

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

6 Calnan, C D (1981) Quinazoline yellow dermatitis (D and C Yellow 11) in an eye cream Contact Dermatitis 7 271

7 Ceriotti, G (1983) Ornithine carbamoyltransferase. Measurement in serum Bergmeyer, H U , Bergmeyer, J , Grassl, M , Eds Methods of Enzymatic Analysis, Vol 3 Enzymes 1 Oxidoreductases, Transferases, 3rd ed Moss, D W , Ed Consultant Verlag Chemie Weinheim, Federal Republic of Germany, p 319

8 Colour Index (1982) 3rd ed Pigments and Solvent Dyes The Society of Dyers and Colourists, pp 147-148

9 The Cosmetic, Toiletry and Fragrance Association (CTFA) Cosmetic Ingredient Dictionary (1982) 3rd ed Estrin, N F , Crosley, P A , Haynes, C R , Eds Washington, DC The Cosmetic, Toiletry and Fragrance Association, Inc , p 72

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

11 El Dareer, S M , Kalin, J R , Tillery, K F , Hill, D L (1988) Disposition of 2-(2-quinolyl)-1,3-indandione (D C Yellow #11) in rats dosed orally or intravenously J Toxicol Environ Health 23 385-393

12 Gershbein, L L (1982) Action of dyes and indicators on rat-liver regeneration. Food Chem Toxicol 20 1-8

13 Hansen, W H , Wilson, D C , Fitzhugh, O G (1960) Subacute oral toxicity of ten D&C coal-tar colors Fed Proc 19 390

14 Hazleton Laboratories, Inc (1962a) Acute Oral Administration--Albino Rats File 9, Entry 130 Falls Church, VA

15 Hazleton Laboratories, Inc (1962b) Acute Oral Administration--Dogs File 9, Entry 131 Falls Church, VA

16 Hazleton Laboratories, Inc (1962c) Six-Week Range Finding Study- Rats File 9, Entry 133 Falls Church, VA

17 Hazleton Laboratories, Inc (1962d) Subacute Dietary Administration Dogs File 9, Entry 137 Falls Church, VA

18 Hazleton Laboratories, Inc (1965) Repeated Dermal Application--Rabbits Final Report D&C Blue 6, D&C Green 6, D&C Red 33, D&C Red 30, and D&C Yellow 11 Falls Church, VA

19 Hazleton Laboratories, Inc (1967a) One-Year Dietary Feeding--Albino Rats Entry 142 Falls Church, VA

20 Hazleton Laboratories, Inc (1967b) One Year Repeated Oral Administration--Dogs Final Report D&C Yellow 11 Falls Church, VA

21 Hazleton Laboratories, Inc (1967c) Lifetime Repeated Topical Application- Mice Final Report D&C Blue 6, D&C Green 6, D&C Red 30, D&C Red 33, and D&C Yellow 11 Falls Church, VA

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

23 Jordan, W P , Jr (1981) Contact dermatitis from D & C Yellow 11 dye in a toilet bar soap J Am Acad Dermatol 4 613-614

24 Kita, S , Kobayashi, T , Kutsuna, H , Kligman, A M (1984) Human maximization testing of D&C Yellow No 10 and Yellow No 11 Contact Dermatitis 11 210-213

25 Lamson, S A , Kong, B M , De Salva, S J (1982) D&C Yellow Nos 10 and 11 Delayed contact hypersensitivity in the guinea pig Contact Dermatitis 8 200-203

26 Marmion, D M (1984) Handbook of U S Colorants for Foods, Drugs, and Cosmetics, 2nd ed New York John Wiley & Sons, Inc , pp 12-15, 88-89

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

28 Matsunaga, K , Hosokawa, K , Suzuki, M , Arima, Y , Hayakawa, R (1988) Occupational allergic contact dermatitis in beauticians Contact Dermatitis 18 94-96

29 Meyer, M , Brock, K , Lawrence, K , Casto, B , Moore, M M (1986) Evaluation of the effect of agar on results obtained in the L5178Y mouse lymphoma assay Environ Mutagen 8 727-740

30 Moore, M M , Allen, J W , Claxton, L D , Westbrook-Collins, B , Doerr, C , Gwaltney, C , Loud, K , Kohan, M , Lawrence, K , Templeton, R (1985) Genotoxicity of C I Solvent Yellow No 33 and a C I Solvent Green No 3--C I Solvent Yellow mixture Environ Mutagen 7(Suppl 3) 66

31 Morrissey, R E , Schwetz, B A , Lamb, J C , IV, Ross, M.D , Teague, J L , Morris, R W (1988) Evaluation of rodent sperm, vaginal cytology, and reproductive organ weight data from National Toxicology Program 13-week studies Fundam Appl Toxicol 11 343-358

32 National Institute for Occupational Safety and Health (NIOSH) (1989) National Occupational Exposure Survey (NOES), 1981-1983

33 National Toxicology Program (NTP) (1990a) Toxicity Studies of 1,2,4,5-Tetrachlorobenzene in F344/N Rats and B6C3F$_1$ Mice NTP Toxicity Report No 7 U S Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC 43 p

34. National Toxicology Program (NTP) (1990b) Toxicity Studies of Pentachlorobenzene in F344/N Rats and B6C3F$_1$ Mice. NTP Toxicity Report No. 6. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. 48 p.

35. Palazzolo, M.J.; DiPasquale, L.C. (1983) The sensitization potential of D & C Yellow No. 11 in guinea pigs. Contact Dermatitis 9:367-371.

36. Rapaport, M.J. (1984) Allergy to yellow dyes. Arch. Dermatol. 120:535-536.

37. Sato, Y.; Kutsuna, H.; Kobayashi, T.; Mitsui, T. (1984) D & C Nos. 10 and 11: Chemical composition analysis and delayed contact hypersensitivity testing in the guinea pig. Contact Dermatitis 10:30-38.

38. Schmidt, E.; Schmidt, F.W. (1983) Glutamate dehydrogenase. Bergmeyer, H.U.; Bergmeyer, J.; Grassl, M., Eds: Methods of Enzymatic Analysis, Vol. 3. Enzymes 1: Oxidoreductases, Transferases, 3rd ed. Moss, D.W., Ed. Consultant. Verlag Chemie: Weinheim, Federal Republic of Germany, p. 216.

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

40. Sun, J.D.; Henderson, R.F.; Marshall, T.C.; Cheng, Y.-S.; Dutcher, J.S.; Pickrell, J.A.; Mauderly, J.L.; Hahn, F.F.; Banas, D.A.; Seiler, F.A.; Hobbs, C.H. (1987) The inhalation toxicity of two commercial dyes: Solvent Yellow 33 and Solvent Green 3. Fundam. Appl. Toxicol. 8:358-371.

41. U.S. Code of Federal Regulations (USCFR) (1988) CFR 21:74.1711.

42. Weaver, J.E. (1983) Dose response relationships in delayed hypersensitivity to quinoline dyes. Contact Dermatitis 9:309-312.

43. Zeiger, E.; Anderson, B.; Haworth, S.; Lawlor, T.; Mortelmans, K. (1988) *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. Environ. Molec. Mutagen. 11 (Suppl. 12):1-158.

APPENDIX

ORGAN WEIGHTS AND SERUM CHEMISTRY DATA IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO. 11

		PAGE
TABLE A1	ORGAN WEIGHTS OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO. 11	29
TABLE A2.	SERUM ENZYME ACTIVITY AND MALE REPRODUCTIVE SYSTEM DATA FOR RATS IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO. 11	30
TABLE A3.	ORGAN WEIGHTS OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO 11	31
TABLE A4.	SERUM ENZYME ACTIVITY AND MALE REPRODUCTIVE SYSTEM DATA FOR MICE IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO. 11	32

TABLE A1. ORGAN WEIGHTS OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO. 11 (a)

Organ	Control	500 ppm	1,700 ppm	5,000 ppm	17,000 ppm	50,000 ppm
MALE						
Body weight (grams)	337 ± 3 6	332 ± 5 8	318 ± 10 8	326 ± 1 9	**316 ± 3 2	**309 ± 2 4
Brain						
Absolute	1,976 ± 17	1,935 ± 27	1,932 ± 20	1,967 ± 30	1,946 ± 9	1,962 ± 17
Relative	5 9 ± 0 08	5 8 ± 0 09	6 1 ± 0 17	6 0 ± 0 08	*6 2 ± 0 08	**6 4 ± 0 07
Heart						
Absolute	1,032 ± 22	1,003 ± 32	934 ± 44	1,055 ± 29	976 ± 26	1,077 ± 69
Relative	3 1 ± 0 07	3 0 ± 0 07	2 9 ± 0 08	3 2 ± 0 08	3 1 ± 0 06	3 5 ± 0 24
Kidney						
Absolute	1,174 ± 18	*1,084 ± 32	1,130 ± 25	1,141 ± 20	(b) 1,112 ± 16	(b) 1,104 ± 19
Relative	3 5 ± 0 04	3 3 ± 0 06	3 6 ± 0 08	3 5 ± 0 05	(b) 3 5 ± 0 05	(b) 3 6 ± 0 06
Liver						
Absolute	13,670 ± 270	*15,010 ± 360	**16,190 ± 730	**17,190 ± 340	**16,760 ± 380	**16,360 ± 270
Relative	40 6 ± 0 92	**45 2 ± 0 59	**50 9 ± 1 00	**52 7 ± 0 80	**52 9 ± 0 73	**52 9 ± 0 74
Lung						
Absolute	1,551 ± 45	1 623 ± 55	1,564 ± 70	1,623 ± 65	*1,779 ± 83	*1 749 ± 81
Relative	4 6 ± 0 16	4 9 ± 0 15	4 9 ± 0 13	*5 0 ± 0 20	**5 6 ± 0 29	**5 7 ± 0 29
Right testis						
Absolute	1,485 ± 36	1,412 ± 39	1,429 ± 16	1,443 ± 21	1,444 ± 19	1,496 ± 42
Relative	4 4 ± 0 08	4 3 ± 0 13	4 5 ± 0 13	4 4 ± 0 06	*4 6 ± 0 07	**4 8 ± 0 15
Thymus						
Absolute	234 ± 13	**326 ± 27	219 ± 27	**318 ± 20	*274 ± 18	**314 ± 19
Relative	0 69 ± 0 041	**0 98 ± 0 075	0 68 ± 0 065	**0 98 ± 0 068	*0 87 ± 0 056	**1 01 ± 0 060
FEMALE						
Body weight (grams)	192 ± 2 6	191 ± 2 6	189 ± 2 3	188 ± 2 6	**180 ± 1 4	**176 ± 2 1
Brain						
Absolute	1 797 ± 27	1,785 ± 19	1,828 ± 16	1,820 ± 22	1,810 ± 16	1,788 ± 17
Relative	9 4 ± 0 17	9 3 ± 0 10	9 7 ± 0 09	9 7 ± 0 08	**10 0 ± 0 12	**10 2 ± 0 11
Heart						
Absolute	644 ± 14	673 ± 16	643 ± 11	677 ± 12	643 ± 13	611 ± 17
Relative	3 4 ± 0 06	3 5 ± 0 07	3 4 ± 0 05	*3 6 ± 0 03	3 6 ± 0 06	3 5 ± 0 08
Kidney						
Absolute	667 ± 13	646 ± 7	648 ± 5	669 ± 16	*623 ± 10	**599 ± 11
Relative	3 5 ± 0 07	3 4 ± 0 05	3 4 ± 0 04	3 6 ± 0 08	3 5 ± 0 04	3 4 ± 0 03
Liver						
Absolute	6,519 ± 149	*6,976 ± 129	**7,833 ± 158	**8,398 ± 145	**8,436 ± 152	**8,151 ± 248
Relative	34 0 ± 0 96	**36 5 ± 0 42	**41 5 ± 0 54	**44 6 ± 0 70	**46 8 ± 0 68	**46 2 ± 1 37
Lung						
Absolute	1 116 ± 28	1,122 ± 26	1,124 ± 22	1,182 ± 56	1,128 ± 37	1,121 ± 31
Relative	5 8 ± 0 12	5 9 ± 0 16	6 0 ± 0 13	6 3 ± 0 30	6 3 ± 0 21	*6 4 ± 0 16
Thymus						
Absolute	250 ± 8	239 ± 12	226 ± 8	239 ± 13	224 ± 5	*224 ± 8
Relative	1 3 ± 0 04	1 2 ± 0 05	1 2 ± 0 05	1 3 ± 0 06	1 3 ± 0 03	1 3 ± 0 05

(a) Mean ± standard error (absolute in milligrams, relative in milligrams per gram unless otherwise specified) for groups of 10 animals unless otherwise specified, P values vs the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977)
(b) Kidneys of nine animals were weighed
*P<0 05
**P<0 01

TABLE A2. SERUM ENZYME ACTIVITY AND MALE REPRODUCTIVE SYSTEM DATA FOR RATS IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO. 11 (a)

Analysis	Control	500 ppm	1,700 ppm	5,000 ppm	17,000 ppm	50,000 ppm
MALE						
Alanine aminotransferase (IU/liter)	68 1 ± 2 29	**47 9 ± 2 76	58 5 ± 5 09	89 1 ± 15 19	53 9 ± 2 25	58 5 ± 4 75
Glutamic dehydrogenase (IU/liter)	1 7 ± 0 50	2 7 ± 0 81	2 2 ± 0 66	4 0 ± 0 64	1 8 ± 0 34	1 9 ± 0 62
Ornithine carbamoyltransferase (IU/liter)	9 5 ± 1 39	5 8 ± 0 65	6 8 ± 1 06	9 6 ± 1 59	7 3 ± 1 08	8 0 ± 1 06
Sorbitol dehydrogenase (IU/liter)	13 1 ± 1 33	13 6 ± 1 03	15 2 ± 1 72	16 0 ± 1 86	14 3 ± 0 76	15 3 ± 1 21
Caudal weight (mg)	173 ± 6	167 ± 5		(b) 170 ± 6		170 ± 8
Right epididymis (mg)	446 ± 8	450 ± 10		(b) 442 ± 12		448 ± 12
Abnormal sperm (percent)	1 08 ± 0 120	1 06 ± 0 123		(b) 0 96 ± 0 109		1 04 ± 0 183
Sperm density ($\times 10^6$)	655 ± 41	709 ± 38		(b) 705 ± 44		662 ± 37
Sperm motility (percent)	97 2 ± 0 35	97 3 ± 0 71		(b) 97 3 ± 0 49		(b) 96 3 ± 0 89
FEMALE						
Alanine aminotransferase (IU/liter)	46 7 ± 3 24	40 1 ± 0 89	58 5 ± 3 76	*64 1 ± 6 12	*63 4 ± 5 42	46 7 ± 3 12
Glutamic dehydrogenase (IU/liter)	(b) 4 0 ± 0 41	3 5 ± 0 58	4 3 ± 0 29	(b) 4 3 ± 0 61	4 4 ± 0 41	4 2 ± 0 44
Ornithine carbamoyltransferase (IU/liter)	5 8 ± 1 28	(b) 6 5 ± 1 24	**18 0 ± 2 82	**14 2 ± 1 76	**13 1 ± 1 48	**9 4 ± 1 81
Sorbitol dehydrogenase (IU/liter)	(b) 10 3 ± 0 78	11 1 ± 0 64	**15 9 ± 1 03	**19 0 ± 2 33	**16 7 ± 2 41	**14 1 ± 1 41

(a) Mean ± standard error for groups of 10 animals unless otherwise specified, P values vs the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977), IU = international units
(b) Nine animals were examined
*P<0 05
**P<0 01

TABLE A3. ORGAN WEIGHTS OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO. 11 (a)

Organ	Control	500 ppm	1,700 ppm	5,000 ppm	17,000 ppm	50,000 ppm
MALE						
Body weight (grams)	31 8 ± 0 88	30 9 ± 0 69	30 1 ± 0 49	30 7 ± 0 71	30 2 ± 0 45	*29 5 ± 0 35
Brain						
Absolute	447 ± 4	440 ± 8	453 ± 3	454 ± 6	449 ± 6	455 ± 7
Relative	14 0 ± 0 40	14 3 ± 0 30	*15 1 ± 0 27	14 8 ± 0 36	14 9 ± 0 31	**15 4 ± 0 29
Heart						
Absolute	163 ± 7	167 ± 5	158 ± 4	168 ± 4	169 ± 4	156 ± 3
Relative	5 2 ± 0 29	5 4 ± 0 18	5 3 ± 0 14	5 5 ± 0 12	5 6 ± 0 13	5 3 ± 0 11
Kidney						
Absolute	292 ± 7	298 ± 5	294 ± 6	*328 ± 9	304 ± 8	294 ± 7
Relative	9 3 ± 0 38	9 7 ± 0 24	9 8 ± 0 13	**10 7 ± 0 28	*10 1 ± 0 21	*10 0 ± 0 23
Liver						
Absolute	1 640 ± 31	**1,839 ± 37	**1 924 ± 33	**2,150 ± 48	**2,186 ± 52	**2,272 ± 62
Relative	51 9 ± 1 45	**59 8 ± 1 43	**64 0 ± 1 12	**70 0 ± 0 82	**72 4 ± 1 20	**76 9 ± 1 46
Lung						
Absolute	213 ± 8	235 ± 10	221 ± 9	214 ± 9	222 ± 12	225 ± 13
Relative	6 7 ± 0 26	7 6 ± 0 38	7 3 ± 0 25	7 0 ± 0 29	7 3 ± 0 33	7 6 ± 0 46
Right testis						
Absolute	123 ± 3	121 ± 6	115 ± 2	118 ± 2	123 ± 2	117 ± 4
Relative	3 9 ± 0 18	3 9 ± 0 17	3 8 ± 0 06	3 9 ± 0 08	4 1 ± 0 05	4 0 ± 0 12
Thymus						
Absolute	40 7 ± 3 62	37 9 ± 2 07	35 7 ± 2 09	39 7 ± 1 79	39 5 ± 1 82	(b) 36 5 ± 1 42
Relative	1 3 ± 0 12	1 2 ± 0 05	1 2 ± 0 06	1 3 ± 0 06	1 3 ± 0 06	(b) 1 2 ± 0 06
FEMALE						
Body weight (grams)	25 4 ± 0 42	26 0 ± 0 33	26 6 ± 0 32	25 7 ± 0 54	26 5 ± 0 48	26 6 ± 0 30
Brain						
Absolute	461 ± 4	464 ± 5	463 ± 4	452 ± 6	464 ± 5	454 ± 8
Relative	18 2 ± 0 25	17 9 ± 0 12	*17 4 ± 0 25	17 6 ± 0 29	17 6 ± 0 34	*17 1 ± 0 32
Heart						
Absolute	128 ± 3	*140 ± 3	**142 ± 3	**149 ± 6	**147 ± 5	**150 ± 5
Relative	5 0 ± 0 15	5 4 ± 0 14	5 3 ± 0 16	**5 8 ± 0.18	*5 5 ± 0 17	*5 6 ± 0 17
Kidney						
Absolute	187 ± 3	*201 ± 5	*202 ± 6	*205 ± 6	*198 ± 3	**206 ± 5
Relative	7 4 ± 0 21	7 8 ± 0 19	7 6 ± 0 24	8 0 ± 0 15	7 5 ± 0 12	7 7 ± 0 15
Liver						
Absolute	1 316 ± 42	**1 599 ± 44	**1,737 ± 33	**1,909 ± 74	**2 134 ± 95	**2,066 ± 56
Relative	51 8 ± 1 44	**61 6 ± 1 83	**65 3 ± 1 04	**74 2 ± 2 19	**80 7 ± 3 39	**77 5 ± 1 52
Lung						
Absolute	195 ± 11	203 ± 10	213 ± 6	218 ± 17	236 ± 19	209 ± 6
Relative	7 7 ± 0 49	7 8 ± 0 38	8 0 ± 0 21	8 5 ± 0 69	8 9 ± 0 62	7 8 ± 0 20
Thymus						
Absolute	4 59 ± 2 88	48 5 ± 1 87	48 4 ± 2 32	45 0 ± 2 85	44 5 ± 3 81	47 1 ± 1 45
Relative	1 8 ± 0 13	1 9 ± 0 07	1 8 ± 0 08	1 8 ± 0 12	1 7 ± 0 14	1 8 ± 0 05

(a) Mean ± standard error (absolute in milligrams, relative in milligrams per gram unless otherwise specified) for groups of 10 animals unless otherwise specified, P values vs the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977)
(b) Thymuses of nine animals were weighed
*P<0 05
**P<0 01

TABLE A4. SERUM ENZYME ACTIVITY AND MALE REPRODUCTIVE SYSTEM DATA FOR MICE IN THE THIRTEEN-WEEK FEED STUDIES OF D&C YELLOW NO. 11 (a)

Analysis	Control	500 ppm	1,700 ppm	5,000 ppm	17,000 ppm	50,000 ppm
MALE						
Number examined (b)	10	10	10	10	8	10
Alanine aminotransferase (IU/liter)	(c) 32.1 ± 2.41	(d) 29 4 ± 1.73	34 1 ± 1.91	(d) 39.5 ± 2.20	34.5 ± 1.81	**46.0 ± 3.25
Ornithine carbamoyltransferase (IU/liter)	(e) 1.5 ± 0.53	(c) 1.9 ± 0 82	(f) 3 0 ± 0.87	(c) 2.7 ± 0.80	(c) 2.2 ± 0.96	(d) 1.5 ± 0.48
Sorbitol dehydrogenase (IU/liter)	(f) 27.0 ± 1.90	(f) 28 4 ± 1.42	31.3 ± 1.11	27.9 ± 1.52	(g) 29.4 ± 2.25	(f) 28.6 ± 1.80
Abnormal sperm (percent)	1 32 ± 0.112	1.44 ± 0 136		(f) 1.58 ± 0.131		1.48 ± 0.147
Caudal weight (mg)	18 ± 1	18 ± 1		18 ± 0		(f) 18 ± 1
Right epididymis (mg)	47 ± 1	48 ± 0		47 ± 1		(f) 48 ± 1
Sperm density ($\times 10^6$)	1,252 ± 62	1,387 ± 97		(f) 1,083 ± 66		1,170 ± 75
Sperm motility (percent)	92 3 ± 0.67	90 5 ± 1 20		*88.7 ± 1.19		**82.3 ± 2.58
FEMALE						
Number examined (b)	10	10	10	9	10	10
Alanine aminotransferase (IU/liter)	(f) 23.7 ± 1.47	*28 1 ± 1.22	**32 0 ± 2.82	**40.1 ± 6.76	**37.9 ± 3.98	**38.9 ± 3.83
Glutamic dehydrogenase (IU/liter)	(h) 2 7 ± 1.17	(i) 3 1 ± 0 84	(j) 2 7 ± 0.09	(i) 2.5 ± 0.23	(e) 3.5 ± 0.58	(d) 2.8 ± 0.21
Ornithine carbamoyltransferase (IU/liter)	1.3 ± 0.20	1 5 ± 0 18	(f) 2 3 ± 0.52	2.4 ± 0.47	**2.9 ± 0.53	*2.1 ± 0.36
Sorbitol dehydrogenase (IU/liter)	24.0 ± 1.32	24 7 ± 1.33	*29 0 ± 1.56	(g) 28.2 ± 1.72	26.3 ± 1.58	*30.9 ± 2.44

(a) Mean ± standard error; P values vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977), IU = international units.
(b) Unless otherwise specified
(c) Seven animals were examined.
(d) Eight animals were examined
(e) Five animals were examined
(f) Nine animals were examined.
(g) Ten animals were examined.
(h) Three animals were examined.
(i) Six animals were examined.
(j) Four animals were examined.
*P<0.05
**P<0 01