NTP TECHNICAL REPORT

ON THE

TOXICITY STUDIES OF

CASTOR OIL

IN F344/N RATS AND B6C3F$_1$ MICE

(DOSED FEED STUDIES)

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NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
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March 1992

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

NOTE TO THE READER

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. In July, 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from the Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

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The studies described in this toxicity study 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.

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TOXICITY STUDIES

of

CASTOR OIL

(CAS No.: 8001-79-4)

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The NTP report on the toxicity studies of castor oil is based on 13-week studies that began in April, 1988, and were completed in July, 1988, at Microbiological Associates, Inc., Bethesda, MD.

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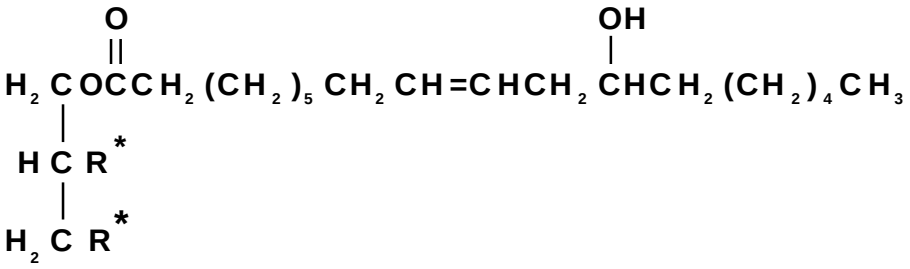
CASTOR OIL



* R indicates an ester of one of the fatty acids listed above.

CAS Number: 8001-79-4

Composition: Triglyceride of fatty acids. Fatty acid composition is approximately 87% ricinoleic, 7% oleic, 3% linoleic, 2% palmitic, 1% stearic, and trace amounts of dihydroxystearic.

Synonyms: Ricinus Oil, oil of Palma Christi, tangantangan oil, phorboyl, Neoloid.

ABSTRACT

Castor oil is a natural oil derived from the seeds of the castor bean, Ricinus communis. It is comprised largely of triglycerides with a high ricinolin content. Toxicity studies with castor oil were performed by incorporating the material at concentrations as high as 10% in diets given to F344/N rats and B6C3F$_1$ mice of both sexes for 13 weeks. Genetic toxicity studies also were performed and were negative for mutation induction in *Salmonella typhimurium*, for induction of sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells, and for induction of micronuclei in the peripheral blood erythrocytes of mice evaluated at the end of the 13-week studies.

Exposure to castor oil at dietary concentrations as high as 10% in 13-week studies did not affect survival or body weight gains of rats or mice (10 per sex and dose). There were no biologically significant effects noted in hematologic analyses in rats. Mild increases in total bile acids and in serum alkaline phosphatase were noted at various times during the studies in rats receiving the higher dietary concentrations of castor oil. Liver weights were increased in male rats receiving the 10% dietary concentration and in male and female mice receiving diets containing 5% or 10% castor oil. However, there were no histopathologic lesions associated with these liver changes, nor were there any compound-related morphologic changes in any organ in rats or mice. No significant changes were noted in a screening for male reproductive endpoints, including sperm count and motility, and no changes were observed in the length of estrous cycles of rats or mice given diets containing castor oil. Thus, no significant adverse effects of castor oil administration were noted in these studies.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of castor oil on November 20, 1990, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members act to determine if the design and conditions of the NTP studies were appropriate and to ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

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SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY STUDIES OF CASTOR OIL

On November 20, 1990, the draft toxicity study report on castor oil received public review by the Technical Reports Review Subcommittee and associated Panel of Experts of the National Toxicology Program's Board of Scientific Counselors. The review meeting was held at the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina.

Dr. Richard Irwin, NIEHS, reviewed the design and findings of the castor oil studies. The panel had no comments other than those concerning editorial matters, and Dr. Scala accepted the report on behalf of the panel.

I. INTRODUCTION

Castor oil (ricinus oil, phorboyl, tangantangan oil) is a natural oil derived from the seeds of the castor bean by cold pressing (for medicinal use) or hot pressing (for industrial purposes). Chemically, castor oil is a triglyceride characterized by a high content of ricinolein (a glyceride of 12-hydroxy-9-octadecenoic acid). It has the approximate fatty acid composition of ricinoleic acid (87%), oleic acid (7%), linoleic acid (3%), palmitic acid (2%), stearic acid (1%), with trace amounts of dihydroxystearic acid (Windholz, 1976).

Well-known medicinally as a cathartic, castor oil has had a wide variety of industrial applications: as a drying oil for paints, varnishes, plastics, and resins; in the manufacture of fatty acids; and as a lubricating oil. It also is an ingredient in numerous cosmetics.

Castor oil is considered minimally toxic when administered orally to humans; the estimated lethal oral dose is 1-2 pints of undiluted oil (Gosselin *et al.*, 1976). Several instances of sensitization to castor oil in cosmetics have been reported, including an allergic reaction to a make-up remover (Brandle *et al.*, 1983), and contact dermatitis caused by use of a lipstick containing castor oil (Sai, 1983). Hypersensitivity reactions such as angioedema, rhinitis, asthma, and scarlatiniform rashes have been reported in factory workers involved in the extraction of castor oil (Blacow, 1972; Juhlin, 1983; Mitchell, 1979), or in association with ingesting it (McGuire *et al.*, 1983).

Relatively few studies of castor oil toxicity have been conducted with experimental animals, and no studies were located concerning its absorption, distribution, metabolism, or excretion. Daily application of 0.5 ml of castor oil to the skin of adult female albino rabbits produced mild irritant reactions, including slight erythema and edema, acanthosis and disorganization of the basal layer, and slight inflammation of the dermis (Rantuccio *et al.*, 1981). Subcutaneous injection of 0.1 ml of castor oil in adult C57Bl/6 mice, daily for 4 weeks, was associated with the presence of electron dense lipid inclusions in parenchymal cells of the zona fasciculata of the adrenal gland (Migally, 1979). Gavage administration of 1 ml/kg to rhesus monkeys, daily for 4 days, caused mild morphological changes in the small intestine, characterized by lipid droplets along the mucosal epithelium and in the underlying lamina propria (Diener and Sparano, 1968). This was considered a possible indication that castor oil had reduced lipid metabolism in the intestinal epithelium.

Because of widespread human exposure, large annual production and use, and the lack of studies characterizing the effect of exposures of moderate duration, the subchronic toxicity of castor oil was evaluated by administering diet formulations to F344/N rats and B6C3F_1 mice for 13 weeks.

II. MATERIALS AND METHODS

Procurement and Characterization of Castor Oil

USP AA grade castor oil was obtained in one lot (#L-5G30-01) from Cas Chemical, Inc. (Bayonne, NJ). Purity and identity analyses were conducted by Midwest Research Institute (MRI) (Kansas City, MO). MRI reports on the analyses performed in support of the castor oil studies are on file at the National Institute of Environmental Health Sciences (Research Triangle Park, NC).

The study material was identified as castor oil by infrared, ultraviolet/visible and nuclear magnetic resonance spectroscopy. All spectra were consistent with the structure and composition of castor oil. The study material was analyzed by Karl Fischer water analysis, thin-layer and high performance liquid chromatography, and a battery of U.S. Pharmacopeia (USP) standard analyses for castor oil. Cumulative data indicated a purity consistent with the USP specifications and the reported composition for castor oil.

The stability of the study material during the toxicology studies was monitored by determination of peroxide content and by high performance liquid chromatography. No deterioration of the castor oil study material was observed over the course of the studies.

Preparation and Characterization of Formulated Diets

Formulated diets were prepared by blending the appropriate amount of castor oil with a small quantity of feed to prepare a premix. The premix then was layered between the required amount of feed in a twin-shell blender and blended for 15 minutes to achieve a uniform mix. The homogeneity of castor oil in feed at 10% (100 mg/g) was determined by gravimetric analysis, and blends at 0.5% (5 mg/g) were determined by HPLC analysis. These concentrations of chemical in feed were found to be homogeneously distributed by this mixing procedure. The stability of the 0.5% dose level was determined using HPLC; it was found to be stable for at least 21 days when stored in the dark at 5°C and for 3 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 5°C; feed hoppers in the animal cages were changed twice weekly.

Periodic analysis of the castor oil-formulated diets was conducted by HPLC at the study and analytical chemistry laboratories. Three complete sets of formulated diet mixtures were analyzed by either the study laboratory or the analytical laboratory during the 13-week studies. All but a single sample were within specifications (±10% of the target concentration) (Table 1). A single low-dose mixture which did not meet specifications was remixed and found to be within specifications before it was given to the animals. The results of the analyses for all dose mixtures given to the animals ranged from 97% to 106% of the target concentrations. A single referee analysis conducted by the analytical laboratory agreed with the results obtained by the study laboratory.

TABLE 1 Results of Analysis of Formulated Diets in the 13-Week Feed Studies of Castor Oil

Target Concentration (% w/w)	Determined Concentration (% w/w) (a)
0.62	0.62 ± 0.06
1.25	1.26 ± 0.09
2.50	2.64 ± 0.09
5.00	4.91 ± 0.05
10.00	9.67 ± 0.54

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

13-Week Study Design

Source and Specifications of Study Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Simonsen Laboratories (Gilroy, CA) and were quarantined and acclimated to laboratory conditions for 14 days prior to study start. Near the end of the quarantine period, 5 randomly selected rats and mice were killed and examined grossly for disease and the presence of ecto and endo parasites; none were found. On day 13 of the quarantine period, the remaining animals were stratified by weight and randomized into dosed and control groups.

Experimental Design

Rats were housed 5 per cage; mice were housed individually. Polycarbonate cages lined with heat-treated hardwood chips and covered with polyester filter sheets were used for both species; the cages were stored on stainless steel racks equipped with an automatic watering system. Temperature in the animal room was maintained within 68-76°F; relative humidity ranged from 42% to 72%. Incoming air was filtered to remove particulates, and a flow rate was maintained to ensure complete exchange at least 10 times per hour. A controlled light cycle of 12 hours of daylight and 12 hours of darkness was maintained. Control feed or diet formulations of castor oil were available *ad libitum*; feeders were changed twice per week throughout the study.

The core study was conducted with groups of 10 rats and 10 mice per sex, each group receiving diets containing 0, 0.62%, 1.25%, 2.5%, 5.0% or 10% castor oil, continuously for 13 weeks. Ten additional rats/sex were included at each dose level for evaluation of hematological and clinical chemistry parameters. At days 5 and 21, these animals were anesthetized with CO_2, and blood was collected from the orbital sinus. These animals were killed following the blood collection on day 21. Blood samples for hematology and clinical chemistry also were collected from core-study rats at study termination.

Clinical Examinations, Supplemental Studies, and Pathology

At the study termination, all core-study animals were euthanized by CO_2 anesthesia, and complete necropsies were performed. Complete histopathology examinations were conducted on all rats and mice from the control and 10% dose groups. Livers were examined from male rats in all other dose groups; histologic sections of gross lesions were examined from all rats. Organ weights were determined to the nearest milligram for the liver, right kidney, right testicle, heart, thymus, and lungs. All tissues were preserved in 10% neutral buffered formalin. Details of pathology procedures are outlined in Table 2.

On days 5 and 21, and at sacrifice, blood samples were collected from the orbital sinus of non-fasted animals under CO_2 anesthesia. Hematology parameters were determined on a Baker 9000 automated hematology analyzer (J.T. Baker, Phillipsburg, NJ). Analyses included red blood cell (RBC) count, red blood cell morphologic assessment, hematocrit (HCT), hemoglobin concentration (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), white blood cell differential count, reticulocyte count (absolute), and platelet counts (absolute). Clinical chemistry assays, performed with a Centrifichem 600 (J.T. Baker, Phillipsburg, NJ) using commercially available kits and standard methods developed for this instrument, included alkaline phosphatase activity (ALP), albumin (ALB), urea nitrogen (UN), creatinine (CREA), alanine aminotransferase activity (ALT), total bile acids (TBA), sorbitol dehydrogenase activity (SDH), total protein (TP), and creatinine kinase (CK).

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, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed; the results were reviewed and evaluated by the NTP.

Reproductive Toxicity Screen

To screen for potential reproductive toxicity, sperm motility and morphology were evaluated at necropsy, and vaginal cytology was evaluated on core-study animals during the week just preceding necropsy, following published procedures (Morrissey *et al*., 1988). For the 12 days prior to termination, females were subject to vaginal lavage with saline. The aspirated cells were air-dried onto slides, stained with Toluidine Blue O, and coverslipped. The relative preponderance of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were used to identify the stages of the estrual cycle.

Sperm motility was evaluated at necropsy as follows: The left epididymis was removed and quickly weighed; the cauda epididymis was removed at the junction of the vas deferens and the corpus epididymis, then weighed. A small cut was made in the distal cauda epididymis. The sperm that were removed from the epididymis were dispersed and the number of moving and non-moving sperm were counted in 5 fields of 30 sperm or less on each slide. After

sperm sampling for motility evaluation, the cauda was placed in phosphate buffered saline (PBS), and gently chopped with a razor blade. The solution was mixed gently and heat-fixed at 65°C. Sperm density was then determined using a hemocytometer.

The left 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% DMSO. Homogenization spermatid nuclei were enumerated using a hemocytometer; the data were expressed as spermatid heads per total testis and per gram of testis.

Statistical Methods

Body weight and organ weight data were statistically analyzed within each sex by one-way Analysis of Variance tests, followed by Dunnett's t-test if pair-wise comparisons were indicated ($p < 0.05$)(Dunnett, 1955).

Quality Assurance

The studies of castor oil were performed in compliance with FDA Good Laboratory Practices Regulations (21 CFR 58). All laboratory methods and procedures were conducted in accordance with the appropriate Quality Assurance Programs of Microbiological Associates, Inc., or Pathology Associates, Inc. The operations of the Quality Assurance Units were monitored by the NTP.

TABLE 2 Summary of Experimental Design and Materials and Methods in the 13-Week Dosed Feed Studies of Castor Oil

Date of Studies
April - July, 1988

Strain and Species
F344/N rats; B6C3F$_1$ mice

Animal Source
Simonsen Laboratories, Gilroy, CA

Method of Animal Distribution
Animals weight-randomized into groups by sex, assigned to cages, and cages assigned to dose groups.

Chemical Source
Cas Chemical, Inc.

Diet
NIH 07; available *ad libitum*

Study Laboratory
Microbiological Associates, Inc., Bethesda, MD

Animal Room Environment
Temp--68-76°F; relative humidity--42-72%; fluorescent light 12 h/d; 10 room air changes/h

Size of Study Groups
10 males and 10 females of each species. Rats were housed 5 per cage, and mice were individually caged.

Time Held Before Study
Rats -- 14 d, mice -- 15 d

Concentrations
0, 0.62, 1.25, 2.5, 5.0, and 10.0% in feed

Age When Placed on Study
6 wk

Type and Frequency of Observation
Observed 2 X d, weighed initially and 1 x wk thereafter.

Age When Killed
19 wk

Necropsy and Histologic Examinations
The following tissues were routinely processed for preparation of histologic sections and microscopic examination: adrenal glands, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testes or ovaries/uterus, esophagus, eyes (if grossly abnormal), femur (including marrow), heart, ileum, jejunum, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pituitary gland, preputial or clitoral glands, rectum, salivary glands, skin, spinal cord and sciatic nerve (if neurologic signs present), spleen, forestomach and glandular stomach, thymus, thyroid gland, trachea, urinary bladder, zymbal glands, and all gross lesions and tissue masses including regional lymph nodes. A complete histopathologic examination was conducted on all rats and mice from the control and 10% dose groups. Liver was examined from male rats in all other dose groups, and histologic sections of gross lesions were examined from all rats.

III. RESULTS

F344/N Rats

Exposure to castor oil in the diet at concentrations up to 10% had no effect on survival of F344/N rats. No significant differences in average food consumption among each sex were observed, although food consumption of male and female rats receiving diets containing 10% castor oil was slightly lower than that of controls (Table 3).

Group mean body weights of rats receiving diets containing castor oil did not differ significantly from controls (Figure 1). Mean body weights of exposed female rats were slightly lower than the mean body weights of controls but the differences were not dose-related.

TABLE 3 Survival and Average Food and Compound Consumption of F344/N Rats in the 13-Week Feed Studies of Castor Oil

Dose (% in feed)	Survival (a)	Mean Body Weight (grams) Initial	Final	Change	Final Weight Relative to Controls (%)	Feed Consumption (b)	Compound Consumption (c)
MALE							
0	10/10	132	364	233		65	0
0.62	10/10	130	346	216	95.0	65	404
1.25	10/10	126	359	233	98.6	65	809
2.5	10/10	131	356	226	97.8	63	1583
5.0	10/10	131	351	220	96.4	61	3067
10.0	10/10	129	353	224	97.0	58	5835
FEMALE							
0	10/10	108	208	100		64	0
0.62	10/10	108	202	95	97.1	65	401
1.25	10/10	107	205	97	98.6	64	797
2.5	10/10	109	202	93	97.1	63	1569
5.0	10/10	110	206	96	99.0	61	3045
10.0	10/10	108	197	89	94.7	57	5725

(a) Number surviving/number initially in group.
(b) Average grams food consumed per kg body weight per day.
(c) Average mg compound consumed per kg body weight per day.

Hematological effects of the castor oil diets among male rats included a slight decrease in MCHC at day 21 in those receiving the 10% diet; a statistically significant decrease in MCV among the 10% group; a decrease in MCH among the 5% and 10% groups; and an increase in platelets among the 1.25%, 5%, and 10% groups. The only change observed among female rats was a statistically significant decrease in reticulocyte counts at day 5 in groups receiving the 0.62% or 10% diets (Table 4). None of these changes was considered biologically significant.

A treatment- and dose-related increase in the activity of serum alkaline phosphatase was observed in male and female rats at days 5 and 21, and at study termination. Total bile acids

were increased among males receiving the higher dietary levels at days 5 and 21 but were not increased at study termination. Other minor changes included increases in albumin observed at study termination in males receiving 5% diets and at day 5 in females receiving 10% diets, and an increase in urea nitrogen at study termination in males that received 0.62% diets and a decrease at day 5 in females that received castor oil at 10% in the diet (Table 5).

TABLE 4 Selected Hematological Data for F344/N Rats in the 13-Week Feed Studies of Castor Oil (a)

Analysis	Dose (% in Feed) 0	0.62	1.25	2.5	5.0	10.0
MALE						
Mean Corpuscular Volume (μm^3)						
Day 21	62.1 ± 1.5	62.8 ± 1.8	62.5 ± 1.3	63.4 ± 1.5	63.6 ± 1.2	63.9 ± 1.5
Day 90	54.2 ± 0.8	53.5 ± 0.6	53.8 ± 0.4	54.1 ± 0.7	53.6 ±1.0	52.7 ± 0.9*
Mean Corpuscular Hemoglobin (pg)						
Day 21	21.6 ± 0.6	21.5 ± 0.7	21.2 ± 0.5	21.7 ± 0.6	21.9 ± 0.6	21.5 ± 0.7
Day 90	18.1 ± 0.4	17.8 ± 0.4	18.0 ± 0.3	18.0 ± 0.5	17.7 ± 0.3*	17.6 ± 0.2*
Mean Corpuscular Hemoglobin Concentration (%)						
Day 21	34.7 ± 0.7	34.3 ± 0.3	34.0 ± 0.6	34.2 ± 0.8	34.5 ± 0.8	33.7 ± 0.6*
Day 90	33.4 ± 0.9	33.4 ± 0.6	33.5 ± 0.6	33.2 ± 1.1	32.9 ± 0.7	33.5 ± 0.7
Platelet Count (x $10^3/mm^3$)						
Day 21	721 ± 50	730 ± 49	735 ± 43	734 ± 44	719 ± 73	702 ± 41
Day 90	557 ± 27	572 ± 36	599 ± 45*	593 ± 30	612 ± 34*	632 ± 2*
FEMALE						
Reticulocytes (x $10^3/mm^3$)						
Day 5	413 ± 93	296 ± 111*	360 ± 77	424 ± 95	328 ± 91	205 ± 52*

(a) Results presented as mean ± standard deviation.
* Statistically significantly different from control group using ANOVA and Dunnett's test ($p < 0.05$).

Absolute liver weights and the liver-to-body-weight ratio were increased in male rats that received diets containing 10% castor oil. Heart-to-body-weight ratios were increased in groups of male rats receiving 0.62%, 2.5%, and 10% diets; however, absolute heart weights were not increased, and the differences in body weight ratios were small and not considered treatment related (Table 6). Using light microscopy, it was determined there were no morphologic changes associated with the slight differences in organ weights between groups.

In male rats, there was a slight decrease in epididymal weight (6-7%) which occurred in the middle- and high-dose groups, but this was not dose-related. There were no effects on any other male rat reproductive endpoint, or on any female rat reproductive endpoint. Although there was some variation in epididymal weights, their small magnitude and the absence of changes in other endpoints suggested that there was little or no evidence of any reproductive toxicity associated with castor oil exposure (Appendix A). Histopathologic examination revealed an absence of compound-related lesions in any organ or tissue of rats exposed to castor oil in the diet.

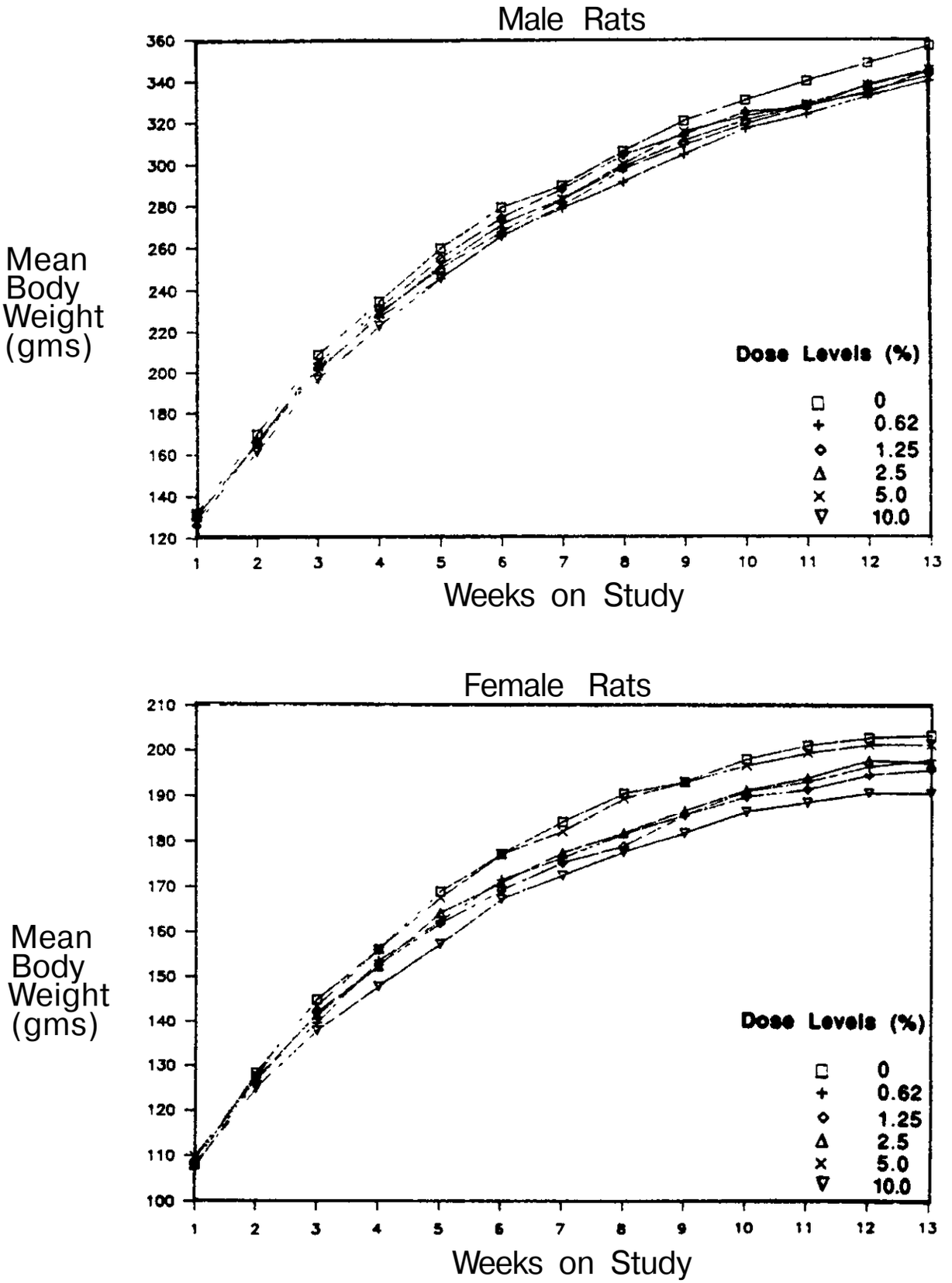

Figure 1 Body Weights of F344/N Rats Exposed to Castor Oil by Dosed Feed For 13 Weeks

TABLE 5 Selected Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Studies of Castor Oil (a)

Analysis	Dose (% in Feed) 0	0.62	1.25	2.5	5.0	10.0
MALE						
Total Bile Acids (µMol/L)						
Day 5	10 ± 1	16 ± 8	18 ± 7*	11 ± 5	20 ± 7*	18 ± 10*
Day 21	7 ± 1	11 ± 4	9 ± 4	6 ± 1	13 ± 6 *	13 ± 7*
Day 90	10 ± 3	6 ± 2	6 ± 3	8 ± 3	9 ± 3	11 ± 7
Alkaline Phosphatase Activity (IU/L)						
Day 5	524 ± 30	548 ± 54	577 ± 51	610 ± 54*	710 ± 65*	795 ± 120*
Day 21	434 ± 38	442 ± 44	465 ± 36	495 ± 40	455 ± 113	539 ± 54*
Day 90	200 ± 25	243 ± 26*	232 ± 15	256 ± 32*	265 ± 35*	298 ± 36*
Albumin (g/dL)						
Day 5	4.3 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.3 ± 0.1	4.3 ± 0.2	4.4 ± 0.2
Day 21	4.4 ± 0.1	4.4 ± 0.1	4.4 ± 0.1	4.4 ± 0.2	4.4 ± 0.1	4.5 ± 0.1
Day 90	4.4 ± 0.1	4.4 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.5 ± 0.1*	4.5 ± 0.1*
Urea Nitrogen (mg/dL)						
Day 5	18.2 ± 2.3	16.9 ± 1.8	18.0 ± 1.8	16.1 ± 2.8	16.6 ± 2.0	15.8 ± 1.6
Day 21	18.6 ± 1.8	18.3 ± 1.9	17.6 ± 2.1	17.3 ± 1.7	18.0 ± 1.7	16.4 ± 2.1
Day 90	19.8 ± 1.2	22.0 ± 1.9*	18.8 ± 1.1	20.8 ± 2.1	19.4 ± 1.2	18.5 ± 1.5
FEMALE						
Total Bile Acids (µMol/L)						
Day 5	11 ± 4	16 ± 9	19 ± 14	12 ± 5	13 ± 5	14 ± 3
Day 21	14 ± 6	15 ± 10	16 ± 12	15 ± 8	17 ± 8	15 ± 8
Day 90	22 ± 19	25 ± 15	22 ± 14	22 ± 14	17 ± 8	27 ± 11
Alkaline Phosphatase Activity (IU/L)						
Day 5	444 ± 35	449 ± 61	485 ± 32	515 ± 54*	574 ± 47*	616 ± 51*
Day 21	348 ± 25	328 ± 36	342 ± 30	360 ± 22	388 ± 27*	420 ± 31*
Day 90	184 ± 19	191 ± 23	221 ± 30*	214 ± 27	253 ± 35*	268 ± 25*
Albumin (g/dl)						
Day 5	4.3 ± 0.2	4.3 ± 0.2	4.4 ± 0.2	4.4 ± 0.2	4.4 ± 0.2	4.5 ± 0.1 *
Day 21	4.5 ± 0.1	4.6 ± 0.1	4.5 ± 0.2	4.5 ± 0.1	4.6 ± 0.2	4.7 ± 0.2
Day 90	4.9 ± 0.2	5.0 ± 0.1	4.9 ± 0.2	4.9 ± 0.4	4.9 ± 0.2	5.0 ± 0.2
Urea Nitrogen(mg/dL)						
Day 5	17.8 ± 1.5	17.9 ± 1.3	18.7 ± 1.4	17.4 ± 2.7	16.7 ± 0.6	14.8 ± 2.2*
Day 21	18.2 ± 1.8	17.7 ± 1.9	17.4 ± 2.2	17.3 ± 1.3	16.6 ± 1.6	16.4 ± 1.8
Day 90	19.1 ± 1.1	20.5 ± 1.9	20.0 ± 2.0	20.3 ± 2.5	19.0 ± 1.4	18.9 ± 1.2

(a) Results presented as mean ± standard deviation.

* Statistically significantly different from control group using ANOVA and Dunnett's test ($p<0.05$).

TABLE 6. Selected Organ Weights of Male F344/N Rats in the 13-Week Feed Studies of Castor Oil (a)

Organ	Dose (% in Feed)					
	0	0.62	1.25	2.5	5.0	10.0
Body weight	364.4 ± 20.3	345.9 ± 13.6	359.2 ± 15.4	356.4 ± 17.2	351.0 ± 19.2	352.9 ± 15.1
Heart						
Absolute	0.99 ± 0.07	1.04 ± 0.05	1.03 ± 0.07	1.03 ± 0.08	1.00 ± 0.07	1.027 ± 0.05
Relative (b)	2.72	3.01	2.87	2.89	2.85	2.91
Liver						
Absolute	12.54 ± 1.15	12.37 ± 0.74	12.81 ± 1.2	12.98 ± 0.88	12.49 ± 1.02	14.31 ± 0.86*
Relative (b)	34.41	35.76	35.66	36.42	35.58	40.55

(a) Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard deviation).
(b) Changes in relative organ weights were not analyzed statistically.
* Statistically significant difference from control group using ANOVA and Dunnett's test ($p \leq 0.05$).

B6C3F$_1$ Mice

Exposure to castor oil in the diet at concentrations up to 10% had no effect on survival of male or female B6C3F$_1$ mice. There were no statistically significant differences in average food consumption among groups of a given sex and species, although food consumption by female mice receiving diets containing 10% castor oil was slightly lower than controls (Table 7).

Mean body weights of exposed male mice generally were lower than controls, while mean body weights of exposed females generally were higher. There were no obvious indications that these differences were related to dietary concentrations of castor oil, except that mean body weights of male mice receiving the 10% castor oil diet were consistently lower than those of control mice from week 3 through the end of the study (Figure 2).

Liver weights were increased in male and female mice that received diets containing 5% or 10% castor oil. Kidney weights were increased in female mice that received 5% or 10% diets (Table 8). Using light microscopy, it was determined that there were no morphologic changes associated with the slight differences between groups in organ weights. Histopathologic examination revealed an absence of compound-related lesions in any organs or tissues of mice exposed to castor oil in the diet.

Castor oil exposure produced no adverse effects on any male (testes weight, epididymal sperm motility, density, or testicular spermatid head count) or female (estrual cycle length, or time spent in each phase of the cycle) reproductive parameter among mice. The low value for sperm motility in control mice was attributed to poor preparative technique (Appendix A).

TABLE 7 Average Food and Compound Consumption of B6C3F$_1$ Mice in the 13-Week Feed Studies of Castor Oil

Dose (% in feed)	Survival (a)	Mean Body Weight (grams) Initial	Final	Change	Final Weight Relative to Controls (%)	Feed Consumption (b)	Compound Consumption (c)
MALE							
0	10/10	22.7	33.7	11.0		146	0
0.62	10/10	22.9	32.7	9.8	97.0	148	917
1.25	10/10	23.0	32.9	9.9	97.6	162	2022
2.5	10/10	22.6	33.8	11.2	101.0	152	3800
5.0	10/10	22.7	32.6	9.9	96.7	157	7823
10.0	10/10	22.7	30.5	7.8	90.5	150	15017
FEMALE							
0	10/10	17.5	26.6	9.1		184	0
0.62	10/10	17.7	28.3	10.6	106.4	186	1153
1.25	9/10 (d)	17.7	29.3	11.6	110.2	183	2282
2.5	10/10	17.2	28.5	11.3	107.1	200	5009
5.0	10/10	17.4	28.0	10.6	105.3	193	9627
10.0	10/10	17.5	28.6	11.1	107.5	168	16786

(a) Number surviving/number initially in group.
(b) Average grams food consumed per kg body weight per day.
(c) Average mg compound consumed per kg body weight per day.
(d) Not a treatment-related death.

TABLE 8 Selected Organ Weights of B6C3F$_1$ Mice in the 13-Week Feed Studies of Castor Oil (a)

Organ	Dose (% in Feed) 0	0.62	1.25	2.5	5.0	10.0
MALE						
Body weight	33.7 ± 3.8	32.7 ± 2.5	32.9 ± 2.6	33.8 ± 3.3	32.6 ± 2.7	30.5 ± 2.7
Liver						
Absolute	1.59 ± 0.17	1.53 ± 0.15	1.64 ± 0.11	1.73 ± 0.14	1.81 ± 0.21*	1.85 ± 0.18*
Relative(b)	47.18	46.79	49.85	51.18	55.52	60.66
FEMALE						
Body weight	26.6 ± 2.4	28.3 ± 2.2	29.3 ± 3.1	28.5 ± 2.5	28.0 ± 1.9	28.6 ± 1.3
Kidney						
Absolute	0.20 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.23 ± 0.02*	0.23 ± 0.02*
Relative (b)	7.52	7.77	7.17	7.37	8.21	8.04
Liver						
Absolute	1.28 ± 0.11	1.39 ± 0.14	1.47 ± 0.15	1.42 ± 0.14	1.57 ± 0.20*	1.71 ± 0.09*
Relative (b)	48.12	49.12	50.17	49.82	56.07	59.79

(a) Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard deviation).
(b) Changes in relative organ weights were not analyzed statistically.
* Statistically significant difference from control group using ANOVA and Dunnett's test ($p \leq 0.05$).

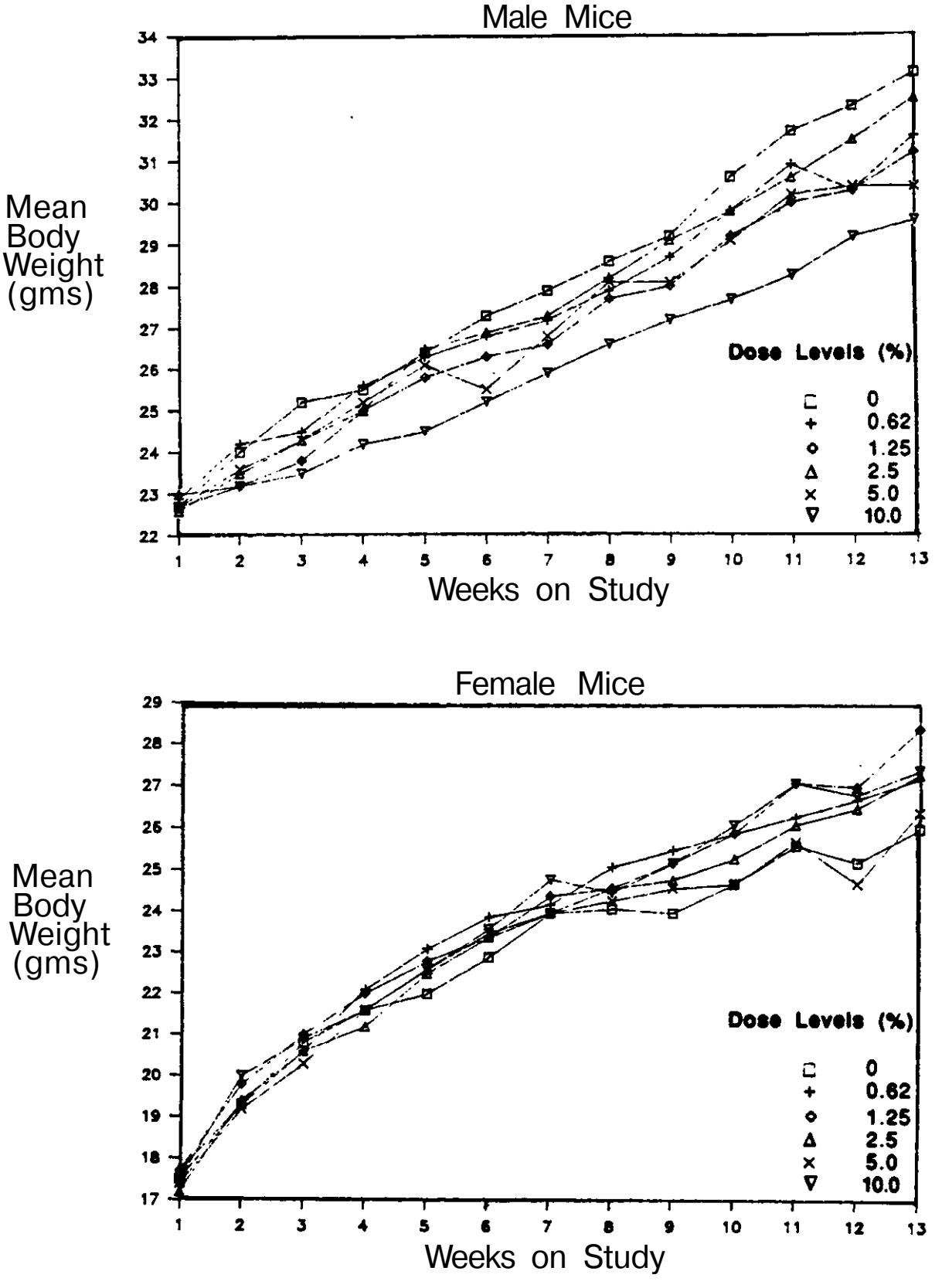

FIGURE 2 Body Weights of B6C3F1 Mice Exposed to Castor Oil by Dosed Feed For 13 Weeks

Genetic Toxicity

In genetic toxicity studies, castor oil (100-10,000 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA97, or TA98 when tested with a preincubation protocol in the presence and the absence of exogenous metabolic activation (S9) (Zeiger *et al.*, 1988) (Appendix B). Castor oil did not induce sister-chromatid exchanges or chromosome aberrations in Chinese hamster ovary cells treated with concentrations up to 5000 µg/ml with and without S9 (Appendix B). No induction of micronuclei was observed in peripheral blood erythrocytes of male and female B6C3F_1 mice sampled at the termination of the 13-week study (Appendix B).

IV. DISCUSSION AND CONCLUSIONS

The cathartic action of orally ingested castor oil traditionally has been attributed to irritant or stimulatory effects of ricinoleic acid on the gastrointestinal smooth muscle; the ricinoleic acid is liberated in the small intestine by the action of pancreatic lipase (Stewart and Bass, 1976). As a purgative, castor oil is ingested as a bolus. Since this would lead to higher concentrations of ricinoleic acid in the gastrointestinal tract than would occur with dietary exposure, it is not surprising that in the present study there were no indications of loose or wet feces.

Exposure to diets containing up to 10% castor oil was associated with only minimal indications of toxicity in F344/N rats and $B6C3F_1$ mice. Absolute liver weights and liver-to-body-weight ratios increased in male rats and in both sexes of mice receiving diets that contained the higher concentrations of castor oil. These increases, however, were not accompanied by corresponding histopathologic lesions or alterations in clinical chemical endpoints that would indicate hepatotoxicity. Since castor oil is composed of triacylglycerols, the increased liver weights could be a reflection of elevated metabolic activity associated with increased lipid absorption, rather than a toxic response. This conclusion is consistent with the observed increases of total bile acids in serum of male rats and of alkaline phosphatase activity in the serum of both sexes of rats. Bile acids and alkaline phosphatase (intestinal form) are both involved with intestinal absorption and metabolism of lipids, and the serum concentrations are normally increased in association with ingestion of a lipid-rich diet (Young *et al*., 1981; Loeb and Quimbey, 1989).

In general, food consumption by groups receiving castor oil diets was comparable to food consumption of controls, with only a slight reduction being observed in the groups receiving the 10% diets. In spite of the elevated intake of dietary lipid, body weights of most dosage groups that received castor oil were not markedly different from body weights of control animals.

Ricinoleic acid, the major fatty acid present in castor oil, has a variety of effects on the gastrointestinal tract, including inhibition of water and electrolyte absorption (Donowitz, 1979), stimulation of water secretion into the intestinal lumen (Ammon and Phillips, 1974), and depression of small bowel contractile activity (Ammon *et al*., 1974). Moreover, absorption of ricinoleic acid occurs incompletely; substantial quantities remain in the gastrointestinal tract after oral administration (Stewart and Bass, 1976). Since diet palatability was not affected by the presence of castor oil, the poor absorption of ricinoleic acid and its potential to reduce absorption of other fatty acids could be responsible for the absence of more substantial body weight gains by rats and mice consuming castor oil-containing diets.

In conclusion, castor oil was found not to be mutagenic or clastogenic in several *in vitro* genetic toxicity assays, and administration of diets containing up to 10% castor oil was not associated with toxicity to any specific organ, organ system, or tissue in this study.

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APPENDIX A

Results of Reproductive System Analyses in the 13-Week Feed Studies of Castor Oil

TABLE A1 Reproductive System Data for F344/N Rats in the 13-Week Feed Studies of Castor Oil

	Percent in Feed			
	0	2.5	5	10
MALE (a)				
Left caudal weight (mg)	151 ± 3	153 ± 7	145 ± 4	153 ± 5
Left epididymal weight (mg)	502 ± 11	498 ± 10	464 ± 10 *	476 ± 7
Left testis (mg)	1,539 ± 21	1,550 ± 31	1,463 ± 53	1,492 ± 29
Sperm count (x106)/gram testis	72.8 ± 0.5	65.9 ± 0.5	71.7 ± .5	77.5 ± 0.7
Sperm motility (percent)	73.6 ± 2.3	65.9 ± 5.6	72.1 ± 4.1	69.8 ± 2.3
FEMALE (b)				
Estrous stage (percent)				
Proestrus	12.5	14.2	15.8	16.7
Estrous	28.3	32.5	25.8	25.8
Metestrous	18.3	19.2	18.3	19.2
Diestrous	40.8	34.2	39.2	38.3
Not clear or no cells observed	0.0	0.0	0.8	0.0
Cycle Length (days)	5.0 ± 0.0	5.1 ± 0.1	5.2 ± 0.1	5.1 ± 0.1

(a) Mean ± standard error for groups of 10 animals; no significant difference vs. the controls by Dunn's test (Dunn, 1964).
(b) Mean for groups of 10 animals unless otherwise specified
* Significantly different from control groups by Shirley's test (Shirley, 1977); $p < 0.05$.

TABLE A1 Reproductive System Data for B6C3F$_1$ Mice in the 13-Week Feed Studies of Castor Oil

	Percent in Feed			
	0	2.5	5	10
MALE (a)				
Left caudal weight (mg)	15 ± 1	13 ± 1	16 ± 1	16 ± 1
Left epididymal weight (mg)	45 ± 1	46 ± 2	46 ± 2	44 ± 1
Left testis (mg)	121 ± 3	120 ± 3	121 ± 2	119 ± 2
Sperm count (x10^6)/gram testis	179.2 ± 0.9	162.4 ± 0.9	170.1 ± 1.0	158.3 ± 1.0
Sperm motility (percent)	39.2 ± 10.0	53.7 ± 8.2	45.4 ± 8.0	52.2 ± 8.2
FEMALE (b)				
Estrous stage (percent)				
Proestrus	12.5	14.2	15.8	16.7
Estrous	28.3	32.5	25.8	25.8
Metestrous	18.3	19.2	18.3	19.2
Diestrous	40.8	34.2	39.2	38.3
Not clear or no cells observed	0.0	0.0	0.8	0.0
Cycle Length (days)	5.0 ± 0.0	5.1 ± 0.1	5.2 ± 0.1	5.1 ± 0.1

(a) Mean ± standard error for groups of 10 animals; no significant difference vs. the controls by Dunn's test (Dunn, 1964).
(b) Mean for groups of 10 animals unless other wise specified

APPENDIX B

Results of Genetic Toxicity Analyses

TABLE B1 Mutagenicity of Castor Oil in *Salmonella typhimurium* (a)

Strain	Dose (µg/plate)	Revertants/plate (b) (-) S9 Trial 1	(-) S9 Trial 2	Hamster S9 10%	Hamster S9 30%	Rat S9 10%	Rat S9 30%
TA100	0	110 ± 8.7	92 ± 10.3	131 ± 14.4	89 ± 7.2	126 ± 5.9	86 ± 3.1
	100	130 ± 14.6	91 ± 4.2	142 ± 3.5	116 ± 7.0	159 ± 4.4	84 ± 9.9
	333	127 ± 13.5	98 ± 6.9	143 ± 11.9	91 ± 5.2	122 ± 14.8	89 ± 1.7
	1000	127 ± 10.7	81 ± 8.7	154 ± 1.5	94 ± 3.5	139 ± 17.8	88 ± 10.5
	3333	109 ± 12.8	98 ± 7.3	139 ± 11.7	86 ± 6.9	132 ± 13.3	84 ± 4.4
	10000	101 ± 5.4	83 ± 6.6	132 ± 13.1	93 ± 3.0	154 ± 3.9	86 ± 15.3
Trial Summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		461 ± 44.5	437 ± 5.5	1245 ± 92.4	555 ± 16.3	614 ± 39.7	524 ± 30.6
TA1535	0	21 ± 2.4	25 ± 4.7	8 ± 0.3	10 ± 1.5	7 ± 0.6	10 ± 1.9
	100	24 ± 1.5	20 ± 2.5	9 ± 0.3	12 ± 2.2	11 ± 2.1	11 ± 1.2
	333	25 ± 2.4	19 ± 4.2	12 ± 0.7	10 ± 2.8	8 ± 0.3	9 ± 2.4
	1000	23 ± 1.0	24 ± 3.3	10 ± 1.2	11 ± 1.5	9 ± 1.5	6 ± 0.3
	3333	24 ± 3.2	22 ± 2.3	9 ± 0.7	8 ± 2.9	9 ± 0.3	4 ± 0.3
	10000	22 ± 1.9	25 ± 3.5	8 ± 0.7	8 ± 2.0	8 ± 0.7	12 ± 0.9
Trial Summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		433 ± 6.1	435 ± 9.3	498 ± 12.2	544 ± 34.6	215 ± 9.9	167 ± 13.5
TA97	0	121 ± 3.2	102 ± 4.7	162 ± 2.3	139 ± 3.5	173 ± 11.7	180 ± 13.7
	100	133 ± 3.2	127 ± 8.4	177 ± 2.7	159 ± 11.8	180 ± 9.5	189 ± 9.4
	333	125 ± 8.0	131 ± 3.2	173 ± 13.9	163 ± 6.4	179 ± 11.3	191 ± 4.5
	1000	141 ± 6.1	137 ± 3.2	156 ± 10.8	172 ± 14.6	152 ± 8.5	171 ± 9.8
	3333	157 ± 6.9	129 ± 6.8	179 ± 11.0	150 ± 8.7	155 ± 10.4	184 ± 4.7
	10000	140 ± 12.0	134 ± 6.1	183 ± 6.4	140 ± 7.0	166 ± 8.4	169 ± 9.0
Trial Summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		902 ± 44.4	1058 ± 81.4	1748 ± 53.5	1163 ± 22.3	1213 ± 19.9	1053 ± 37.0
TA98	0	15 ± 0.6	14 ± 2.3	30 ± 0.6	25 ± 2.3	26 ± 2.9	30 ± 1.2
	100	12 ± 1.0	12 ± 0.3	28 ± 2.2	26 ± 3.2	30 ± 1.9	27 ± 1.2
	333	14 ± 2.6	11 ± 1.2	27 ± 2.3	19 ± 1.5	30 ± 3.8	27 ± 2.8
	1000	15 ± 0.3	13 ± 2.7	32 ± 2.4	19 ± 0.7	31 ± 2.6	22 ± 4.8
	3333	14 ± 2.3	15 ± 0.9	31 ± 3.5	17 ± 2.0	27 ± 3.2	29 ± 0.6
	10000	14 ± 1.2	12 ± 0.9	32 ± 5.0	22 ± 2.5	26 ± 6.6	26 ± 0.6
Trial Summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		872 ± 8.5	619 ± 41.0	959 ± 17.6	282 ± 30.2	333 ± 11.8	298 ± 13.2

(a) Study performed at SRI, International. The detailed protocol and these data are presented in Zeiger *et al.* (1988). Cells and study compound or solvent (dimethylsulfoxide) 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 to 10 mg/plate; 0 µg/plate dose is the solvent control.

(b) Revertants are presented as mean ± the standard error from 3 plates.

(c) Positive control; 2-aminoanthracene was used for all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-o-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA97.

TABLE B2 Induction of Sister-Chromatid Exchanges in Chinese Hamster Ovary Cells by Castor Oil (a)

Dose (µg/ml)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo some	SCEs/ Cell	Hrs in BrdU	Increase Over Control % (b)
-S9 (c)							
Summary: Negative							
Dimethylsulfoxide							
	50	1049	394	0.37	7.9	26.0	
Castor oil							
160	50	1050	379	0.36	7.6	26.0	-3.90
500	50	1049	429	0.40	8.6	26.0	8.88
1600	50	1050	422	0.40	8.4	26.0	7.00
5000	50	1050	430	0.40	8.6	26.0	9.03
Mitomycin-C (e)							
0.0005	50	1048	509	0.48	10.2	26.0	29.31
0.0050	10	210	278	1.32	27.8	26.0	252.46
			TREND (f):	1.78			
			PROBABILITY:	0.037			
+S9 (d)							
Summary: Negative							
Dimethylsulfoxide							
	50	1049	404	0.38	8.1	26.0	
Castor oil							
160	50	1050	402	0.38	8.0	26.0	-0.59
500	50	1049	363	0.34	7.3	26.0	-10.15
1600	50	1050	427	0.40	8.5	26.0	5.59
5000	50	1049	399	0.38	8.0	26.0	-1.24
Cyclophosphamide (e)							
0.10	50	1049	531	0.50	10.6	26.0	31.44
0.60	10	210	228	1.08	22.8	26.0	181.91
			TREND (f):	0.244			
			PROBABILITY:	0.403			

(a) Study performed at Environmental Health Research & Testing, Inc. SCE = sister-chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway *et al.* (1985, 1987). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethylsulfoxide) 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/chromosome of culture exposed to study chemical relative to those of culture exposed to solvent.

(c) In the absence of S9, cells were incubated with study compound or solvent for 2 h at 37°C. Then BrdU was added and incubation was continued for 24 h. Cells were washed, fresh medium containing BrdU and colcemid was added, and incubation was continued for 3 h.

(d) In the presence of S9, cells were incubated with study compound or solvent for 2 h at 37°C. The cells were then washed, and medium containing BrdU without study compound was added. Cells were incubated for a further 26 h, with colcemid present for the final 2-3 h. S9 was from the livers of Aroclor 1254-induced male Sprague Dawley rats.

(e) Positive controls.

(f) Statistics performed on SCE/chromosome values.

TABLE B3 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Castor Oil (a)

-S9 (b)					+S9 (c)				
Dose (mg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (mg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Harvest time: 12.0 hours					**Harvest time: 13.0 hours**				
Dimethylsulfoxide					Dimethylsulfoxide				
	200	2	0.01	1.0		200	3	0.02	1.5
Castor oil					Castor oil				
1600	200	1	0.01	0.5	1600	200	4	0.02	2.0
3000	200	2	0.01	1.0	3000	200	4	0.02	2.0
5000	200	1	0.01	0.5	5000	200	3	0.02	1.5
Summary: Negative					Summary: Negative				
Mitomycin-C (d)					Cyclophosphamide (d)				
0.0625	200	48	0.24	15.5	2.50	200	44	0.22	16.0
0.2500	50	16	0.32	26.0	7.50	50	18	0.36	30.0
TREND(e):	-0.344				TREND(e):	0.012			
PROBABILITY:	0.634				PROBABILITY:	0.495			

(a) Study performed at Environmental Health Research & Testing, Inc. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al.* (1985, 1987). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethylsulfoxide) 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 10 h at 37°C. Cells were then washed and fresh medium containing colcemid was added for an additional 3 h followed by harvest.

(c) In the presence of S9, cells were incubated with study compound or solvent for 2 h at 37°C. Cells were then washed, medium without test compound was added, and incubation was continued for 10 h. Colcemid was added for the last 3 h of incubation before harvest. S9 was from the livers of Aroclor 1254-induced male Sprague Dawley rats.

(d) Positive controls.

(e) Statistics performed on % cells with Abs.

TABLE B4 Frequency of Micronuclei in Peripheral Blood Erythrocytes of B6C3F$_1$ Mice Exposed to Castor Oil in Dosed Feed for 13 Weeks (a)

Percent in feed	% Normochromatic erythrocytes with micronuclei (b)	% Polychromatic erythrocytes with micronuclei (b)	Number of mice
Male mice:			
0	0.11 ± 0.02	1.20 ± 0.08	10
0.6	0.13 ± 0.02	1.18 ± 0.10	10
1.3	0.11 ± 0.01	1.16 ± 0.09	10
2.5	0.13 ± 0.01	1.24 ± 0.10	10
5.0	0.09 ± 0.02	1.40 ± 0.11	9
10.0	0.09 ± 0.01	1.21 ± 0.08	10
Female mice:			
0	0.10 ± 0.01	1.18 ± 0.07	10
0.6	0.09 ± 0.01	1.21 ± 0.10	10
1.3	0.07 ± 0.01	1.11 ± 0.08	9
2.5	0.09 ± 0.02	1.11 ± 0.08	10
5.0	0.09 ± 0.01	1.49 ± 0.18	10
10.0	0.06 ± 0.01	1.00 ± 0.10	10
Urethane (c)			
0.2%	1.68 ± 0.25 (d)	1.71 ± 0.25 (d)	3

(a) Smears were prepared from peripheral blood samples obtained by cardiac puncture of dosed and control animals at the termination of the 13 week study. Slides were stained with Hoechst 33258/pyronin Y (MacGregor *et al.*, 1983). At least 2000 PCE and 10000 NCE from each animal were scored for micronuclei. No significant elevation in the frequency of micronucleated erythrocytes was observed in either male or female mice administered castor oil in dosed feed.
(b) Values are Mean ± Standard Error of the Mean.
(c) Positive control: Male mice treated for 4 weeks with urethane in the drinking water (0.2%). These animals were not part of the 13-week study, but were added as a measure of quality control for the assay.
(d) Significantly different from control groups by Shirley's test (Shirley, 1977), $p < 0.05$. (None noted)