NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 415

TOXICOLOGY AND CARCINOGENESIS STUDIES OF POLYSORBATE 80 (CAS NO. 9005-65-6) IN F344/N RATS AND B6C3F$_1$ MICE (FEED STUDIES)

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

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

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

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF POLYSORBATE 80

(CAS NO. 9005-65-6)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
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CONTRIBUTORS

National Toxicology Program

C.J. Alden, Ph.D.
G.A. Boorman, D.V.M., Ph.D.
D.A. Bridge, B.S.
S.L. Eustis, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
R.A. Griesemer, D.V.M., Ph.D.
J.K. Haseman, Ph.D.
C.W. Jameson, Ph.D.
M.P. Jokinen, D.V.M.
J. Mahler, D.V.M.
G.N. Rao, D.V.M., Ph.D.
C.C. Shackelford, D.V.M., M.S., Ph.D.
D.B. Walters, Ph.D.
K.L. Witt, M.S., Oak Ridge Associated Universities

Southern Research Institute

Conducted studies, evaluated pathology findings

J.D. Prejean, Ph.D., Principal Investigator
C.H. Farr, Ph.D.

Integrated Laboratory Systems

Prepared quality assurance audits

S.L. Smith, J.D., Principal Investigator

Experimental Pathology Laboratories, Inc.

Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator

Biotechnical Services, Inc.

Prepared Technical Report

L.G. Cockerham, Ph.D., Principal Investigator
G.F. Corley, D.V.M.
T.A. King-Hunter, B.S.
W.D. Sharp, B.A., B.S.

NTP Pathology Working Group

Evaluated slides, prepared pathology report for rats (19 July 1990)

M.A. Stedham, D.V.M., Chair
Pathology Associates, Inc.
K.M. Ayers, D.V.M.
Burroughs Wellcome
M.P. Jokinen, D.V.M.
National Toxicology Program
J. Mahler, D.V.M.
National Toxicology Program
M.M. McDonald, D.V.M., Ph.D.
National Toxicology Program
T. Monticello, D.V.M., Ph.D. (observer)
CIIT
A. Pinter, M.D., Ph.D.
National Institute of Hygiene, Hungary
S.A. Stefanski, D.V.M.
National Toxicology Program

Evaluated slides, prepared pathology report for mice (21 June 1990)

J.R. Leininger, D.V.M., Ph.D., Chair
Pathology Associates, Inc.
G.A. Boorman, D.V.M., Ph.D.
National Toxicology Program
J.F. Hardisty, D.V.M.
Experimental Pathology Laboratories, Inc.
M.P. Jokinen, D.V.M.
National Toxicology Program
S.A. Klumpp, D.V.M., M.S.
Yerkes Regional Primate Research Center
A.W. Macklin, D.V.M., Ph.D.
Burroughs Wellcome

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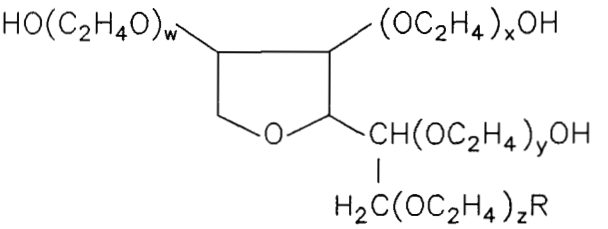
ABSTRACT



[Sum of w, x, y, and z is 20; R is $(C_{17}H_{33})COO$]

POLYSORBATE 80

CAS No. 9005-65-6

Chemical Formula: $\approx C_{64}H_{124}O_{26}$ Molecular Weight: ≈1,308

Synonyms: Glycol; sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl) derivatives; polyoxyethylene (20) sorbitan mono-oleate; sorethytan (20) mono-oleate; polyethylene oxide sorbitan mono-oleate

Trade names: Alkamuls PSMO-20; Armotan PMO-20; Capmul POE-O; Drewmulse POE-SMO; Emsorb 2722; Glycosperse O-20; Glycosperse O20 Veg; Glycosperse O20X; Hetsorb O20; Industrol O20S; Laxan ESO; Liposorb O-20; Lonzest SMO-20; Montanox 80; Nikkol TO-10; Protasorb O-20; Sorbitan mono-oleate polyoxyethylene; Sorlate; Tween 80; Monitan; Olothorb; Sorbimacrogol Oleate 300; T-Maz 80

Polysorbate 80 is a nonionic surfactant used widely as an additive in foods, pharmaceutical preparations, and cosmetics as an emulsifier, dispersant, or stabilizer. Toxicity and carcinogenicity studies were conducted by administering polysorbate 80 (which met all compendial specifications) in feed to groups of F344/N rats and $B6C3F_1$ mice of each sex for 14 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*.

14-Day Studies

Groups of five rats and five mice of each sex received diets containing 0, 3,000, 6,000, 12,500, 25,000, or 50,000 ppm polysorbate 80. All animals survived to the end of the studies. The mean body weight change of male rats that received 50,000 ppm was significantly lower than that of the controls. The mean body weight changes in all other groups of dosed rats and in all groups of dosed mice were similar to those of the respective controls. No clinical findings or changes in absolute or relative organ weights in rats or mice were related to polysorbate 80 administration.

13-Week Studies

Groups of 10 rats and 10 mice of each sex received diets containing 0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm polysorbate 80. All animals survived to the end of the studies. The final mean body weights of dosed rats and mice were similar to those of the controls. No clinical findings, changes in absolute or relative organ weights, or gross or microscopic lesions in rats or mice were related to polysorbate 80 administration.

2-Year Studies

Doses for the 2-year studies were selected based on the lack of observed compound-related effects at the dose levels used in the 13-week studies. Groups of 60 rats and 60 mice of each sex received diets containing 0, 25,000, or 50,000 ppm polysorbate 80 for up to 103 weeks.

15-Month Interim Evaluations
Interim evaluations were performed on 7 to 10 rats and mice from each dose group at 15 months. There were no significant changes in absolute or relative organ weights. Incidences of hyperplasia and inflammation of the forestomach were increased in female mice that received 50,000 ppm. No other chemical-related lesions occurred in rats or male mice evaluated at 15 months.

Body Weights, Clinical Findings, and Survival in the 2-Year Studies
The mean body weights in male and female rats and male mice administered polysorbate 80 were similar to those of the controls throughout the studies. The final mean body weight of female mice receiving 50,000 ppm was 11% lower than that of the controls. No clinical findings were associated with administration of polysorbate 80. The survival of dosed male rats was lower than that of the controls (0 ppm, 29/50; 25,000 ppm, 18/50; 50,000 ppm, 18/50); the survival of dosed female rats and male and female mice was similar to that of the respective controls (female rats: 23/50, 25/50, 25/50; male mice: 33/49, 34/50, 32/50; female mice: 30/50, 28/50, 26/50).

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies
The incidence of adrenal medulla pheochromocytoma was marginally increased in high-dose male rats (21/50, 19/50, 29/50). The incidence of hyperplasia of the adrenal medulla was increased in low-dose male rats but not in high-dose male rats (11/50, 22/50, 12/50).

No chemical-related increases in the incidences of neoplasms occurred in male or female mice. The incidences of squamous hyperplasia and inflammation of the forestomach were significantly increased in high-dose male and female mice; forestomach ulcers were significantly increased in high-dose females.

Genetic Toxicology
Polysorbate 80 was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 with or without exogenous metabolic activation (S9).

Conclusions
Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity** for polysorbate 80 in male F344/N rats based on an increased incidence of pheochromocytomas of the adrenal medulla. There was *no evidence of carcinogenic activity* for polysorbate 80 in female F344/N rats or in male or female B6C3F$_1$ mice given 25,000 or 50,000 ppm.

Administration of polysorbate 80 was associated with inflammation and squamous hyperplasia of the forestomach in male and female mice, and with ulcers of the forestomach in female mice.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 10.

Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of Polysorbate 80

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses			
0, 25,000, or 50,000 ppm in feed	0, 25,000, or 50,000 ppm in feed	0, 25,000, or 50,000 ppm in feed	0, 25,000, or 50,000 ppm in feed
Body weights			
Dosed groups similar to controls	Dosed groups similar to controls	Dosed groups similar to controls	High-dose group 11% lower than controls
2-Year survival rates			
29/50, 18/50, 18/50	23/50, 25/50, 25/50	33/49, 34/50, 32/50	30/50, 28/50, 26/50
Nonneoplastic effects			
None	None	Forestomach: squamous hyperplasia (3/48, 4/50, 19/50); inflammation (0/48, 4/50, 12/50)	Forestomach: squamous hyperplasia (4/49, 8/50, 26/49); inflammation (4/49, 4/50, 16/49); ulcer (1/49, 0/50, 7/49)
Neoplastic effects			
None	None	None	None
Uncertain findings			
Adrenal medulla: pheochromocytoma (21/50, 19/50, 29/50) hyperplasia (11/50, 22/50, 12/50)	None	None	None
Level of evidence of carcinogenic activity			
Equivocal evidence	No evidence	No evidence	No evidence
Genetic toxicology			
Salmonella typhimurium gene mutation:	Negative with or without S9 in strains TA100, TA1535, TA1537, and TA98		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on polysorbate 80 on July 10, 1991, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing NTP studies:

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

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

Paul T. Bailey, Ph.D., Principal Reviewer
Toxicology Division
Mobil Oil Corporation
Princeton, NJ

Louis S. Beliczky, M.S., M.P.H.
Department of Industrial Hygiene
United Rubber Workers International Union
Akron, OH

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

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

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

Jay I. Goodman, Ph.D.
Department of Pharmacology and Toxicology
Michigan State University
East Lansing, MI

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

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

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

Ellen K. Silbergeld, Ph.D.*
University of Maryland Medical School
Baltimore, MD

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

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On July 10, 1991, the draft Technical Report on the toxicology and carcinogenesis studies of polysorbate 80 received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. M.P. Jokinen, NIEHS, introduced the toxicology and carcinogenesis studies of polysorbate 80 by discussing the uses and rationale for the studies, describing the chemistry and experimental design, and commenting on compound-related neoplastic and nonneoplastic lesions in mice and rats. The proposed conclusions were *equivocal evidence of carcinogenic acitivity* in male rats and *no evidence of carcinogenic activity* in female rats or in male or female mice.

Dr. Garman, a principal reviewer, agreed with the proposed conclusions. He asked for clarification of the criterion for distinguishing between benign and malignant pheochromocytomas. Dr. Jokinen said the criterion for malignancy is invasion through the capsule. Dr. Garman also asked for clarification of the actual historical control range for pheochromocytomas in male rats. Dr. J. Haseman, NIEHS, explained that 48% is the upper limit on the current database while 65% was cited from an earlier NTP study to give perspective to the fact that rates had been higher in the past.

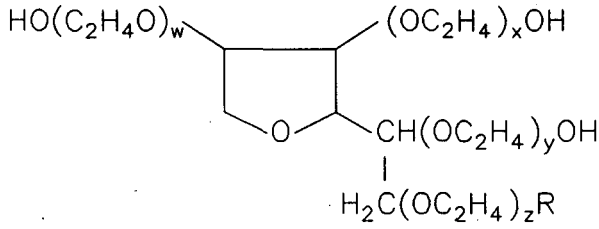
Dr. Bailey, the second principal reviewer, agreed with the proposed conclusions. He commented on the large temperature excursion in the animal room during the 2-year study, noting that it occurred only during a 2-day period, and asked if there was any possible impact on the results of the study. Dr. Jokinen agreed to add a statement as to the limited time of the excursion and the likelihood that there was no effect on the study results (p. 18).

Dr. Davis, the third principal reviewer, agreed with the proposed conclusions. He inquired about the significance of the adrenal hyperplasia in low-dose male rats or, if adrenal hyperplasia is part of a continuum toward neoplasia, why there were not increases in the high-dose group. Dr. S. Eustis, NIEHS, said that if both hyperplasia and a neoplasm were present in the same adrenal gland only the neoplasm was diagnosed and reported. This was mainly a matter of practicality because the small size of the adrenal medulla made it difficult to determine whether hyperplasia was part of the pheochromocytoma or a separate lesion.

Dr. Carlson commented on the occurrence of splenic sarcomas in two high-dose male rats, an incidence which exceeded the current historical control range for dosed feed studies. Dr. Eustis said the small number of tumors along with the lack of preneoplastic lesions did not support this being a chemical-related effect.

Dr. Garman moved that the Technical Report on polysorbate 80 be accepted with the revisions discussed and with the conclusions as written. Dr. Hayden seconded the motion, which was unanimously accepted with ten votes.

INTRODUCTION



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Trade names: Alkamuls PSMO-20; Armotan PMO-20; Capmul POE-O; Drewmulse POE-SMO; Emsorb 2722; Glycosperse O-20; Glycosperse O20 Veg; Glycosperse O20X; Hetsorb O20; Industrol O20S; Laxan ESO; Liposorb O-20; Lonzest SMO-20; Montanox 80; Nikkol TO-10; Protasorb O-20; Sorbitan mono-oleate polyoxyethylene; Sorlate; Tween 80; Monitan; Olothorb; Sorbimacrogol Oleate 300; T-Maz 80

PHYSICAL AND CHEMICAL PROPERTIES

Polysorbate 80 is a lemon- to amber-colored, translucent, viscous, oily liquid with a distinctive odor and a somewhat bitter taste. It is soluble in water and in a variety of vegetable oils or organic solvents and has a pH of 5 to 7 in a 5% aqueous solution (*Merck Index*, 1983).

Polysorbate 80 is a commercial compound and is one of a series of polysorbates (polyoxyethylene sorbitan esters) which act as hydrophilic, nonionic surfactants. It is produced by reacting sorbitol and its anhydrides with ethylene oxide followed by esterification of the reaction product with oleic acid. The reaction with ethylene oxide is a polymerization reaction which produces polyoxyethylene chains of varying lengths; the average number of molecules of ethylene oxide in the side chains of a polysorbate molecule is 20. Consequently, polysorbate 80 is a complex mixture consisting mainly of the oleate ester of sorbitol and its anhydrides, copolymerized with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides (*Kirk-Othmer*, 1983; *Merck Index*, 1983).

PRODUCTION, USE, AND HUMAN EXPOSURE

Polysorbate 80 is used primarily as an emulsifier, and as such has a broad range of applications (CFR, 1979; FDA, 1981; CTFA, 1984). Polysorbate 80 has been approved by the Food and Drug Administration (FDA) for numerous uses as a direct or indirect food additive for human consumption and is widely employed in the food industry as a flavoring agent, emulsifier, defoamer, dough stabilizer, dispersant, and surfactant. The FDA has set allowable levels of polysorbate 80 in foods, dependent upon the type of food product in which it is used. Levels are generally low, less than 1,000 ppm, but can be as high as 10,000 ppm when used as an emulsifier in shortenings and edible oils.

The Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives set the maximum allowable level of daily intake at 25 mg/kg (FAO/WHO, 1974). As an ingredient in numerous prescription and nonprescription pharmaceutical products, polysorbate 80 suspends insoluble compounds and modifies absorption of active ingredients. It is approved for use in vitamin-mineral preparations containing both oil- and water-soluble vitamins at levels which will result in a maximum daily intake of 175 to 475 mg. Polysorbate 80 is also widely used in the manufacture of cosmetics, as a wetting agent in ophthalmic solutions, as an ingredient in dental care products, as a therapeutic agent in the treatment of lipid malabsorption in humans, and as an additive in animal feeds. In addition, because of its surfactant properties, polysorbate 80 has many industrial applications. One of the more important applications is in textile manufacture, where polysorbate 80 is used for a variety of purposes including fiber lubrication, as an antistatic agent, and as an emulsifier for processing oils; it has also been used as a dispersant on oil spills (*Kirk-Othmer*, 1983). From a survey conducted from 1981-1983, NIOSH has estimated that 162,120 workers may have been exposed to polysorbate 80 (NIOSH, 1990). Thus, there is large-scale consumer and occupational exposure to polysorbate 80.

METABOLISM AND CHEMICAL DISPOSITION

The metabolism of radiolabeled polysorbates in rats has been studied. After oral administration, the ester linkage of the polysorbate molecule is hydrolyzed by pancreatic lipase and the released fatty acid is absorbed and metabolized; essentially all of the fatty acid in polysorbate 80 fed as 10% of the diet is hydrolyzed and absorbed (Oser and Oser, 1957a). The sorbitol-polyoxyethylene moiety is poorly absorbed and most is excreted in the feces. In metabolism studies conducted with rats fed polysorbate 80 containing radiolabeled sorbitol, 91% of the radioactivity was found in the feces, 2.1% was found in the urine, and 1.6% was found in the carcass. None was found in the liver, kidney, adrenal glands, spleen, brain, or fat (Treon *et al.*, 1967). Following intravenous administration of polysorbates to rats, the ester linkage of the polysorbates is hydrolyzed by blood lipases. Intravenous studies using radiolabeled polysorbate 20 in rats demonstrated that the fatty acid portion was metabolized and excreted mostly as expired carbon dioxide, with lesser amounts appearing in the urine and feces; the sorbitol-polyoxyethylene moiety was not catabolized and was excreted mainly in the urine, although a small amount was found in the feces, indicating some biliary excretion; trace amounts were detected in the carcass and liver (Nelson *et al.*, 1966; Treon *et al.*, 1967).

Metabolism of polysorbates in humans appears to be similar to that in rats. Analysis of urine and feces from human volunteers who consumed 4.5 g of polysorbate 80 daily showed that 95% of the polyoxyethylene portion was excreted in the feces and 5% in urine; apparently, none was retained in the body (Culver *et al.*, 1951).

The effects of polysorbate 80 on cellular metabolism and function have been studied extensively. Many of these effects have been detected *in vitro*; because the intact molecule is rapidly broken down *in vivo*, the relevance of these *in vitro* findings to possible *in vivo* effects of polysorbate 80 is uncertain. The compound has been shown to affect the activity of a number of microsomal enzymes *in vitro*. In particular, polysorbate 80 has been reported to inhibit the *in vitro* activity of rat hepatic dimethylnitrosamine demethylase; however, polysorbate 80 was found to have the opposite effect *in vivo* of inducing the activity of this enzyme (Argus *et al.*, 1980). Polysorbate 80 has been reported to increase DNA synthesis of isolated hamster kidney cells (Burke and Pearson, 1979), to cause a dose-dependent suppression of DNA excision repair in suspensions of mouse splenic leukocytes (Tuschl *et al.*, 1975), and to increase the incorporation of amino acids in cultures of normal embryonic chicken or hamster cells (Artamonova *et al.*, 1972). *In vitro* mitochondrial respiration is stimulated by low concentrations and inhibited by high concentrations of polysorbate 80 (Carreau and Mazliak, 1977). The effect on mitochondrial respiration is presumably due to the ability of the polysorbates to activate cytochrome C oxidase, apparently by helping to attain the most active conformation of the enzyme (Green and Fry, 1980).

The surfactant properties of polysorbates cause them to affect the function of cellular membranes. Polysorbate 80 produced lysis of human erythrocytes (Zaslavsky *et al.*, 1978) and caused a dose-dependent change in erythrocyte osmotic resistance (Pelle,

1975). Injection of polysorbate 80 in normal saline into the anterior chambers of the eyes of rabbits rapidly produced corneal edema, indicating that polysorbate 80 increased the permeability of the corneal endothelium (Quiroga and Klintworth, 1967). Polysorbate 80 increased transmural potential differences and decreased tissue resistance in the isolated rat jejunum, demonstrating that polysorbate 80 affects transmembrane movement of electrolytes (Feldman *et al.*, 1975). Polysorbate 80 has also been reported to bind and transport sodium, potassium, calcium, and ammonium ions through a model (CH_2Cl_2) membrane (Thoman, 1986). Results from studies on neuromuscular effects of polysorbates have been inconclusive. Polysorbate 80 stimulated colonic muscular activity in rabbits (Falconer *et al.*, 1978), but inhibited muscular contraction in the guinea pig ileum (Gaginella *et al.*, 1975). In other studies, polysorbate 80 inhibited the ability of acetylcholine to produce muscular contraction in the guinea pig duodenum (Caujolle *et al.*, 1967), but had no effect on the inhibitory activity of epinephrine and isoproterenol on the rabbit jejunum (Sabir *et al.*, 1972). Polysorbates can also influence membrane transport of large molecules, which, in turn, can affect the activity of drugs and other compounds. Polysorbate 80 was found to decrease the acute oral toxicity of several compounds in mice (Berezovskaya and Rudzit, 1967), to inhibit the absorption of drugs injected intramuscularly (Kobayashi *et al.*, 1977), and to increase percutaneous absorption of topically applied medications in mice (Sarpotdar and Zatz, 1987). The effect of polysorbates on the transport of large molecules may be mediated through a generalized increase in membrane permeability or through interaction with membrane-bound proteins (Yasuhara *et al.*, 1979).

Other effects of polysorbate 80 have been described. Polysorbate 80 was reported to increase intestinal absorption of fat in rats fed high fat diets; this may be due to the stimulation of bile secretion by polysorbate 80 (Croce and Ferrini, 1973), or it may occur through a mechanism similar to that of bile acids with the production of micelles in the intestinal lumen to enhance fat absorption (El-Gorab *et al.*, 1975). Polysorbate 80 given intravenously caused a slight blood pressure decrease in dogs, cats, rabbits, and monkeys; decreased blood pressure was not seen in these species after oral administration (Krantz *et al.*, 1951). Perfusion of polysorbate 80 into isolated rabbit or guinea pig hearts led to dilation of coronary vessels and increased cardiac output (Correia da Silva and Paiva, 1970). Polysorbate 80 (10 mg/kg) injected intravenously in human patients produced an increase in cardiac output and a decrease in peripheral vascular resistance (CTFA, 1984). Polysorbates have been reported to be histamine releasers (Yamasaki *et al.*, 1969). Intravenous injection of 10 mg/kg polysorbate 80 into dogs produced a marked increase in plasma histamine levels and a severe hypotension that could be prevented by treatment with antihistamines (Masini *et al.*, 1985); in the same study, it was found that polysorbate 80 released histamine from rat peritoneal mast cells *in vitro*. Intraperitoneal injection of polysorbate 80 into mice prior to immunization with ovalbumin led to inhibition of the IgE response (Bryant and Barnett, 1979) and suppression of the IgG response (Barnett and Bryant, 1980), while mice immunized with sheep red blood cells following intraperitoneal injection of polysorbate 80 had significantly reduced numbers of IgM-producing cells in the spleen (Barnett, 1981).

TOXICITY

Numerous studies of the potential toxic effects of polysorbate 80 have been conducted in laboratory animals and in humans. The general conclusion from these studies is that polysorbate 80 is essentially nontoxic.

Acute oral toxicity studies of polysorbate 80 in rats and mice indicate the compound is relatively nontoxic by this route of administration. Reported oral LD_{50} values for rats range from approximately 33.8 g/kg to approximately 54.5 g/kg, while the oral LD_{50} for mice is greater than 25 g/kg (CTFA, 1984). Clinical experience with humans who have ingested large quantities of polysorbate 80 indicates that the compound is essentially nontoxic by the oral route, with the estimated lethal dose being 15 g/kg (Gosselin *et al.*, 1976).

Long-term oral toxicity studies ranging from 3 months to 2 years in duration, including some multiple-generation studies, have been conducted in rats. The most comprehensive study was a 2-year, four-generation study conducted by Oser and Oser (1956a,b; 1957a,b) using groups of 12 male and 20 female rats. Doses of 5%, 10%, and 20% (50,000, 100,000, and 200,000 ppm) were administered in the feed. Composition of the diet was

altered for different dose levels to compensate for the decrease in caloric value caused by addition of polysorbate 80. Diarrhea was seen in animals fed 10% or 20% polysorbate 80, and was presumably due to the osmotic effect of the large amounts of unabsorbed sorbitol-polyoxyethylene moiety in the intestinal tract. There was some reduction in growth rate, postnatal survival of pups, lactation and breeding efficiency, and longevity in rats fed the diet containing 20% polysorbate 80. Hematologic examination showed no abnormalities and no treatment-related histologic lesions were seen upon examination of kidneys, livers, and gross lesions. No significant findings were reported in a three-generation study using groups of 30 rats fed 2% polysorbate 80 (Krantz and Carr, 1969). Degeneration of the heart, liver, and kidney were reported in groups of 12 Charles Foster rats administered a daily dose of 1.5 mL of a solution of 1%, 2%, or 3% polysorbate 80 for 3 months (Nityanand and Kapoor, 1979). Similar effects were not reported in other studies using higher doses for longer periods, so the significance of the findings of this single study is debatable. Daily administration of 1 g polysorbate 80 to each of two monkeys for 17 months produced no compound-related gross or microscopic lesions (FAO/WHO, 1974). In a study conducted to evaluate the effect of polysorbate 80 on the intestinal mucosa, Oshumi *et al.* (1980) administered a 10% solution of polysorbate 80 in drinking water to rats for 3 months. Observed effects in the intestinal mucosa were slight; histological effects included hypersecretion of mucus by goblet cells and deformation of villi, while there was ultrastructural evidence of mitochondrial changes and microvilli destruction.

A study has been reported in which germ-free and conventional male CD-1 mice were fed a diet containing polysorbate 80 for 10 weeks (Reyniers *et al.*, 1985). The exact dose level was not given, but was described as being "equivalent to that deemed acceptable for medicinal use in humans." Histologically, hepatocytes in both the germ-free and conventional mice were swollen and vacuolated due to the cytoplasmic accumulation of lipid, as confirmed by special stains. No other abnormalities were reported.

Evidence indicates that the long-term ingestion of polysorbate 80 by humans does not result in toxicity. Long-term consumption (one or more years) of 4.5 to 6 g polysorbate 80 per day by human patients for the treatment of lipid malabsorption syndromes has been reported to cause no adverse effects (Krantz *et al.*, 1951). The consumption of doses as high as 15 g/day for several months caused no clinical indications of toxicity (Jones *et al.*, 1948).

The reported LD_{50} values for polysorbate 80 injected intravenously are 1.8 g/kg for rats and 5.8 g/kg for mice, while the values for intraperitoneal injection are 8 to 9 mL/kg for rats and 7.6 mg/kg for mice (CTFA, 1984). Subcutaneous injection of polysorbate 80 given to rats over a period of 40 weeks caused the death of the animals when a cumulative dose of 49 g/kg was reached (NIOSH, 1977).

The potential of polysorbates to produce skin irritation has been extensively evaluated. In 24-hour rabbit skin-patch tests, polysorbate 80 was found to produce minimal or no irritation (CTFA, 1984). Daily application of 100% solutions of polysorbate 80 to the backs of rabbits for 30 days produced histologic changes in the skin, including mild to moderate inflammation, acanthosis, and necrosis (Mezei *et al.*, 1966). Application of 1% or 2.5% aqueous solutions of polysorbate 80 to the backs of mice for up to 9 days produced no gross or microscopic lesions (Lansdown and Grasso, 1972). Repeated intradermal injections of polysorbate 80 into guinea pigs did not produce skin sensitization, and studies conducted in humans did not result in skin irritation or sensitization (CTFA, 1984).

CARCINOGENICITY

A number of carcinogenicity studies have been conducted with several of the polysorbates (CTFA, 1984). Most of the studies were conducted using an oral or dermal route of exposure, although a few used subcutaneous injection. Study duration was usually from 6 months to 1 year, but one 2-year subcutaneous injection study of polysorbate 60 was carried out in rats. There was no evidence of carcinogenicity associated with oral dosing of any of the polysorbates. Some reports from dermal studies suggest the polysorbates may produce benign reversible skin tumors; however, after performing a comprehensive evaluation of the available data and conducting additional studies, Setala (1960) concluded that polysorbates were not carcinogenic when applied to the skin.

A few studies have been conducted with polysorbate 80. In one study, 28 mice were fed diets containing 100 mg polysorbate 80 daily for 51 weeks; no neoplasms occurred as the result of treatment (Wong *et al.*, 1959). Two dermal exposure studies have been conducted in mice. In one study, a 100% solution of polysorbate 80 was applied to the skin of 50 mice 6 days a week for 24 weeks; no neoplasms occurred as a result of polysorbate 80 administration (Setala *et al.*, 1957). The second dermal exposure study was conducted using the same study design but with a treatment duration of 52 weeks; a single benign skin tumor was observed in a dosed animal (Setala, 1956). In a subcutaneous injection study, 10 mice were administered 0.1 mL of a 0.5% solution of polysorbate 80 in saline once weekly for 15 weeks; the study was terminated at 270 days, and no neoplasms occurred as a result of treatment (Shirasu, 1963). A subcutaneous injection study was also performed in rats (Grasso *et al.*, 1971). Twenty rats were administered 2 mL doses of a 6% aqueous solution of polysorbate 80 three times a week for 40 weeks, and 11 rats developed fibrosarcomas at the injection site. The authors suggested that the surface-active properties of the compound produced extensive cellular damage at the injection site, leading to a prolonged connective tissue proliferative response that eventually progressed to sarcoma. In a lifetime intratracheal instillation study, 50 Syrian hamsters were administered 0.2 mL of a 5% solution of polysorbate 80 once a week for life; no neoplasms occurred as a result of treatment (Farrell, 1974).

By current standards for chronic carcinogenicity studies, it appears that the dose levels employed, numbers of animals used, or duration of the reported studies may not have been adequate to ensure proper testing of the carcinogenic potential of polysorbate 80.

There are many reports of tumor-enhancing effects of polysorbates administered to animals in conjunction with known carcinogens (CTFA, 1984). Most of these studies are concerned with the production of skin neoplasms through dermal application or gastric neoplasms through oral administration. Proposed mechanisms by which polysorbates may increase the incidence of carcinogen-induced neoplasms include: 1) inhibition of DNA repair, 2) cellular membrane effects which cause tissue damage and increased cellular proliferation, 3) enhancement of cellular uptake of the carcinogen, and 4) increased absorption of the carcinogen from the skin surface or gastrointestinal tract lumen. There are a few reports of such studies using polysorbate 80, and the results have been contradictory. Bock and Tso (1974) found that dermal application of the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) to the skin of mice followed by the application of polysorbate 80 increased the incidence of skin tumors over that in mice treated with DMBA alone; the authors did not describe the nature of the tumors produced. Wong *et al.* (1959) reported that the concurrent feeding of the carcinogen methylcholanthrene and polysorbate 80 to mice increased the incidence of gastric and lung tumors over that of mice fed methylcholanthrene only; feeding polysorbate 80 only had no effect on tumor incidence. In contrast, when the carcinogen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was administered to rats via dosed water, the addition of polysorbate 80 had no effect on tumor incidence as compared with the incidence in animals receiving only MNNG (Fukushima *et al.*, 1974). Intratracheal instillation of a mixture of benzo(a)pyrene and polysorbate 80 into hamsters resulted in respiratory tract neoplasms in 12 of 50 treated animals, while neoplasms were seen in 24 of 50 animals treated with benzo-(a)pyrene only (Farrell, 1974). Apparently, the effects of polysorbate 80 on the incidence of induced neoplasms may depend in part upon the nature of the carcinogen and the route of administration.

There is also some evidence that polysorbates can inhibit tumor growth. The intraperitoneal injection of polysorbate 80 into mice inoculated with Ehrlich ascites cancer cells was associated with a decrease in the size and number of tumors, with a resultant increase in the survival time of the tumor-bearing animals (Kubis *et al.*, 1979). Likewise, intraperitoneal injection of polysorbate 80 into female SJL/J mice, which develop a high incidence of spontaneous reticulum cell sarcoma (RCS), resulted in a reduction in the incidence of RCS to approximately one half that seen in corresponding controls (Crispens and Sorenson, 1988). Weekly injection of polysorbate 80 and the chemotherapeutic agent cyclophosphamide into SJL/J mice reduced the incidence of RCS to 0% as opposed to 85% in controls (Crispens and Sorenson, 1989). The addition of polysorbate 80 to cell cultures containing transformed mouse cells resistant to the antimitotic activity of the drugs colcemid, colchicine, and

vinblastine resulted in increased sensitivity of the cells to the effects of these drugs (Stavrovskaya *et al.*, 1975). In the same study, it was found that polysorbate 80 increased cellular uptake of radiolabeled colchicine, so the authors concluded polysorbate 80 exerted its action by increasing membrane permeability to the drugs.

GENETIC TOXICITY

Polysorbate 80 has not been tested extensively, but the available evidence indicates that the chemical is not mutagenic *in vitro* or *in vivo*. No induction of gene mutations was observed in *Salmonella typhimurium* (Sugimura *et al.*, 1976; Lockard *et al.*, 1982; Mortelmans *et al.*, 1986) or *Escherichia coli* (Sugimura *et al.*, 1976). A review of Japanese mutagenicity studies which were conducted in the 1970's (Kawachi *et al.*, 1980) lists polysorbate 80 as negative in the *Bacillus subtilis* rec assay and the *Bombyx mori* gene mutation assay. In addition, negative results were reported in tests for the induction of chromosomal aberrations in human lymphocyte and fetal lung cell cultures (Stenchever *et al.*, 1976) and Chinese hamster fibroblast cells (Ishidate and Odashima, 1977). Polysorbate 80 did not induce sister chromatid exchanges in human lymphocytes treated *in vitro* (Lockard *et al.*, 1982). The only *in vitro* studies to provide dosing information were those of Lockard *et al.* (1982) and Mortelmans *et al.* (1986); several of the studies used polysorbate 80 as a solvent and its mutagenicity was reported as the solvent control data.

Negative results were reported for induction of chromosome aberrations in Syrian hamster bone marrow cells *in vivo* following subcutaneous injection of polysorbate 80 in saline (Joneja and Kaiserman, 1978). No induction of micronuclei was observed in mouse bone marrow cells *in vivo* following intraperitoneal injection of polysorbate 80 (Jenssen and Ramel, 1980; Richardson *et al.*, 1982).

Genetic toxicity information is available on the structural analogue polysorbate 60, which also shows no evidence of genotoxic activity. Negative results were reported for induction of DNA damage in repair-deficient *B. subtilis* rec assay (Inoue *et al.*, 1979; Morita *et al.*, 1981), and for induction of gene mutations in *S. typhimurium*, with and without S9 (Inoue *et al.*, 1980; Morita *et al.*, 1981; Sunakawa *et al.*, 1981). In addition, no induction of chromosome aberrations was observed in Chinese hamster fibroblasts treated with 2×10^{-5} M polysorbate 60 (Ishidate and Odashima, 1977).

STUDY RATIONALE

Because of the widespread human exposure and the lack of information on the effects of long-term oral exposure, polysorbate 80 was nominated for study by the National Institute of Occupational Safety and Health for the evaluation of carcinogenic potential.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Polysorbate 80 was obtained from McKesson Chemical Company (Kansas City, MO) in two lots (lots 250-1 and 7230-C). Because polysorbate 80 is a commercial product consisting of a complex mixture mainly of the oleate ester of sorbitol and its anhydrides copolymerized with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides, a purity estimate, in the conventional sense, is not appropriate. Conformance to established compendial specifications is the criteria upon which acceptability of the material was judged. Characterization and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (MRI; Kansas City, MO), and are described in Appendix G. The study chemical, a translucent, pale yellow, viscous fluid, was identified as polysorbate 80 by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The material was further characterized by elemental analyses, Karl Fischer water analysis, titration, colorimetric assay, and thin-layer chromatography. The colorimetric assay was used to estimate relative amounts of sorbitan polyethylene glycols fatty acid esters (which conform to the basic structure of polysorbate 80) and sorbitan polyethylene glycols present in the samples. Both lots had approximately 85% sorbitan polyethylene glycols fatty acid esters and 15% sorbitan polyethylene glycols. Free fatty acids, saponification values, and hydroxyl values were within United States Pharmacopeia and Food Chemicals Codex specifications for both lots. The composition of both lots was consistent with that expected for polysorbate 80. While some of the National Formulary (NF) tests were performed on lot 250-1, the complete battery of NF tests was performed on lot 7230-C. All test results indicated that the lots met the NF requirements for polysorbate 80.

Stability studies performed by titration for free fatty acids and colorimetric assay indicated that polysorbate 80 was stable as a bulk chemical for 2 weeks at temperatures up to 60° C when protected from light. To ensure stability, the bulk chemical was stored in the dark at 5° C throughout the studies. The stability of the bulk chemical was monitored periodically by infrared spectroscopy and titration to determine free fatty acids and peroxide during all phases of the studies. No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing appropriate amounts of polysorbate 80 with feed (Table G1). Studies were conducted by the analytical chemistry laboratory to determine the homogeneity and stability of polysorbate 80 in feed. Homogeneity of the dose formulations was confirmed; stability was confirmed for at least 14 days when stored in the dark at temperatures up to 5° C. Dose formulations were prepared once for the 14-day studies and weekly for the 13-week and 2-year studies. During the 14-day, 13-week, and 2-year studies, the dose formulations were stored in the dark at 5° C for no longer than 2 weeks. The study laboratory conducted periodic analyses of the polysorbate 80 dose formulations using ultraviolet/visible spectroscopy as described in Appendix G. All dose formulations analyzed for the 14-day studies were within 10% of the target concentrations (Table G2). During the 13-week studies, 79% (11/14) of the dose formulations analyzed were within 10% of the target concentrations (Table G3). In the 2-year studies, the first set of dose formulations and one of every eight subsequent sets were analyzed; 97% (84/87) were found to be within 10% of the target concentrations (Table G4). Results of periodic referee analyses of the dose formulations performed by the analytical chemistry laboratory were in agreement with the results from the study laboratory (Table G5).

14-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Harlan Industries (Indianapolis, IN) and observed for 16 days before the studies began.

Rats were 45 days old and mice were 58 days old at the beginning of the studies. Groups of five rats and five mice of each sex received feed containing 0, 3,000, 6,000, 12,500, 25,000, or 50,000 ppm polysorbate 80 (Table 1). All groups received dosed feed for 14 days, followed by a 1-day observation period when the animals were given only undosed feed. Animals were housed five per cage; water and feed were available *ad libitum*. Clinical observations were conducted and recorded twice daily and at the end of the studies. Animals were weighed at the start of the studies, on day 8, and day 15. Feed consumption was determined weekly. Complete necropsies were performed on all animals. The brain, heart, right kidney, liver, lung, and thymus of all animals were weighed at necropsy. Complete histopathology was performed on all animals in the 0 and 50,000 ppm dose groups. The liver was examined from male rats receiving 3,000, 6,000, 12,500, or 25,000 ppm and female rats receiving 25,000 ppm. Further details are presented in Table 1.

13-WEEK STUDIES

The 13-week studies were conducted to determine the cumulative toxic effects of repeated exposure to polysorbate 80 and to determine appropriate chemical concentrations to be used in the 2-year studies. The experimental design of the 13-week studies is summarized in Table 1.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories (Kingston, NY) and observed for 19 days before the studies began. Rats were 51 days old and mice were 58 days old at the beginning of the studies. Groups of 10 rats or 10 mice of each sex were given 0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm of polysorbate 80 in feed for 13 weeks. Animals were housed five per cage; water and feed were available *ad libitum*. Clinical observations were made twice each day and recorded weekly. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix J). Animals were weighed weekly and at the end of the study. Feed consumption was measured weekly. Further experimental details are presented in Table 1.

Complete necropsies were performed on all animals. The brain, heart, right kidney, liver, lung, right testis, and thymus were weighed at necropsy. Complete histopathology was performed on all animals that received 0 or 50,000 ppm. Additional information about histologic examination is provided in Table 1.

2-YEAR STUDIES

Study Design

Groups of 60 rats and 60 mice of each sex were administered 0, 25,000, or 50,000 ppm polysorbate 80 in feed for 7 days a week for up to 103 weeks. After 15 months of polysorbate 80 administration, 7 to 10 animals from each group were evaluated.

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Frederick Cancer Research Facility (Frederick, MD) for use in the 2-year studies and were observed for 15 to 19 days before the studies began. Five rats and mice per sex were randomly selected and killed for parasite evaluation and gross observation of disease. Serology samples were collected for viral screens. Male rats and mice were approximately 56 days old and female rats and mice were approximately 64 days old when the studies began. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program.

Animal Maintenance

Rats and mice were housed five per cage. Cages were rotated within racks and racks were rotated within rooms every 2 weeks. Feed and water were available *ad libitum*. Further details of animal maintenance are given in Table 1. Temperature and relative humidity levels in the animal rooms experienced a few significant excursions from the normal range around month 5 and month 19 of the studies due to difficulties with the environmental control system. However, for the remainder of the studies temperature and relative humidity levels were at or near the recommended ranges of 72° ± 2° F and 40% to 60%, respectively. These isolated excursions had no apparent adverse effects on animal health. Information on feed composition is provided in Appendix I.

Clinical Examinations and Pathology

Clinical observations were made twice daily; findings were recorded weekly for 13 weeks, then monthly or as necessary thereafter. Animals were weighed at study initiation, weekly for 13 weeks, and monthly thereafter. Feed consumption was measured once a month (Appendix H).

The brain, right kidney, and liver of each animal evaluated at 15 months were weighed at necropsy. Further details of the interim evaluations are presented in Table 1.

Animals found in a moribund state, selected for the 15-month interim evaluations, or surviving to the end of the 2-year studies were killed. Necropsies were performed on all animals. At necropsy, all organs and tissues were examined for gross lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. At the 15-month interim evaluation, a complete histopathologic examination was performed on rats and mice that received 0 or 50,000 ppm; tissues with grossly visible lesions were examined from male and female rats and male mice receiving 25,000 ppm, and gross lesions and stomachs were examined from female mice that received 25,000 ppm. Complete histopathology was performed on all animals dying or killed in a moribund condition prior to the end of the studies, on all rats, and on all control and high-dose mice that survived to the end of the studies. Stomachs and tissues with grossly visible lesions were examined from male and female mice that received 25,000 ppm. Tissues examined are listed in Table 1.

Upon completion of the microscopic evaluation by the study laboratory pathologist, the pathology data were entered into the Toxicology Data Management System. The microscope 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 quality assessment laboratory. The individual animal records and pathology tables were compared for accuracy, slide and tissue counts were verified, and histotechnique was evaluated by the quality assessment laboratory. The spleen from male and female rats, the adrenal gland (medulla) and the mediastinal and mesenteric lymph nodes from male rats, the liver from female rats, and the stomach and forestomach from male and female mice were reviewed microscopically by the quality assessment pathologist for neoplasms or nonneoplastic lesions.

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected tissues and any other tissues for which there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnosis between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chair to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without knowledge of dose groups or previously rendered diagnoses. When the consensus opinion of the PWG differed from that of the laboratory pathologist, the diagnosis was changed to reflect the PWG consensus. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of pathology data, the diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses at the time they were found dead of other than natural causes or were found to be missing; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidence of neoplasms or nonneoplastic lesions is given as the ratio of the number of animals

bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators include only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions had multiple potential sites of occurrence (e.g., mononuclear cell leukemia), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence

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

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

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

Historical Control Data

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

Analysis of Continuous Variables

Organ and body weight data, which have approximately normal distributions, were analyzed using the multiple comparison procedures of Williams (1971, 1972) and Dunnett (1955). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams' test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-response (Dunnett's test).

QUALITY ASSURANCE METHODS

The 13-week and 2-year studies were conducted in compliance with FDA Good Laboratory Practice Regulations (CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, they were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and preliminary review draft of the NTP Technical Report were conducted. Audit procedures and findings are presented in the reports, which are on file at the NIEHS. The audit findings were reviewed and assessed by NTP staff so that all had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICITY

The genetic toxicity of polysorbate 80 was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*. The protocol for this study and tabular presentations of the findings are in Appendix E.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Polysorbate 80

14-Day Studies	13-Week Studies	2-Year Studies
Study Laboratory		
Southern Research Institute (Birmingham, AL)	Same as 14-day studies	Same as 14-day studies
Strain and Species		
Rats: F344/N Mice: $B6C3F_1$	Same as 14-day studies	Same as 14-day studies
Animal Source		
Harlan Industries (Indianapolis, IN)	Charles River Breeding Laboratories (Kingston, NY)	Frederick Cancer Research Facility (Frederick, MD)
Size of Study Groups		
5 males and 5 females	10 males and 10 females	60 males and 60 females
Doses		
0, 3,000, 6,000, 12,500, 25,000, or 50,000 ppm of polysorbate 80 in feed	0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm polysorbate 80 in feed	0, 25,000, or 50,000 ppm polysorbate 80 in feed
Time Held Before Study		
16 days	19 days	20 days
Average Age When Placed on Study		
Rats: 45 days Mice: 58 days	Rats: 51 days Mice: 58 days	Rats: 57 days (males) 64 days (females) Mice: 57 days (males) 64 days (females)
Date of First Dose		
19 March 1981	16 June 1981	Rats: 25 August 1982 Mice: 18 August 1982
Duration of Dosing		
14 days	Day 2 to day of sacrifice (days 91-94)	103 weeks (7 days/week, from day 2)
Date of Last Dose		
1 April 1981	14-17 September 1981	Rats: 14 August 1984 Mice: 7 August 1984
Average Age When Killed		
Rats: 64 days Mice: 77 days	Rats: 144 days Mice: 151 days	Rats: Interim - 519 or 527 days Terminal - 788 or 796 days Mice: Interim - 514 or 522 days Terminal - 789 or 796 days
Animals per Cage		
10 during quarantine, 5 during testing	Same as 14-day studies	Same as 14-day studies

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Polysorbate 80 (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Method of Animal Distribution		
Animals were grouped by body weight and assigned to cages. Cages were assigned to treatment groups using a table of random numbers.	Same as 14-day studies	Same as 14-day studies
Method of Animal Identification		
Ear mark	Ear mark	Ear mark and toe clip
Diet		
NIH-07 Open-Formula Mash (Zeigler Bros., Inc., Gardners, PA), available *ad libitum*	Same as 14-day studies	Same as 14-day studies
Water		
Tap water via automatic watering system (Edstrom Industries, Inc., Waterford, WI), checked daily, flushed every two weeks, available *ad libitum*	Same as 14-day studies	Same as 14-day studies
Cages		
Solid-bottom polycarbonate (Lab Products, Inc., Garfield, NJ), changed twice weekly	Same as 14-day studies	Same as 14-day studies
Bedding		
BetaChips® hardwood laboratory bedding (Northeastern Products Corp., Warrensburg, NY), changed twice weekly	Same as 14-day studies	Same as 14-day studies
Cage Filters		
Reemay spun-bonded polyester (Snow Filtration, Cincinnati, OH), changed once every 2 weeks	Same as 14-day studies	Same as 14-day studies
Racks		
Stainless steel (Lab Products, Inc., Garfield, NY), changed once every 2 weeks	Same as 14-day studies	Same as 14-day studies
Animal Room Environment		
Temperature: 71°-75° F Relative humidity: 40%-56% Fluorescent light: 12 hours/day Room air changes: minimum of 15 changes/hour	Temperature: 70°-77° F Relative humidity: 46%-59% Fluorescent light: 12 hours/day Room air changes: minimum of 15 changes/hour	Temperature: 64°-92° F Relative humidity: 18%-82% Fluorescent light: 12 hours/day Room air changes: minimum 15 changes/hour
Type and Frequency of Observation		
Observed twice/day; weighed initially and once/week; clinical observations recorded twice/day and at study termination; feed consumption once/week by cage	Observed twice/day; weighed once/week and at study termination; clinical observations recorded once/week; feed consumption once/week by cage	Observed twice/day; weighed once/week for 13 weeks, once/month thereafter; clinical observations recorded once/week for 13 weeks, once/month thereafter; feed consumption measured once/month

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Polysorbate 80 (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Necropsy Necropsy performed on all animals. The following organs were weighed: brain, heart, right kidney, liver, lung, and thymus.	Necropsy performed on all animals. The following organs were weighed: brain, heart, right kidney, liver, lung, right testis, and thymus.	Necropsy performed on all animals. Organs weighed for all animals at 15-month interim evaluation: brain, right kidney, and liver.
Histopathology Complete histopathology performed on animals receiving 0 or 50,000 ppm. Tissues examined included: adrenal gland, bone and marrow (femur), brain, clitoral or preputial gland (rats), colon, esophagus, heart, kidney, liver, lung and bronchi, mammary gland, mandibular lymph node, mesenteric lymph node, nasal cavity, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine, spleen, stomach, testis, thigh muscle, thymus, thyroid gland, trachea, urinary bladder, and uterus. Livers were examined from male rats in all other dose groups and from female rats in the 25,000 ppm dose group.	Complete histopathology on all animals that received 0 or 50,000 ppm. Tissues examined included: adrenal gland, bone and marrow (femur), brain, clitoral or preputial gland (rats), colon, esophagus, gallbladder (mice), gross lesions and tissue masses with regional lymph nodes, heart, kidney, liver, lung and mainstem bronchi, mammary gland, mandibular or mesenteric lymph node, nasal cavity and turbinates, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, small intestine, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.	Complete histopathology performed on all animals that died or were killed moribund prior to study termination, all animals from the 15-month interim evaluation that received 0 or 50,000 ppm, all rats from all dose groups and all mice from the 0 and 50,000 ppm dose groups that survived to study termination. At the interim evaluation, gross lesions and stomachs were examined from female mice receiving 25,000 ppm. At study termination, gross lesions and stomachs were examined from male and female mice that received 25,000 ppm. Tissues examined included: adrenal gland, bone and bone marrow, brain (frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons), clitoral or preputial gland (rats), esophagus, gallbladder (mice), gross lesions and tissue masses, heart, kidney, large intestine (cecum, colon, and rectum), liver, lungs and mainstem bronchi, mammary gland, mandibular or mesenteric lymph nodes, nasal cavity and turbinates, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, and ileum), spleen, stomach, testis (with epididymis), thymus, thyroid gland, trachea, urinary bladder, and uterus.

RESULTS

RATS

14-Day Studies

All rats survived to the end of the studies (Table 2). The mean body weight change of males receiving 50,000 ppm was significantly lower than that of the controls. There were no clinical findings related to polysorbate 80 administration.

Changes in absolute and relative organ weights were not considered biologically significant (Table F1). Slight clear vacuolation of hepatocyte cytoplasm was observed in several dosed males. No gross or microscopic lesions in dosed females were considered related to chemical administration.

TABLE 2
Survival, Mean Body Weights, and Feed Consumption of Rats in the 14-Day Feed Studies of Polysorbate 80

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Average Weekly Feed Consumption (g)
		Initial	Final	Change		
Male						
0	5/5	128 ± 2	201 ± 6	73 ± 4		118
3,000	5/5	124 ± 3	193 ± 5	69 ± 3	96	117
6,000	5/5	127 ± 5	191 ± 5	64 ± 4	95	111
12,500	5/5	125 ± 3	187 ± 4	61 ± 3	93	110
25,000	5/5	126 ± 3	192 ± 4	65 ± 3	95	112
50,000	5/5	134 ± 1	196 ± 2	62 ± 2*	98	116
Female						
0	5/5	106 ± 3	135 ± 2	29 ± 2		88
3,000	5/5	106 ± 2	139 ± 2	34 ± 2	103	81
6,000	5/5	107 ± 1	140 ± 2	34 ± 2	104	83
12,500	5/5	107 ± 3	143 ± 3*	36 ± 2	106	90
25,000	5/5	107 ± 2	144 ± 3*	36 ± 2	106	88
50,000	5/5	109 ± 2	142 ± 2*	32 ± 2	105	109

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 14 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

13-Week Studies

All rats survived to the end of the studies (Table 3). Final mean body weights and mean body weight gains of dosed groups were similar to those of the control groups. Average feed consumption of dosed groups was similar to that of the controls. There were no clinical findings associated with chemical administration. Changes in absolute or relative organ weights were not considered biologically significant (Table F2). No gross or microscopic lesions in dosed male or female rats were related to polysorbate 80 administration.

Dose Selection Rationale: No compound-related effects occurred in rats that received 50,000 ppm, the highest dose evaluated in the 13-week studies. Thus, 50,000 ppm was selected as the high dose and 25,000 ppm was selected as the low dose for male and female rats in the 2-year studies. Doses greater than 50,000 ppm were considered too high for the 2-year studies. It has been reported in previous studies that levels of polysorbate 80 greater than 50,000 ppm can cause diarrhea. In addition, the substitution of greater than 50,000 ppm (5%) of the diet with polysorbate 80 would have reduced the caloric value of the diet and may also have interfered with the availability of some essential vitamins and minerals. This may have caused nutritional deficiencies that could have led to difficulties in the interpretation of study results.

TABLE 3
Survival and Mean Body Weights of Rats in the 13-Week Feed Studies of Polysorbate 80

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	137 ± 3	348 ± 8	211 ± 6	
3,100	10/10	138 ± 3	364 ± 6	226 ± 5	105
6,200	10/10	137 ± 2	350 ± 3	213 ± 3	100
12,500	10/10	139 ± 4	351 ± 4	212 ± 5	101
25,000	10/10	138 ± 3	344 ± 6	206 ± 5	99
50,000	10/10	137 ± 3	347 ± 4	210 ± 4	100
Female					
0	10/10	116 ± 2	206 ± 3	90 ± 3	
3,100	10/10	116 ± 2	207 ± 3	91 ± 2	101
6,200	10/10	117 ± 2	207 ± 3	90 ± 2	101
12,500	10/10	117 ± 2	202 ± 2	85 ± 3	98
25,000	10/10	118 ± 2	205 ± 1	87 ± 2	100
50,000	10/10	117 ± 2	205 ± 4	88 ± 3	100

[a] Number of animals surviving at 13 weeks/number initially in group. Differences from the control group were not significant by Williams' or Dunnett's test.
[b] Weights and weight changes are given as mean ± standard error.

2-Year Studies

15-Month Interim Evaluations

No significant changes in absolute or relative organ weights occurred (Table F3). Neoplasms observed during the 15-month interim evaluations are listed in Table 4. No neoplasms or nonneoplastic lesions were considered related to the administration of polysorbate 80.

Body Weights, Feed Consumption, and Clinical Findings

The mean body weights of dosed and control males and females were similar throughout the studies (Tables 5 and 6 and Figure 1). Average feed consumption of male and female rats in all dose groups was similar to that of the controls (Tables H1 and H2). There were no clinical findings associated with the administration of polysorbate 80.

TABLE 4
Incidences of Neoplasms in Rats at the 15-Month Interim Evaluations in the 2-Year Feed Studies of Polysorbate 80[a]

	0 ppm	50,000 ppm
Male		
Pituitary gland, pars distalis		
Adenoma	1/10	0/9
Skin, subcutis		
Lipoma	1/10	0/10
Testis, interstitial cell		
Adenoma	9/10	9/10
Thyroid gland, follicular cell		
Adenoma	0/10	1/10
Female		
Mammary gland		
Fibroadenoma	1/10	1/10
Pituitary gland, pars distalis		
Adenoma	1/10	0/10
Pituitary gland, pars intermedia		
Adenoma	1/10	0/10
Thyroid gland, C-cell		
Adenoma	0/10	1/10
Uterus		
Stromal polyp	3/10	0/10
Stromal sarcoma	1/10	0/10

[a] Incidences given as number of lesions/number of tissues examined and reflect consensus of study pathologist and PWG.

TABLE 5
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of Polysorbate 80

Weeks on Study	0 ppm		25,000 ppm			50,000 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	149	60	151	101	60	149	100	60
2	185	60	184	100	60	186	100	60
3	205	60	207	101	60	207	101	60
4	223	60	224	100	60	223	100	60
5	242	60	241	100	60	239	99	60
6	252	60	252	100	60	246	98	60
7	266	60	268	101	60	263	99	60
8	277	60	278	100	60	276	100	60
9	292	60	292	100	60	290	100	60
10	303	60	305	101	60	302	100	60
11	311	60	311	100	60	309	99	60
12	321	60	323	100	60	320	100	60
13	330	60	333	101	60	327	99	60
14	339	60	344	101	60	338	100	60
17	360	60	361	100	59	356	99	60
21	384	60	387	101	59	384	100	60
25	406	60	406	100	59	402	99	60
29	420	60	420	100	59	416	99	60
33	436	60	434	100	59	430	99	60
37	446	60	447	100	59	444	100	60
41	452	60	450	100	59	447	99	59
45	456	60	458	101	59	452	99	59
49	459	60	463	101	59	453	99	59
53	451	60	464	103	59	457	101	59
57	458	60	466	102	59	456	100	59
61	461	60	467	102	59	459	100	58
65	459	60	473	103	59	457	100	58
69[a]	469	47	472	101	49	465	99	45
73	472	46	479	102	47	465	99	43
77	471	46	479	102	47	465	99	43
81	469	46	481	103	46	470	100	43
85	480	42	478	100	46	473	99	40
89	481	41	473	98	42	467	97	39
93	476	38	474	100	34	470	99	36
97	450	37	452	100	31	444	99	32
101	434	32	441	102	22	437	101	21
104	430	29	436	101	18	429	100	18
Terminal sacrifice		29			18			18
Mean for weeks								
1-13	258		259	100		257	100	
14-52	416		417	100		412	99	
53-104	462		467	101		458	99	

[a] Interim evaluation occurred during week 66.

TABLE 6
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of Polysorbate 80

Weeks on Study	0 ppm		25,000 ppm			50,000 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	122	60	124	101	60	122	100	60
2	138	60	138	100	60	137	99	60
3	145	60	144	99	60	144	100	60
4	150	60	150	100	60	150	100	60
5	160	60	158	99	60	158	99	60
6	164	60	163	99	60	162	99	60
7	170	60[a]	168	99	60	168	99	60
8	174	60	172	99	60	172	99	60
9	179	60	177	99	60	177	99	60
10	183	60	183	100	60	182	100	60
11	184	60	184	100	60	183	100	60
12	188	60	189	101	60	187	99	60
13	191	60	191	100	60	189	99	60
14	195	60	195	100	60	191	98	60
17	202	60	202	100	60	200	99	60
21	212	60	214	101	60	213	100	60
25	222	60	224	101	60	222	100	60
29	227	59	231	102	60	227	100	60
33	237	59	238	101	60	233	98	60
37	246	59	251	102	60	247	100	60
41	254	59	254	100	59	251	99	60
45	262	59	264	101	59	256	98	60
49	276	59	278	101	58	266	96	59
53	284	59	285	100	58	275	97	59
57	297	58	296	100	58	284	96	59
61	305	58	302	99	58	292	96	59
65	316	58	313	99	58	305	97	58
69[b]	326	48	323	99	45	314	96	48
73	332	48	333	100	45	324	98	48
77	341	47	337	99	44	331	97	48
81	347	45	339	98	44	335	97	47
85	354	43	347	98	43	343	97	44
89	353	42	349	99	42	346	98	40
93	351	35	349	100	35	351	100	34
97	347	31	347	100	34	345	99	34
101	348	28	348	100	31	345	99	29
104	354	23	353	100	25	346	98	25
Terminal sacrifice		23			25			25
Mean for weeks								
1-13	165		165	100		164	99	
14-52	233		235	101		231	99	
53-104	333		330	99		324	97	

[a] The number of animals weighed for this week is fewer than the number of animals surviving.
[b] Interim evaluation occurred during week 66.

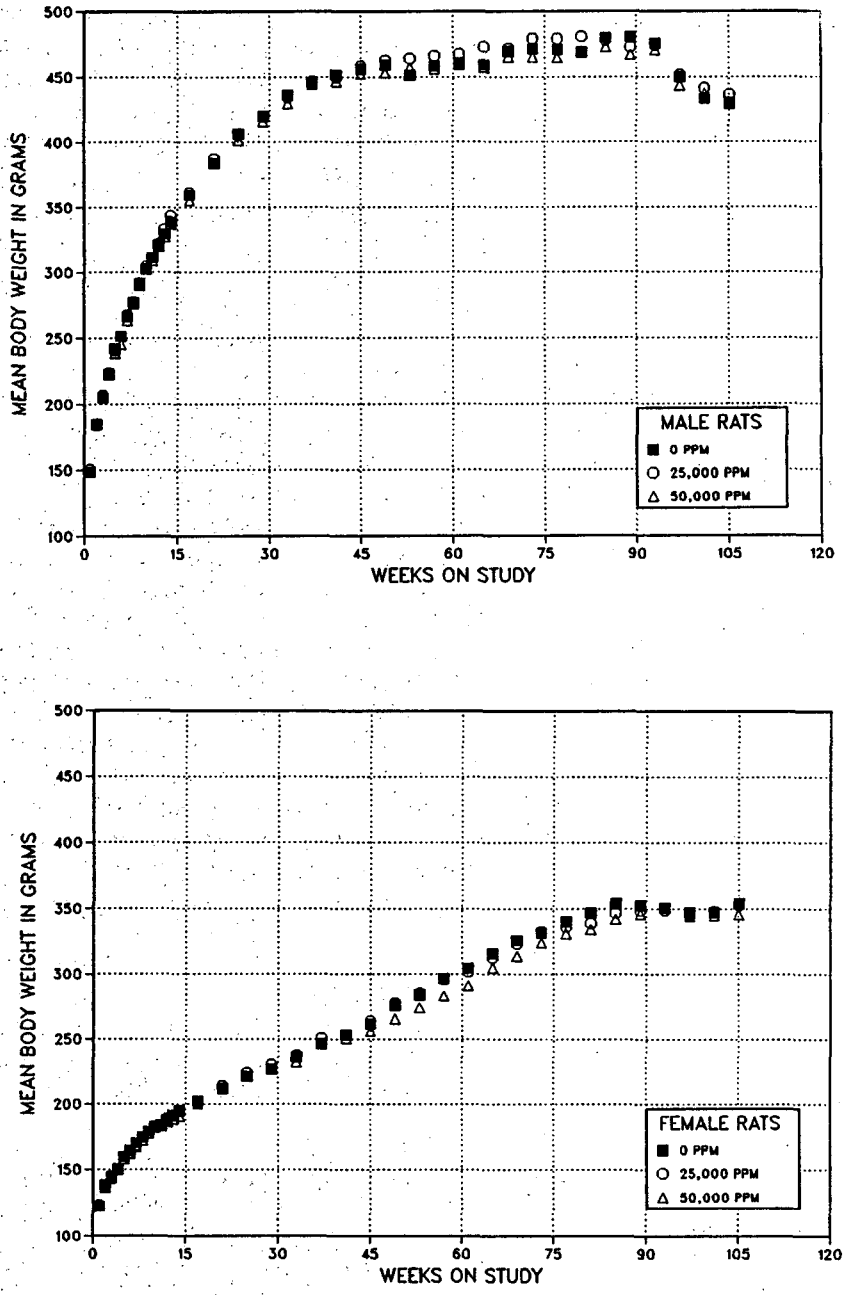


FIGURE 1
Growth Curves for Rats Administered Polysorbate 80 in Feed for 2 Years

Survival

The survival of dosed female rats was similar to that of the controls (Table 7 and Figure 2). Survival of dosed male rats was lower than that of the controls primarily due to an increased number of moribund animals killed after week 93. The animals were killed mainly because of the presence of one of a variety of neoplasms commonly seen in aging F344/N rats, including mononuclear cell leukemia, pituitary gland adenoma, preputial gland carcinoma, mammary gland fibroadenoma, Zymbal's gland carcinoma, and mesothelioma.

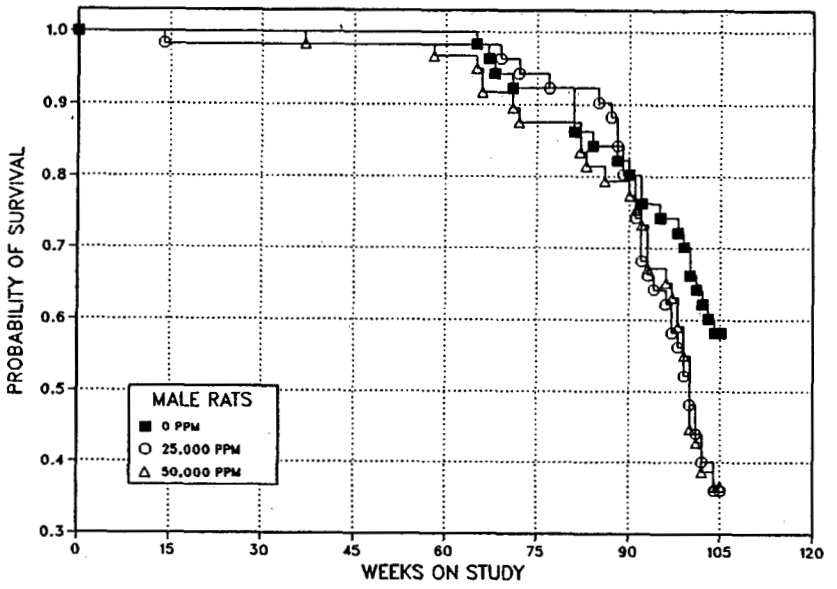
Sentinel Animals

Positive serological titers for pneumonia virus of mice were found in sentinel rats at 6, 12, 18, and 24 months (Table J1). However, there was no clinical or histopathologic evidence of disease.

TABLE 7
Survival of Rats in the 2-Year Feed Studies of Polysorbate 80

	0 ppm	25,000 ppm	50,000 ppm
Male			
Animals initially in study	60	60	60
15-month interim evaluation[a]	10	10	10
Natural deaths	7	5	7
Moribund kills	14	27	25
Animals surviving to study termination	29	18	18
Percent survival at end of study[b]	58	36	37
Mean survival (days)[c]	645	631	623
Survival analysis[d]	P=0.043	P=0.058	P=0.048
Female			
Animals initially in study	60	60	60
15-month interim evaluation[a]	10	10	10
Natural deaths	2	3	4
Moribund kills	25	22	21
Animals surviving to study termination	23	25	25
Percent survival at end of study[b]	46	50	50
Mean survival (days)[c]	633	633	640
Survival analysis[d]	P=0.779N	P=0.780N	P=0.843N

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



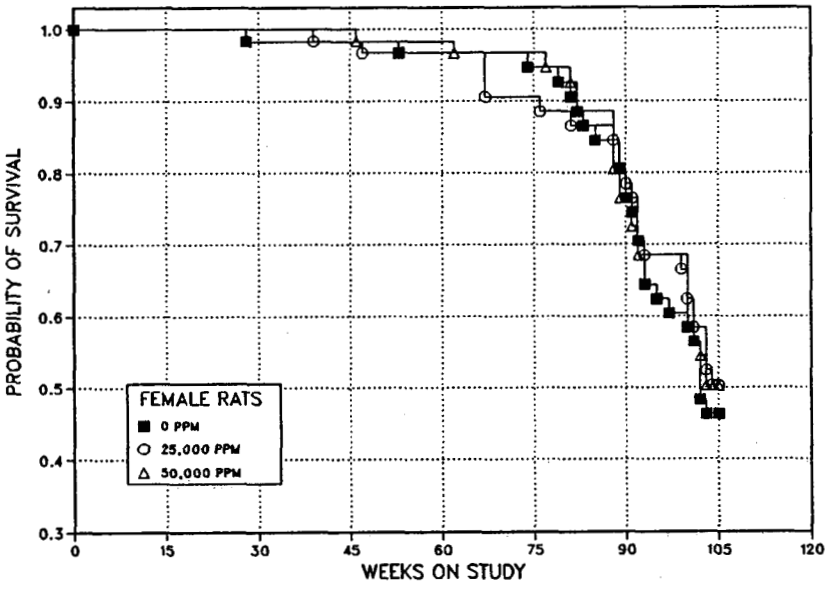


FIGURE 2
Kaplan-Meier Survival Curves for Rats Administered Polysorbate 80 in Feed for 2 Years

Pathology and Statistical Analysis of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplastic or nonneoplastic lesions of the adrenal medulla, hematopoietic system, and spleen in rats.

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

Adrenal Medulla: The incidence of benign or malignant pheochromocytoma (combined) was significantly increased in the high-dose male group (Table 8). The incidence in the high-dose group (58%) exceeds the historical control range of 22% to 48% for male rats from current NTP 2-year feed studies (Table A4). Nearly all of the pheochromocytomas in all groups occurred in a single gland. The incidences of bilateral neoplasms (4/50, 4/50, 7/50) and malignant neoplasms were low and were similar across treatment groups. Thus, the increased incidence of pheochromocytomas in high-dose males was due to an increase in the number of benign pheochromocytomas occurring in a single gland.

TABLE 8
Adrenal Medulla Lesions in Male Rats in the 2-Year Feed Study of Polysorbate 80[a]

	0 ppm	25,000 ppm	50,000 ppm
Hyperplasia			
Overall rates[a]	11/50 (22%)	22/50 (44%)°	12/50 (24%)
Benign Pheochromocytoma			
Overall rates	21/50 (42%)	16/50 (32%)	28/50 (56%)
Adjusted rates[b]	57.0%	52.5%	86.8%
Terminal rates[c]	14/29 (48%)	6/18 (33%)	14/18 (78%)
First incidence (days)	561	613	570
Logistic regression tests[d]	P=0.030	P=0.256N	P=0.032
Malignant Pheochromocytoma			
Overall rates	1/50 (2%)	4/50 (8%)	1/50 (2%)
Adjusted rates	3.4%	16.5%	2.0%
Terminal rates	1/29 (3%)	2/18 (11%)	0/18 (0%)
First incidence (days)	729 (T)	644	400
Logistic regression tests	P=0.584N	P=0.154	P=0.690N
Benign or Malignant Pheochromocytoma[e]			
Overall rates	21/50 (42%)	19/50 (38%)	29/50 (58%)
Adjusted rates	57.0%	61.6%	87.0%
Terminal rates	14/29 (48%)	8/18 (44%)	14/18 (78%)
First incidence (days)	561	613	400
Logistic regression tests	P=0.023	P=0.499N	P=0.027

(T)Terminal sacrifice

° Significantly different ($P<0.05$) from the control group by the logistic regression tests

[a] Number of lesion-bearing animals/number of animals necropsied or examined microscopically at site

[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard tumors in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in a dose group is indicated by N.

[e] Historical incidence for 2-year studies with untreated control groups in NTP feed studies (mean ± SD): 306/788 (38.8% ± 8.4%), range 22%–48%

The incidence of adrenal medulla hyperplasia, which is generally considered to be a precursor to pheochromocytoma, was increased in the low-dose group but not in the high-dose group. At the 15-month interim evaluation, no pheochromocytomas were observed in control or dosed male rats, and adrenal medulla hyperplasia was observed in only one control male (Table 4).

There was a morphologic continuum from adrenal medulla hyperplasia to benign pheochromocytoma to malignant pheochromocytoma. Hyperplasias were microscopic lesions consisting of clusters of cells which were smaller and more basophilic than normal adrenal medulla cells. Hyperplasias had poorly defined borders and caused little or no compression of the adjacent parenchyma. Benign pheochromocytomas often were small lesions, somewhat larger than hyperplasias and composed of similar-appearing cells, and were distinguished from hyperplasias by the fact that they had discrete borders and produced distinct compression of the adjacent parenchyma. Some benign pheochromocytomas, however, were large and replaced the medullary or cortical parenchyma or both. Malignant pheochromocytomas were large neoplasms that had replaced much of the adrenal gland parenchyma and had invaded through the capsule into adjacent tissues. The presence of invasion was required in order for a pheochromocytoma to be considered malignant.

Hematopoietic System: The incidences of mononuclear cell leukemia in dosed female rats were significantly lower than that of the controls (26/50, 10/50, 13/50; Table B3). The incidences in all groups were within the range of historical controls for female rats from NTP 2-year feed studies (213/800 or 26.6%, range 14%-52%). Incidences in the dosed groups are similar to the average incidence of the historical controls. The incidence in the control group, however, was equal to the upper limit of the historical range. Thus, the significant differences between the incidences in the control and dosed groups appear to be a result of the unusually high incidence in the control group and not related to polysorbate 80 administration.

Spleen: There was a slight but significant ($P<0.01$) increase in the average severity of hematopoietic cell proliferation involving the erythroid cells in the spleens of high-dose male rats; the incidence of hematopoietic cell proliferation was slightly increased in the high-dose group (30/50, 31/50, 37/50). Based on a scale of 1=minimal, 2=mild, 3=moderate, and 4=marked, the average severity grades for affected animals were: control=1.6, low-dose=1.9, and high-dose=2.2. The severity of hematopoietic cell proliferation was graded based on the percentage of red pulp occupied by clusters of erythropoietic cells as follows: minimal, up to 10%; mild, 10% to 25%; moderate, 25% to 50%; marked, 50% or greater. A number of spleens from control and dosed males were evaluated by the Pathology Working Group (PWG); the consensus opinion of the PWG was that the differences between spleens from control and treated animals were slight and were not considered to indicate a chemical-related effect. In addition, no potential cause for increased erythropoiesis was seen in dosed male rats, and there was no corresponding increase in erythropoiesis in dosed females. Consequently, the significance of the slight increase in the severity of splenic erythropoiesis in dosed male rats is unclear. Sarcomas occurred in the spleens of two high-dose male rats; none occurred in the control or low-dose males. Splenic sarcomas are uncommon in control male rats from current NTP 2-year feed studies, occurring with an incidence of 2/796 and with no more than one sarcoma in a group. Although the incidence in this study just exceeds the historical control range for dosed feed studies, the total incidence is still very low. In addition, there was no increase in potentially preneoplastic lesions, such as splenic fibrosis, in treated males. Consequently, the presence of these two splenic sarcomas was not considered to be due to polysorbate 80 administration.

MICE

14-Day Studies

All mice survived to the end of the studies (Table 9). The final mean body weight and mean body weight gain of males in the 25,000 ppm dose group were lower than those of the controls due to marked weight loss in one animal. If the weight of this animal is excluded, the final mean body weight and mean body weight gain of this group are similar to those of the controls. Final mean body weights and mean body weight gains in other dose groups of males and females were similar to those of the controls. There were no clinical findings related to polysorbate 80 administration.

There were no biologically significant changes in absolute or relative organ weights (Table F4). No compound-related gross or microscopic lesions in males or females were related to polysorbate 80 administration.

TABLE 9
Survival, Mean Body Weights, and Feed Consumption of Mice in the 14-Day Feed Studies of Polysorbate 80

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Average Weekly Feed Consumption (g)
		Initial	Final	Change		
Male						
0	5/5	24.4 ± 0.9	25.6 ± 1.2	1.2 ± 0.7		48
3,000	5/5	23.0 ± 0.8	26.0 ± 1.2	3.0 ± 0.6	102	55
6,000	5/5	23.8 ± 0.6	26.4 ± 0.8	2.6 ± 0.2	103	53
12,500	5/5	25.2 ± 0.8	27.6 ± 0.9	2.4 ± 0.2	108	54
25,000	5/5	24.2 ± 0.7	22.4 ± 1.5	-1.8 ± 2.1	87	50
50,000	5/5	25.0 ± 0.5	27.0 ± 0.9	2.0 ± 0.7	106	51
Female						
0	5/5	17.4 ± 0.4	19.6 ± 0.5	2.2 ± 0.4		54
3,000	5/5	17.8 ± 0.2	19.4 ± 0.2	1.6 ± 0.2	99	54
6,000	5/5	17.6 ± 0.2	19.4 ± 0.4	1.8 ± 0.4	99	53
12,500	5/5	18.4 ± 0.7	20.8 ± 0.8	2.4 ± 0.5	106	56
25,000	5/5	17.6 ± 0.6	19.6 ± 0.7	2.0 ± 0.3	100	54
50,000	5/5	18.4 ± 0.2	20.0 ± 0.3	1.6 ± 0.5	102	50

[a] Number of animals surviving at 14 days/number initially in group. Differences from the control group were not significant by Williams' or Dunnett's test.
[b] Weights and weight changes are given as mean ± standard error.

13-Week Studies

All mice survived to the end of the studies (Table 10). Body weight gain was significantly lower in the 25,000 ppm male group. Final mean body weights in dosed groups were similar to those of the controls. Average feed consumption by dosed mice was similar to that of the controls throughout the studies. There were no clinical findings related to polysorbate 80 administration. Changes in absolute or relative organ weights were not considered biologically significant (Table F5). No gross or microscopic lesions in males or females were related to the administration of polysorbate 80.

Dose Selection Rationale: No compound-related effects occurred in mice that received 50,000 ppm, the highest dose evaluated in the 13-week studies. Thus, 50,000 ppm was selected as the high dose and 25,000 ppm was selected as the low dose for the 2-year studies. Doses greater than 50,000 ppm were not considered appropriate for the 2-year studies, because diets containing more than 50,000 ppm polysorbate 80 could lead to nutritional deficiencies in the study animals.

TABLE 10
Survival and Mean Body Weights of Mice in the 13-Week Feed Studies of Polysorbate 80

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	23.3 ± 0.5	34.7 ± 0.8	11.4 ± 0.5	
3,100	10/10	23.3 ± 0.5	33.7 ± 0.8	10.4 ± 0.4	97
6,200	10/10	23.6 ± 0.5	34.5 ± 1.0	10.9 ± 1.3	99
12,500	10/10	24.1 ± 0.6	34.5 ± 1.0	10.4 ± 0.5	99
25,000	10/10	24.0 ± 0.5	32.8 ± 0.9	8.8 ± 0.6*	94
50,000	10/10	23.5 ± 0.4	34.0 ± 0.7	10.5 ± 0.6	98
Female					
0	10/10	18.1 ± 0.3	26.2 ± 0.7	8.1 ± 0.5	
3,100	10/10	17.5 ± 0.2	26.0 ± 0.5	8.5 ± 0.5	99
6,200	10/10	17.7 ± 0.3	26.1 ± 0.7	8.4 ± 0.7	100
12,500	10/10	17.6 ± 0.3	24.6 ± 0.7	7.0 ± 0.5	94
25,000	10/10	17.8 ± 0.3	27.3 ± 0.5	9.5 ± 0.3	104
50,000	10/10	17.8 ± 0.2	25.9 ± 0.3	8.1 ± 0.3	99

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

2-Year Studies

15-Month Interim Evaluations

No significant changes in absolute or relative organ weights occurred (Table F6). Compound-related lesions, which occurred in 5 of 10 high-dose female mice, included mild hyperplasia of the forestomach epithelium and inflammation of the forestomach. Neoplasms observed during the 15-month interim evaluations are listed in Table 11. There were no compound-related neoplasms in males or females and no compound-related nonneoplastic lesions in males.

Body Weights, Feed Consumption, and Clinical Findings

Mean body weights of dosed male mice were similar to those of controls (Table 12 and Figure 3). The final mean body weight of high-dose females was 11% lower than that of controls, while the final mean body weight of the low-dose females was similar to that of the controls (Table 13). Average feed consumption of dosed males and females was similar to that of the controls (Tables H3 and H4). No clinical findings were associated with the administration of polysorbate 80 to male or female mice.

TABLE 11
Incidences of Neoplasms in Mice at the 15-Month Interim Evaluations in the 2-Year Feed Studies of Polysorbate 80[a]

	0 ppm	25,000 ppm	50,000 ppm
Male			
Liver			
Hepatocellular adenoma	0/7	0/7	1/10
Female			
Lung			
Alveolar/bronchiolar adenoma	0/9	1/10	1/10
Ovary			
Cystadenoma	1/9	0/10	0/10
Spleen			
Lymphoma, malignant mixed	1/9	0/10	0/10
Uterus			
Stromal polyp	0/9	1/10	2/10

[a] Incidences given as number of lesions/number of tissues examined.

TABLE 12
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of Polysorbate 80

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm Number of Survivors	25,000 ppm Av. Wt. (g)	25,000 ppm Wt. (% of controls)	25,000 ppm Number of Survivors	50,000 ppm Av. Wt. (g)	50,000 ppm Wt. (% of controls)	50,000 ppm Number of Survivors
1	23.1	60	23.3	101	60	23.1	100	60
2	25.1	60	25.6	102	60	25.5	102	60
3	26.0	60	27.0	104	60	25.6	99	60
4	27.0	60	27.5	102	60	26.5	98	60
5	27.5	60	28.0	102	60	27.5	100	60
6	28.4	60	29.0	102	60	28.7	101	60
7	29.3	60	30.0	102	60	29.5	101	60
8	27.9	60	29.6	106	60	29.7	107	60
9	29.8	60	30.5	102	60	30.3	102	60
10	30.1	60	31.2	104	60	30.8	102	60
11	30.1	60	31.3	104	60	30.9	103	60
12	30.0	60	31.3	104	60	30.6	102	60
13	31.2	59	31.8	102	60	31.6	101	60
16	32.7	59	33.5	102	60	32.9	101	60
20	34.4	59	34.6	101	60	34.4	100	60
25	35.0	58[a]	35.7	102	60	34.9	100	57
29	34.3	58	35.5	104	60	35.1	102	57
33	35.8	58	36.8	103	60	35.8	100	57
37	35.8	58	35.4	99	60	35.8	100	57
41	36.6	56	37.4	102	60	36.3	99	56
45	37.7	55	36.8	98	59	36.7	97	56
49	38.9	55[a]	38.4	99	59	38.4	99	56
53	38.3	55	38.3	100	58	37.7	98	56
58	37.3	55	37.4	100	58	37.5	101	56
62	38.5	55	36.2	94	58	37.5	97	56
66[b]	38.3	46	38.4	100	50	38.3	100	46
70	37.6	45	36.8	98	50	37.2	99	46
74	38.3	44	38.5	101	50	38.2	100	46
78	39.0	43	38.3	98	48	37.7	97	46
82	38.9	43	37.5	96	48	37.0	95	44
86	37.4	41	36.1	97	45	36.4	97	44
90	37.6	41	37.7	100	45	36.9	98	40
94	37.5	36	36.4	97	42	36.9	98	37
98	37.6	36	36.5	97	40	37.6	100	36
102	36.2	34	36.2	100	34	35.0	97	34
Terminal sacrifice		33			34			32
Mean for weeks								
1-13	28.1		28.9	103		28.5	101	
14-52	35.7		36.0	101		35.6	100	
53-104	37.9		37.3	98		37.2	98	

[a] The number of animals weighed for this week is fewer than the number of animals surviving.
[b] Interim evaluation occurred during week 66.

TABLE 13
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of Polysorbate 80

Weeks on Study	0 ppm		25,000 ppm			50,000 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	17.4	60	17.0	98	60	17.1	98	60
2	18.9	60	18.7	99	60	18.9	100	60
3	20.1	60	19.9	99	60	19.8	99	60
4	20.5	60	20.3	99	60	20.3	99	60
5	20.2	60	20.6	102	60	20.3	101	60
6	21.7	60	21.5	99	60	21.3	98	60
7	21.2	60	21.0	99	60	22.3	105	60
8	22.1	60	20.3	92	60	21.6	98	60
9	21.9	60	23.0	105	60	22.8	104	60
10	22.8	60	23.4	103	60	23.3	102	60
11	22.6	60	23.1	102	60	22.7	100	60
12	22.6	60	22.7	100	60	22.6	100	60
13	23.0	60	22.8	99	60	22.8	99	60
16	24.9	60	24.2	97	60	24.7	99	60
20	26.4	60	26.1	99	59	25.3	96	60
25	27.7	60	27.5	99	59	26.6	96	60
29	28.6	60	28.0	98	58	27.7	97	60
33	29.6	60	29.3	99	58	28.1	95	60
37	30.2	59	29.2	97	57	28.2	93	60
41	31.1	59	30.9	99	57	29.4	95	60
45	30.9	59	30.7	99	57	28.8	93	60
49	32.6	59	32.1	99	57	30.7	94	60
53	33.3	59	33.0	99	57	30.8	93	59
58	33.2	58	32.9	99	57	31.3	94	58
62	34.8	58	35.0	101	57	32.9	95	57
66[a]	36.4	49	35.8	98	45	33.1	91	44
70	36.9	49	36.1	98	45	33.5	91	41
74	37.6	49	36.0	96	43	34.2	91	40
78	40.0	49	37.0	93	42	34.4	86	40
82	39.9	47	37.7	95	42	34.4	86	39
86	40.4	44	37.0	92	39	34.7	86	36
90	39.9	42	36.6	92	37	34.4	86	35
94	38.9	38	37.0	95	33	35.1	90	33
98	39.5	37	37.2	94	32	34.7	88	33
102	40.0	30	38.1	95	28	35.6	89	28
Terminal sacrifice		30			28			26
Mean for weeks								
1-13	21.2		21.1	100		21.2	100	
14-52	29.1		28.7	99		27.7	95	
53-104	37.8		36.1	96		33.8	89	

[a] Interim evaluation occurred during week 66.

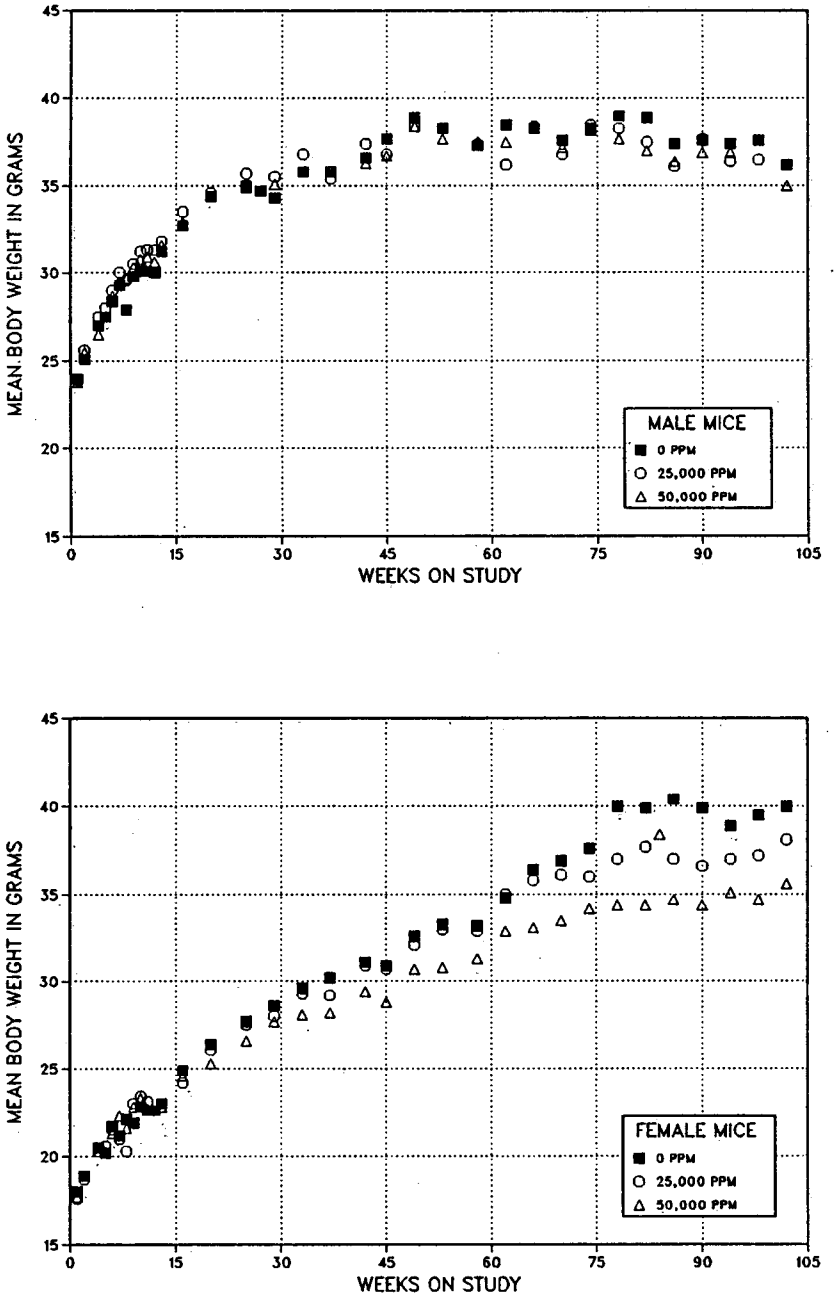


FIGURE 3
Growth Curves for Mice Administered Polysorbate 80 in Feed for 2 Years

Survival

The survival in the dosed groups was similar to that of the controls (Table 14 and Figure 4). Survival was greater than 50% in all groups. Three control males, three low-dose males, and one control female died prior to the 15-month interim evaluations. These early deaths were included in survival analyses but these mice were not included in the 15-month histopathologic examinations.

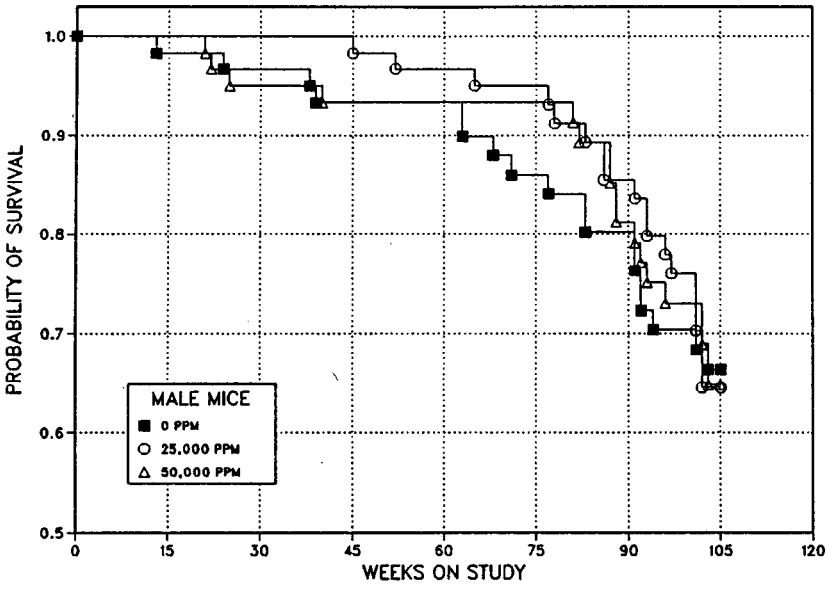
Sentinel Animals

Positive serological titers for pneumonia virus of mice were found in sentinel animals at 6, 12, and 18 months (Table J1). However, there was no clinical or histopathologic evidence of disease.

TABLE 14
Survival of Mice in the 2-Year Feed Studies of Polysorbate 80

	0 ppm	5,000 ppm	10,000 ppm
Male			
Animals initially in study	60	60	60
15-month interim evaluation[a]	7	7	10
Natural deaths	9	8	9
Moribund kills	9	11	9
Missing[a]	1	0	0
Missexed[a]	1	0	0
Animals surviving to study termination	33[b]	34	32
Percent survival at end of study[c]	66	65	65
Mean survival (days)[d]	626	658	628
Survival analysis[e]	P=1.000N	P=1.000N	P=1.000N
Female			
Animals initially in study	60	60	60
15-month interim evaluation[a]	9	10	10
Natural deaths	13	15	15
Moribund kills	8	7	9
Animals surviving to study termination	30	28	26
Percent survival at end of study[c]	59	57	53
Mean survival (days)[d]	648	617	617
Survival analysis[e]	P=0.388	P=0.740	P=0.431

[a] Censored from survival analyses
[b] Includes one animal that died during the last week of the study
[c] Kaplan-Meier determinations. Survival rates adjusted for interim evaluation, missing, and missexed animals.
[d] Mean of all deaths (uncensored, censored, terminal sacrifice)
[e] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A negative trend or lower mortality in a dose group is indicated by N.



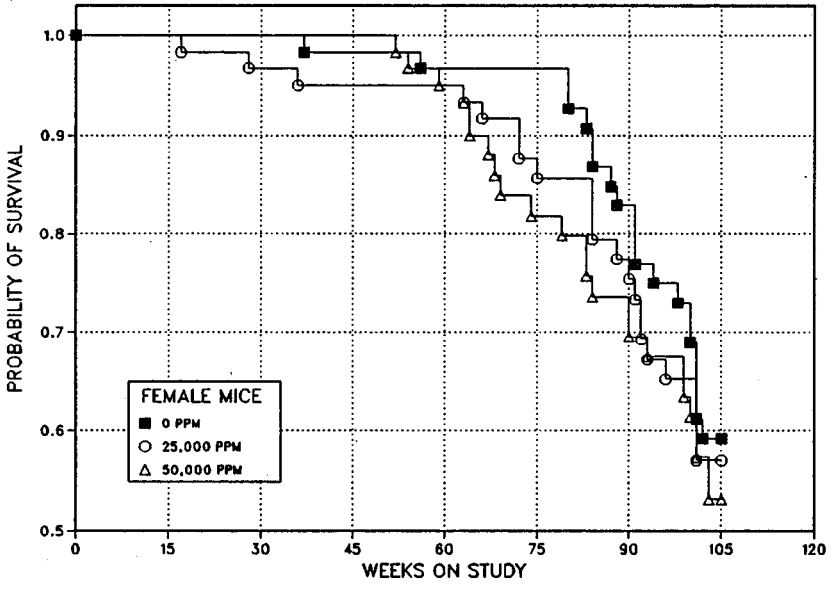


FIGURE 4
Kaplan-Meier Survival Curves for Mice Administered Polysorbate 80 in Feed for 2 Years

Pathology and Statistical Analysis of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the forestomach in mice. At no site in male or female mice was the incidence of neoplasms significantly increased.

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

Forestomach: The incidences of hyperplasia of the forestomach epithelium (hyperplasia, squamous) and inflammation of the forestomach were increased in males and females that received 50,000 ppm (Table 15). Seven high-dose females also had ulcers of the forestomach epithelium which were associated with the areas of hyperplasia and inflammation. One squamous papilloma in a control male and one squamous cell carcinoma in a low-dose male were the only forestomach neoplasms observed in the studies. The hyperplasias were focal, broad-based lesions usually located near the limiting ridge between the forestomach and glandular stomach. Minimal to mild hyperplasia consisted of slight thickening and folding of the epithelium (Plate 1). As the severity of the hyperplasia increased, there was progressively more thickening and folding of the epithelium, producing elevated lesions (Plate 2); in marked lesions the thickened epithelium sometimes formed multiple projections into the lumen. Some hyperplasias were focal, elevated thickenings surrounding an ulcer. Hyperplasias were typically more extensive in high-dose than in low-dose animals. In a number of mice, aggregates of small to moderate numbers of neutrophils (inflammation, acute) were present in the forestomach wall. In several animals, chronic active inflammation, a scattering of a small to moderate number of mixed lymphocytes, macrophages, and neutrophils, often accompanied by varying amounts of fibrosis, were present in the forestomach wall beneath areas of hyperplasia (Plate 3). The forestomach squamous hyperplasia and inflammation in high-dose animals and the ulcers in high-dose females were considered related to polysorbate 80 administration.

Genetic Toxicity

Polysorbate 80 (100-10,000 μg/plate) was tested for gene mutation induction in four strains (TA100, TA1535, TA1537, and TA98) of *Salmonella typhimurium* in a preincubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9; no induction of mutations was observed (Table F1; Mortelmans *et al.*, 1986).

TABLE 15
Incidences of Selected Forestomach Lesions in Mice in the 2-Year Feed Studies of Polysorbate 80[a]

	0 ppm	25,000 ppm	50,000 ppm
Male			
Hyperplasia, squamous	3/48 (6%)	4/50 (8%)	19/50 (38%)**
Inflammation, acute	0/48 (0%)	3/50 (6%)	5/50 (10%)*
Inflammation, chronic active	0/48 (0%)	1/50 (2%)	7/50 (14%)**
Female			
Hyperplasia, squamous	4/49 (8%)	8/50 (16%)	26/49 (53%)**
Inflammation, acute	2/49 (4%)	4/50 (8%)	3/49 (6%)
Inflammation, chronic active	2/49 (4%)	0/50 (0%)	13/49 (27%)**
Ulcer	1/49 (2%)	0/50 (0%)	7/49 (14%)*

* Significantly different ($P<0.05$) from the control group by the logistic regression tests
** $P<0.01$
[a] Number of lesion-bearing animals/number of animals examined at site

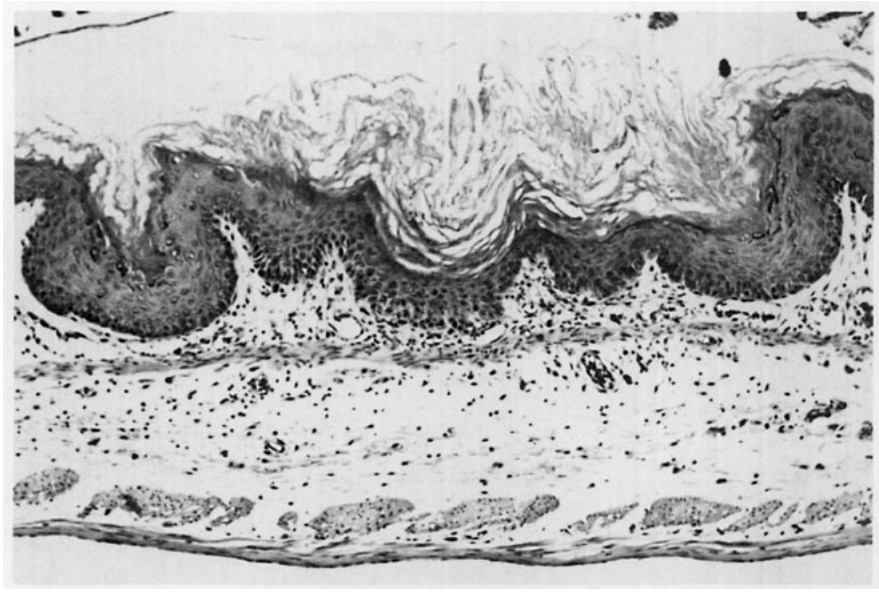


Plate 1
Forestomach: Mild squamous hyperplasia in a female B6C3F$_1$ mouse administered 25,000 ppm polysorbate 80 in the feed for two years. The epithelium is two to three times its normal thickness. ×75

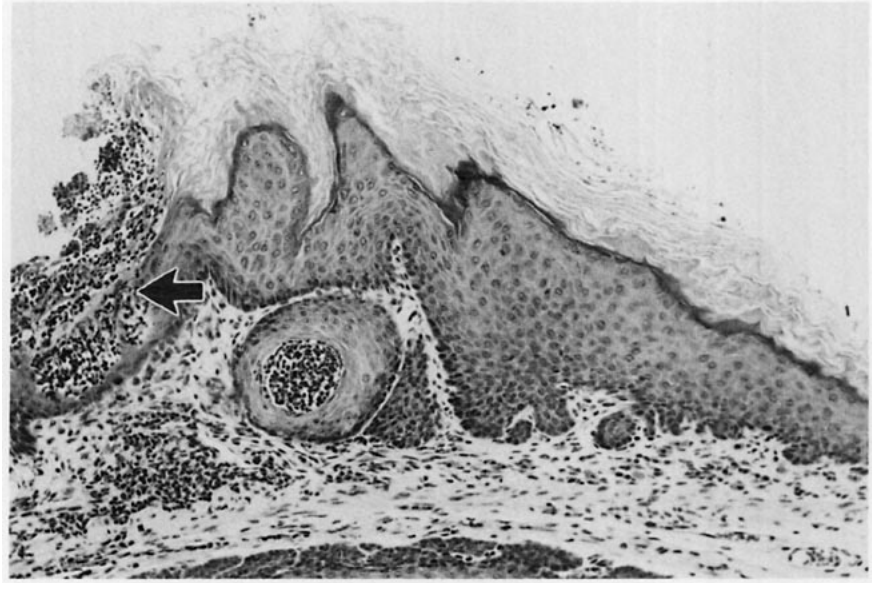


Plate 2
Forestomach: Moderate squamous hyperplasia and inflammation in a male B6C3F$_1$ mouse administered 25,000 ppm polysorbate 80 in the feed for two years. The epithelium is irregularly thickened and folded producing a slightly elevated lesion. Inflammatory cells (arrow) are present in the epithelium and overlying keratin layer, and in the underlying stomach wall. ×100

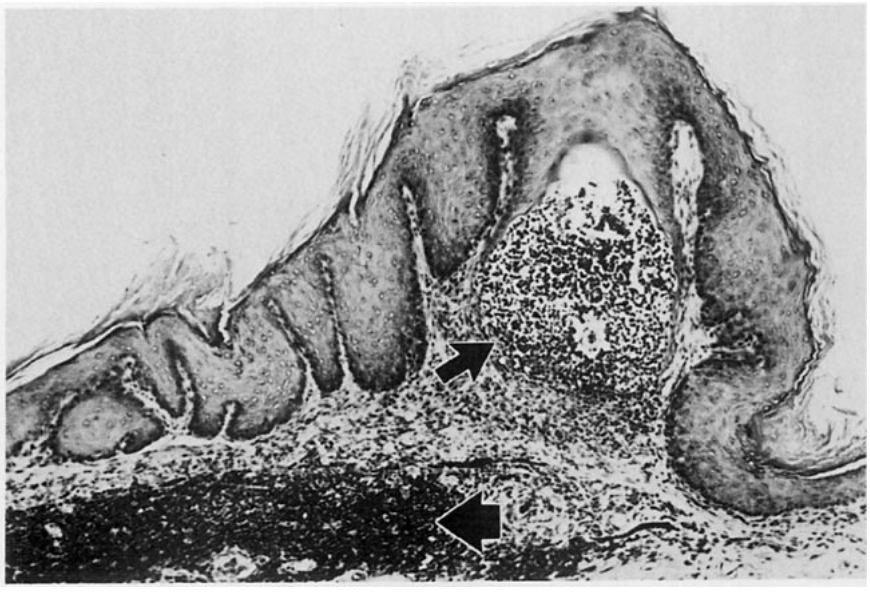


Plate 3

Forestomach: Marked squamous hyperplasia and inflammation in a male $B6C3F_1$ mouse administered 50,000 ppm polysorbate 80 in the feed for 2 years. The markedly thickened epithelium produces a broad elevated lesion. The mass of inflammatory cells (small arrow) within the epithelium indicates the edge of an ulcer, while the aggregate of cells (large arrow) in the underlying stomach wall represents chronic active inflammation. ×75

DISCUSSION AND CONCLUSIONS

Polysorbate 80 is a nonionic surfactant which is used primarily as an oil/water emulsifier. It is an ingredient in a great variety of foods, as well as in numerous types of oral pharmaceutical products; thus, there is widespread human consumption of polysorbate 80. In addition, there is substantial occupational exposure to polysorbate 80. Because of the high degree of human exposure to this compound and the lack of data concerning the possible adverse effects of long-term exposure, polysorbate 80 was evaluated by the NTP for potential toxicity and carcinogenicity in F344/N rats and B6C3F$_1$ mice. The oral route of compound administration, using dosed feed, was chosen for these studies because the most common route of exposure in humans is through the ingestion of foods.

The 14-day and 13-week studies were conducted using doses of up to 50,000 ppm in the feed for rats and mice. Other than slight cytoplasmic vacuolation of hepatocytes in a few dosed male rats during the 14-day study, there were no compound-related effects in any of the short-term studies. Therefore, 50,000 ppm was selected as the high dose for the 2-year studies, and 25,000 ppm was selected as the low dose. Despite the lack of compound-related findings in the short-term studies, the use of dose levels greater than 50,000 ppm was not considered appropriate for the 2-year studies. It is known from the results of previous studies that levels greater than 50,000 ppm can cause diarrhea. In addition, the long-term consumption of diets containing more than 50,000 ppm (5%) polysorbate 80 could lead to nutritional deficiencies in the study animals which could interfere with the interpretation of the study results.

In the 2-year rat studies, polysorbate 80 had no effect on mean body weights of dosed rats. The survival of female rats was similar to that of the controls. The terminal survival of dosed male rats was lower than that of the controls; however, the survival in dosed male rats was similar to that of the controls through week 93 of the study. Therefore, the decreased terminal survival was not considered to affect the evaluation of the carcinogenic potential of polysorbate 80 in the male rats. The decreased survival in dosed male rats resulted from an increased number of animals killed in a moribund condition due to a variety of neoplasms commonly seen in aging male F344/N rats and was not considered to be related to polysorbate 80 administration.

The only potential carcinogenic effect in the 2-year studies occurred in the adrenal medulla of male rats. There was a marginal but statistically significant increase in the incidence of benign and malignant pheochromocytomas in high-dose males (0 ppm, 21/50; 25,000 ppm, 19/50; 50,000 ppm, 29/50). The incidence in the high-dose group (58%) exceeded the highest incidence (24/50, 48%) of pheochromocytoma seen in control male rats from recent NTP 2-year feed studies. However, in an evaluation of historical control incidences in male F344/N rats based upon a broader range of NTP studies than those included in the recent historical control data, Haseman *et al.* (1990) found incidences of pheochromocytoma in untreated male rats as high as 65%. Thus, the slight increase in incidence of pheochromocytoma in the high-dose male rat group was judged to be of questionable significance. In addition, however, there was also an increase in the incidence of adrenal medulla hyperplasia, a lesion generally considered to be the precursor to pheochromocytoma, in the low-dose group, but not in the high-dose group (11/50, 22/50, 12/50). The marginal increased incidence of pheochromocytoma in combination with the increased incidence of hyperplasia were considered to be an equivocal finding.

The relationship between the increase in proliferative adrenal medulla lesions in male rats in the present study and polysorbate 80 is uncertain. Experimental studies in rats suggest that diets high in sorbitol, a sugar which is a component of polysorbate 80, can influence the incidence of proliferative lesions of the adrenal medulla. Baer (1988) has reported producing hyperplasia and neoplasia of the adrenal medulla in rats by feeding diets containing high levels of one of several different sugar alcohols, including sorbitol. The

sugar alcohols did not produce these lesions in the adrenal medullas of dogs or mice. Feeding diets containing 20% sorbitol to Wistar rats produced adrenal medulla hyperplasia or neoplasia in all males; 78% of the control males had lesions. In contrast, no proliferative lesions occurred in the control or dosed females. When diets containing 20% sorbitol were fed to Sprague-Dawley rats, adrenal medulla lesions occurred in 16% of the control males, 35% of the dosed males, 7% of the control females, and 21% of the dosed females. It was concluded from these results that strains with a high spontaneous rate of adrenal medulla lesions would be more responsive to the effects of sugar alcohols. From these findings, it also appears that there may be a sex-related difference in that males have a higher spontaneous rate of adrenal medulla lesions than females, and males also show a stronger dose-related response to the effects of sugar alcohols.

In the present study, there was no effect in the adrenal glands of female rats or male or female mice. Female F344/N rats have a lower control incidence of proliferative lesions in the adrenal medulla than males. The average historical control incidence of pheochromocytomas in female rats from current NTP 2-year feed studies is 6%, while the average historical control incidence for males is 39%. Likewise, B6C3F$_1$ mice have a low spontaneous incidence of proliferative lesions of the adrenal medulla; the average historical control incidence of pheochromocytoma in control male and female mice from current NTP 2-year dosed feed studies is only 2%. Thus, of the animals in this study, only the male rats have a high incidence of spontaneous adrenal medulla neoplasms, and this is the group in which the effect on the adrenal medulla was seen. This result is in accordance with the findings reported by Baer (1988).

The mechanism by which sorbitol and the other sugar alcohols increase the incidence of adrenal medulla lesions in rats is unclear. Based upon experimental findings, Baer (1988) suggested that high levels of sugar alcohols in the diet are associated with interference with calcium homeostasis and increased activity of the adrenal medulla. Sugar alcohols and other poorly digested carbohydrates increase intestinal absorption of divalent cations, with the absorption of calcium being increased the most. Baer reported that in rats fed xylitol there was an increase in calcium absorption (as indicated by increased excretion of calcium in the urine) accompanied by an increase in epinephrine levels in the adrenal gland. When the levels of dietary calcium were decreased, thereby reducing the absorption of calcium, the levels of epinephrine in the adrenal gland were also reduced. The exact nature of the chemical link between increased calcium absorption, increased activity of the adrenal medulla, and increased incidences of proliferative lesions in the adrenal medulla, if indeed a link exists, has not been determined.

It is not known what effect, if any, the sorbitol contained in polysorbate 80 may have on calcium absorption from the intestine. Results of metabolism studies show that after ingestion the fatty acids are cleaved from the polysorbate molecule, leaving nearly all of the sorbitol bound to the polyoxyethylene moiety, and it is not known whether the bound sorbitol can exert the same effect as free sorbitol. Moreover, the sorbitol content of the diets used in this study was quite low, only 0.7% at the 50,000 ppm dose level, as compared with the 20% levels used in the studies by Baer. However, it has also been reported that *in vitro* polysorbate 80 can affect the transport of ions, including calcium, across membranes (Thoman, 1986). This raises the possibility that the intact polysorbate 80 molecule, rather than just the sorbitol portion, may influence intestinal absorption of calcium, but in the absence of specific data indicating that polysorbate 80 can affect the intestinal absorption of calcium *in vivo*, it is not possible to draw any firm conclusions.

In the 2-year studies of mice, the final mean body weight of female mice that received 50,000 ppm polysorbate 80 was 11% lower than that of the controls. The final mean body weights of dosed males and low-dose females were similar to those of the controls. The survival of dosed male and female mice was similar to that of the control groups. No increased incidences of neoplasms occurred in dosed mice of either sex. Compound-related lesions of the forestomach occurred in all dosed mouse groups. These included squamous hyperplasia of the epithelium, inflammation, and, in high-dose females, ulcers. The incidences and severity of stomach lesions were usually greater in high-dose than in low-dose groups.

Results of dermal toxicity studies in rabbits have shown that polysorbate 80 applied to the skin once daily for a month produces inflammation, acanthosis,

and necrosis (Mezei *et al.*, 1966). The epithelium of the skin and the forestomach is of the stratified squamous type, and the skin lesions that occurred in rabbits are morphologically similar to the forestomach lesions of mice in the present studies. This suggests that a similar mechanism of epithelial damage may be involved in both cases. Mezei (1970) studied the biochemical effects on rabbit skin resulting from the application of the nonionic surfactants polysorbate 85 and polyoxyethylene ether 96 and found they increased synthesis of epidermal phospholipids and the cellular content of RNA and DNA. Mezei concluded that the increase in phospholipid synthesis indicated an effort to repair cellular membrane damage, while the increase in levels of cellular RNA was evidence of this increased synthetic activity by the cells. The increased amounts of cellular DNA indicated cellular proliferation was occurring as part of the attempt to repair the surfactant-induced injury. Thus, the author proposed that the surfactants produced skin injury by damaging the membranes of the epithelial cells. This suggests that the forestomach lesions seen in the present studies may have been caused by direct effects of the polysorbate 80 in the feed upon the forestomach epithelium.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity*° for polysorbate 80 in male F344/N rats based on increased incidences of pheochromocytomas of the adrenal medulla. There was *no evidence of carcinogenic activity* for polysorbate 80 in female F344/N rats or in male or female B6C3F$_1$ mice given 25,000 or 50,000 ppm.

Administration of polysorbate 80 was associated with hyperplasia of the adrenal medulla in male rats, with inflammation and squamous hyperplasia of the forestomach in male and female mice, and with ulcers of the forestomach in female mice.

° Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 10.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR FEED STUDY OF POLYSORBATE 80

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Polysorbate 80[a]

	0 ppm	25,000 ppm	50,000 ppm
Disposition Summary			
Animals initially in study	60	60	60
15-month interim evaluation	10	10	10
Early deaths			
Natural death	7	5	7
Moribund	14	27	25
Survivors			
Terminal sacrifice	29	18	18
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large, cecum	(50)	(49)	(48)
Intestine large, colon	(49)	(50)	(49)
Intestine large, rectum	(48)	(49)	(49)
Intestine small, duodenum	(49)	(50)	(49)
Hemangiosarcoma, metastatic, spleen			1 (2%)
Intestine small, ileum	(49)	(47)	(49)
Intestine small, jejunum	(48)	(50)	(49)
Liver	(50)	(50)	(50)
Hepatocellular adenoma	2 (4%)		2 (4%)
Mesentery	(4)	(10)	(12)
Pancreas	(50)	(50)	(48)
Acinus, adenoma	1 (2%)	2 (4%)	1 (2%)
Salivary glands	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)
Stomach, glandular	(49)	(50)	(50)
Tongue	(1)		
Papilloma squamous	1 (100%)		
Cardiovascular System			
Heart	(50)	(50)	(50)
Endocrine System			
Adrenal gland	(50)	(50)	(50)
Pheochromocytoma malignant			1 (2%)
Adrenal gland, cortex	(50)	(50)	(50)
Adenoma	1 (2%)		
Carcinoma			1 (2%)
Adrenal gland, medulla	(50)	(50)	(50)
Pheochromocytoma malignant	1 (2%)	4 (8%)	1 (2%)
Pheochromocytoma benign	17 (34%)	12 (24%)	21 (42%)
Bilateral, pheochromocytoma benign	4 (8%)	4 (8%)	7 (14%)
Islets, pancreatic	(50)	(48)	(48)
Adenoma	4 (8%)	5 (10%)	3 (6%)
Carcinoma		1 (2%)	
Pituitary gland	(50)	(49)	(50)
Pars distalis, adenoma	30 (60%)	23 (47%)	26 (52%)
Pars intermedia, adenoma	1 (2%)	1 (2%)	
Thyroid gland	(50)	(50)	(50)
C-cell, adenoma	6 (12%)	2 (4%)	2 (4%)
Follicle, adenocarcinoma			1 (2%)
Follicle, adenoma		1 (2%)	1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Polysorbate 80
(continued)

	0 ppm	25,000 ppm	50,000 ppm
General Body System			
None			
Genital System			
Epididymis	(49)	(50)	(50)
Preputial gland	(48)	(50)	(49)
Adenoma	5 (10%)	1 (2%)	2 (4%)
Carcinoma	5 (10%)	6 (12%)	5 (10%)
Squamous cell carcinoma		1 (2%)	1 (2%)
Prostate	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)
Testes	(49)	(50)	(50)
Interstitial cell, adenoma	39 (80%)	35 (70%)	41 (82%)
Hematopoietic System			
Blood	(2)	(4)	(1)
Bone marrow	(50)	(50)	(49)
Lymph node	(49)	(50)	(50)
Lymph node, mandibular	(49)	(50)	(48)
Lymph node, mesenteric	(49)	(50)	(49)
Spleen	(50)	(50)	(50)
Sarcoma			2 (4%)
Thymus	(47)	(40)	(49)
Neoplasm NOS		1 (3%)	
Thymoma NOS	1 (2%)		
Integumentary system			
Mammary gland	(47)	(39)	(48)
Fibroadenoma	1 (2%)	2 (5%)	
Neurofibrosarcoma, deep invasion, metastatic, skin	1 (2%)		
Skin	(49)	(50)	(50)
Basal cell carcinoma		2 (4%)	1 (2%)
Keratoacanthoma	2 (4%)	2 (4%)	5 (10%)
Papilloma squamous	2 (4%)		1 (2%)
Subcutaneous tissue, fibroma	1 (2%)	3 (6%)	4 (8%)
Subcutaneous tissue, lipoma		1 (2%)	2 (4%)
Subcutaneous tissue, neurofibroma			1 (2%)
Subcutaneous tissue, neurofibrosarcoma	1 (2%)		1 (2%)
Subcutaneous tissue, sarcoma			1 (2%)
Musculoskeletal System			
Bone	(50)	(50)	(50)
Cranium, osteosarcoma	1 (2%)		
Skeletal muscle		(2)	
Nervous System			
Brain	(50)	(50)	(50)
Astrocytoma NOS			1 (2%)
Hemangioma	1 (2%)		
Neuroblastoma malignant	1 (2%)		

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Respiratory System			
Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)		
Alveolar/bronchiolar carcinoma			1 (2%)
Nose	(50)	(50)	(50)
Mucosa, squamous cell carcinoma			1 (2%)
Special Senses System			
Ear	(1)	(2)	(2)
Canal, squamous cell carcinoma			2 (100%)
Pinna, neurofibroma		1 (50%)	
Eye	(10)	(11)	(14)
Lids, squamous cell carcinoma	1 (10%)		
Zymbal's gland	(1)		
Squamous cell carcinoma	1 (100%)		
Urinary System			
Kidney	(50)	(50)	(50)
Carcinoma	1 (2%)		
Urinary bladder	(49)	(50)	(49)
Systemic Lesions			
Multiple organs[b]	(50)	(50)	(50)
Leukemia mononuclear	23 (46%)	21 (42%)	26 (52%)
Lymphoma malignant lymphocytic	1 (2%)		
Lymphoma malignant mixed		1 (2%)	
Mesothelioma benign			1 (2%)
Mesothelioma malignant		2 (4%)	1 (2%)
Tumor Summary			
Total animals with primary neoplasms[c]	50	49	48
Total primary neoplasms	156	134	167
Total animals with benign neoplasms	48	47	47
Total benign neoplasms	119	95	120
Total animals with malignant neoplasms	31	31	34
Total malignant neoplasms	36	38	46
Total animals with secondary neoplasms[d]	1		1
Total secondary neoplasms	1		1
Total animals with neoplasms uncertain-benign or malignant	1	1	1
Total uncertain neoplasms	1	1	1

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Number of animals with any tissue examined microscopically
[c] Primary tumors: all tumors except metastatic tumors
[d] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80: 0 ppm

Number of Days on Study	4 5 5	4 6 4	4 7 0	4 9 3	5 6 1	5 6 1	5 6 7	5 8 5	6 1 4	6 3 0	6 4 0	6 4 0	6 6 2	6 8 1	6 8 8	6 9 8	7 0 0	7 0 1	7 1 2	7 1 7	7 2 3	7 2 9	7 2 9	7 2 9
Carcass ID Number	1 1 1	0 9 1	0 6 1	1 1 2	0 3 1	0 4 1	0 9 2	0 1 1	0 9 3	0 7 1	1 1 3	1 2 1	1 3 2	0 7 2	0 9 2	0 2 4	0 6 1	0 2 2	1 2 2	1 1 4	0 3 3	0 1 2	0 1 3	0 1 4	0 1 5
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	M	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																X	X							
Mesentery									+							+		+						
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																						X		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																								
Papilloma squamous																								
Cardiovascular System																								
Blood vessel																							+	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																					X			
Pheochromocytoma benign					X			X			X			X		X	X				X		X	X
Bilateral, pheochromocytoma benign													X											
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																						X		X
Parathyroid gland	+	+	+	+	M	+	M	M	+	M	+	+	+	+	+	+	+	+	+	M	+	M	+	+

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80:
0 ppm (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 2 3	0 2 4	0 2 5	0 3 4	0 3 5	0 4 2	0 4 3	0 4 4	0 4 5	0 6 3	0 6 4	0 6 5	0 7 3	0 7 4	0 7 5	0 8 1	0 8 2	0 8 3	0 8 4	0 8 5	0 9 5	1 1 5	1 2 3	1 2 4	1 2 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																				+						2
Mesentery																										4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tongue											+															1
Papilloma squamous											X															1
Cardiovascular System																										
Blood vessel								+					+		+	+					+					6
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma										X																1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma benign	X					X		X		X	X	X		X										X		17
Bilateral, pheochromocytoma benign							X		X											X						4
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma									X		X															4
Parathyroid gland	M	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	M	+	+	+	M	+	+	+	39

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80: 0 ppm (continued)

Number of Days on Study	4 5 5	4 6 4	4 7 0	4 9 3	5 6 1	5 6 1	5 6 7	5 8 5	6 1 4	6 3 0	6 4 0	6 4 0	6 6 2	6 8 1	6 8 8	6 9 8	7 0 0	7 0 1	7 1 2	7 1 7	7 2 3	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	1 1 1	0 9 1	0 6 1	1 1 2	0 3 1	0 4 1	0 9 2	0 1 1	0 9 3	0 7 1	1 1 3	1 2 1	1 3 2	0 7 2	0 9 2	0 2 4	0 6 1	0 2 2	1 2 2	1 1 4	0 3 3	0 1 2	0 1 3	0 1 4	0 1 5
Endocrine System (continued)																									
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X			X	X		X	X	X					X			X	X	X		X				X
Pars intermedia, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																				X					
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Adenoma						X							X									X			
Carcinoma											X									X					
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma			X							X		X	X	X		X	X	X	X	X	X	X	X	X	X
Hematopoietic System																									
Blood										+			+												
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+
Thymoma NOS																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	M	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																		X							
Neurofibrosarcoma, deep invasion, metastatic, skin																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Keratoacanthoma																						X			
Papilloma squamous																									
Subcutaneous tissue, fibroma														X											
Subcutaneous tissue, neurofibrosarcoma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80:
0 ppm (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 2 3	0 2 4	0 2 5	0 3 4	0 3 5	0 4 2	0 4 3	0 4 4	0 4 5	0 6 3	0 6 4	0 6 5	0 7 3	0 7 4	0 7 5	0 8 1	0 8 2	0 8 3	0 8 4	0 8 5	0 9 5	1 1 5	1 2 3	1 2 4	1 2 5	Total Tissues/ Tumors
Endocrine System (continued)																										
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X		X	X	X				X	X	X	X	X		X			X	X	X	X	X	X	X	30
Pars intermedia, adenoma										X																1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma		X												X			X			X				X		6
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Adenoma		X											X													5
Carcinoma												X		X							X					5
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Interstitial cell, adenoma		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	39
Hematopoietic System																										
Blood																										2
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Thymoma NOS													X													1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Fibroadenoma																										1
Neurofibrosarcoma, deep invasion, metastatic, skin											X															1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Keratoacanthoma																				X						2
Papilloma squamous				X													X									2
Subcutaneous tissue, fibroma																										1
Subcutaneous tissue, neurofibrosarcoma											X															1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80: 0 ppm (continued)

Number of Days on Study	4 5 5	4 6 4	4 7 0	4 9 3	5 6 1	5 6 1	5 6 7	5 8 5	6 1 4	6 3 0	6 4 0	6 4 0	6 6 2	6 8 1	6 8 8	6 9 8	7 0 0	7 0 1	7 1 2	7 1 7	7 2 3	7 2 9	7 2 9	7 2 9
Carcass ID Number	1 1 1	0 9 1	0 6 1	1 1 2	0 3 1	0 4 1	0 9 2	0 1 1	0 9 3	0 7 1	1 1 3	1 2 1	0 3 2	0 7 2	0 9 2	0 2 4	6 1 2	2 2 2	2 1 4	1 3 3	3 1 2	1 1 3	1 1 4	0 1 5
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cranium, osteosarcoma	X																							
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																								
Neuroblastoma malignant							X																	
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma					X																			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Ear																								
Eye							+							+		+					+			
Lids, squamous cell carcinoma																								
Zymbal's gland											+													
Squamous cell carcinoma											X													
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																X								
Urethra	+						+		+															
Urinary bladder	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X	X			X		X		X	X		X	X		X	X			X				X
Lymphoma malignant lymphocytic																								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80:
0 ppm (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 2 3	0 2 4	0 2 5	0 3 4	0 3 5	0 4 2	0 4 3	0 4 4	0 4 5	0 6 3	0 6 4	0 6 5	0 7 3	0 7 4	0 7 5	0 8 1	0 8 2	0 8 3	0 8 4	0 8 5	0 9 5	1 1 5	1 2 3	1 2 4	1 2 5	Total Tissues/ Tumors
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cranium, osteosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma								X																		1
Neuroblastoma malignant																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																	+									1
Eye												+		+					+			+		+	+	10
Lids, squamous cell carcinoma																									X	1
Zymbal's gland																										1
Squamous cell carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Urethra																										3
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X		X	X		X				X	X		X				X			X	X			X		23
Lymphoma malignant lymphocytic							X																			1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm

Number of Days on Study	0 9 8	4 7 7	5 0 3	5 3 5	5 8 9	6 0 6	6 1 3	6 1 6	6 1 7	6 1 8	6 3 2	6 3 5	6 3 7	6 3 8	6 4 0	6 4 4	6 4 6	6 5 8	6 6 7	6 7 5	6 7 7	6 8 3	6 8 7	6 9 2	6 9 5
Carcass ID Number	2 5 1	2 5 2	2 9 1	3 3 1	3 0 1	2 9 2	2 6 1	3 2 1	3 3 2	3 2 2	3 0 2	3 6 2	3 1 1	3 6 1	3 8 1	2 5 3	3 4 1	3 3 3	3 3 4	3 0 4	3 1 2	3 2 3	3 6 3	3 4 2	2 6 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery													+	+			+								
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																X					X				
Pheochromocytoma benign							X					X					X				X		X		
Bilateral, pheochromocytoma benign											X			X											
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma								X				X						X						X	
Carcinoma																	X								
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma		X		X	X			X		X	X	X	X				X	X	X	X				X	X
Pars intermedia, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
Follicle, adenoma								X																	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm (continued)

Number of Days on Study	7 0 0	7 0 1	7 0 1	7 0 8	7 1 0	7 2 2	7 2 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	2 5 4	2 9 3	3 6 4	2 8 2	3 4 3	3 1 3	3 0 3	2 5 5	2 6 3	2 6 4	2 6 5	2 8 3	2 8 4	2 8 5	2 9 4	2 9 5	3 0 5	3 1 4	3 1 5	3 2 4	3 2 5	3 3 5	3 4 4	3 4 5	3 6 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery		+					+						+	+					+				+		+	10
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma																X							X			2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant												X						X								4
Pheochromocytoma benign		X						X						X			X					X	X		X	12
Bilateral, pheochromocytoma benign	X						X																			4
Islets, pancreatic	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma							X																			5
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	47
Pituitary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma	X	X		X				X			X					X						X	X	X		23
Pars intermedia, adenoma							X																			1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma													X	X												2
Follicle, adenoma																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm (continued)

Number of Days on Study	0 9 8	4 7 7	5 0 3	5 3 5	5 8 9	6 0 6	6 1 3	6 1 6	6 1 7	6 1 8	6 3 2	6 3 5	6 3 7	6 3 8	6 4 0	6 4 4	6 4 6	6 5 8	6 6 7	6 7 5	6 7 7	6 8 3	6 8 7	6 9 2	6 9 5
Carcass ID Number	2 5 1	2 5 2	2 9 1	3 3 1	3 0 1	2 9 2	2 6 1	3 2 1	3 3 2	3 2 2	3 0 2	3 6 2	3 1 1	3 6 1	3 8 1	2 5 3	3 4 1	3 3 3	3 3 4	3 0 4	3 1 2	3 2 3	3 6 3	3 4 2	2 6 2
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								X	
Carcinoma		X												X	X										
Squamous cell carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma			X			X	X	X	X					X	X	X	X		X		X		X	X	X
Hematopoietic System																									
Blood		+									+								+	+					
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+
Neoplasm NOS																	X								
Integumentary System																									
Mammary gland	M	+	+	+	+	+	+	+	+	M	+	M	M	M	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																				X					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																X									
Keratoacanthoma																			X						
Subcutaneous tissue, fibroma								X															X		
Subcutaneous tissue, lipoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+														+								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm (continued)

Number of Days on Study	7 0 0	7 0 1	7 0 1	7 0 8	7 1 0	7 2 2	7 2 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	2 5 4	2 9 3	3 6 4	2 8 2	3 4 3	3 1 3	3 0 3	2 5 5	2 6 3	2 6 4	2 6 5	2 8 3	2 8 4	2 8 5	2 9 4	2 9 5	3 0 5	3 1 4	3 1 5	3 2 4	3 2 5	3 3 5	3 4 4	3 4 5	3 6 5	Total Tissues/ Tumors
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Carcinoma				X												X						X				6
Squamous cell carcinoma							X																			1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma		X	X	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X		X	35
Hematopoietic System																										
Blood																										4
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	M	+	M	M	+	+	M	+	+	+	M	+	M	+	+	M	+	+	+	+	+	+	+	+	M	40
Neoplasm NOS																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	M	M	+	+	+	+	+	M	+	M	+	+	M	+	+	+	+	+	M	+	39
Fibroadenoma		X																								2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell carcinoma																				X						2
Keratoacanthoma							X																			2
Subcutaneous tissue, fibroma																			X							3
Subcutaneous tissue, lipoma			X																							1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm (continued)

Number of Days on Study	0	4	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	9	7	0	3	8	0	1	1	1	1	3	3	3	3	4	4	4	5	6	7	7	8	8	9	9
	8	7	3	5	9	6	3	6	7	8	2	5	7	8	0	4	6	8	7	5	7	3	7	2	5
Carcass ID Number	2	2	2	3	3	2	2	3	3	3	3	3	3	3	2	2	3	3	3	3	3	3	3	3	2
	5	5	9	3	0	9	6	2	3	2	0	6	1	6	8	5	4	3	3	0	1	2	6	4	6
	1	2	1	1	1	2	1	1	2	2	2	2	1	1	1	3	1	3	4	4	2	3	3	2	2
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+																	+							
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear								+										+							
Pinna, neurofibroma								X																	
Eye		+						+			+								+						
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urethra	+						+																		
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X			X	X		X		X					X		X	X		X	X	X	X	X
Lymphoma malignant mixed																									
Mesothelioma malignant													X				X								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80:
25,000 ppm (continued)

Number of Days on Study	7 0 0	7 0 1	7 0 1	7 0 8	7 1 0	7 2 2	7 2 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	2 5 4	2 9 3	3 6 4	2 8 2	3 4 3	3 1 3	3 0 3	2 5 5	2 6 3	2 6 4	2 6 5	2 8 3	2 8 4	2 8 5	2 9 4	2 9 5	3 0 5	3 1 4	3 1 5	3 2 4	3 2 5	3 3 5	3 4 4	3 4 5	3 6 5	Total Tissues/ Tumors
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										2
Pinna, neurofibroma																										1
Eye	+		+		+		+	+									+	+								11
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urethra																										2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X			X	X	X		X			X	X				X										21
Lymphoma malignant mixed																							X			1
Mesothelioma malignant																										2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm

Number of Days on Study	258	400	454	458	461	494	503	570	574	575	597	625	637	644	646	647	647	678	689	682	682	688	689	699	699
Carcass ID Number	241	181	131	141	201	171	202	221	203	142	151	232	231	191	152	153	172	154	243	132	242	133	192	173	174
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, spleen						X																			
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma		X																							
Mesentery					+	+									+	+							+	+	
Pancreas	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth										+	+							+							
Cardiovascular System																									
Blood vessel					+																				
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant		X																							
Pheochromocytoma benign								X						X			X			X	X	X			
Bilateral, pheochromocytoma benign																X			X						
Islets, pancreatic	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X				X	X		X	X	X	X		X	X					X		X	X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
Follicle, adenocarcinoma																									
Follicle, adenoma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	6 9 9	6 9 9	7 0 0	7 0 1	7 0 9	7 1 0	7 2 4	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	1 1 8 2	1 1 8 3	2 1 3 2	1 2 4 3	2 2 2 3	2 2 0 4	1 0 9 3	1 3 3 4	1 3 3 5	1 4 4 4	1 4 4 5	1 5 5 5	1 7 5 5	1 8 4 4	1 8 5 5	1 8 8 5	1 9 4 4	1 9 9 5	2 0 5 5	2 2 4 4	2 2 2 5	2 3 3 3	2 3 3 4	2 3 5 5	2 4 4 4	2 4 4 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma, metastatic, spleen																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma				X																						2
Mesentery				+	+						+									+				+	+	12
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Acinus, adenoma													X													1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																	+									4
Cardiovascular System																										
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																				X						1
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma					X																					1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma benign			X		X	X	X		X			X	X	X	X	X	X			X	X			X	X	21
Bilateral, pheochromocytoma benign	X	X						X			X												X			7
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma									X							X		X								3
Parathyroid gland	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma						X	X	X	X		X	X	X					X	X	X	X	X	X	X			26
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma																	X								X		2
Follicle, adenocarcinoma																		X									1
Follicle, adenoma			X																								1

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	258	400	454	458	461	494	503	570	574	575	597	625	637	644	646	646	647	668	679	682	682	688	689	699	699
Carcass ID Number	241	181	131	141	201	171	202	221	203	142	151	222	231	191	152	153	172	154	243	132	242	133	192	173	174
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Adenoma																				X					
Carcinoma																					X	X			
Squamous cell carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma						X			X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Hematopoietic System																									
Blood															+										
Bone marrow	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma						X																			
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																									
Keratoacanthoma											X				X										
Papilloma squamous																									
Subcutaneous tissue, fibroma																	X			X					X
Subcutaneous tissue, lipoma								X																X	
Subcutaneous tissue, neurofibroma																									
Subcutaneous tissue, neurofibrosarcoma																									
Subcutaneous tissue, sarcoma				X																					

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	669	690	700	701	701	701	702	703	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	
Carcass ID Number	18212	18323	12232	14233	12233	20244	19233	13244	33255	34244	44255	45255	47255	48244	48255	49244	49255	50255	02244	02255	23253	23344	23355	24244	24255	Total Tissues/ Tumors
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma									X																	2
Carcinoma		X		X																			X			5
Squamous cell carcinoma											X															1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	41
Hematopoietic System																										
Blood																										1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma																			X							2
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	48
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell carcinoma	X																									1
Keratoacanthoma									X			X					X									5
Papilloma squamous																		X								1
Subcutaneous tissue, fibroma												X														4
Subcutaneous tissue, lipoma																										2
Subcutaneous tissue, neurofibroma												X														1
Subcutaneous tissue, neurofibrosarcoma				X																						1
Subcutaneous tissue, sarcoma																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	2 5 8	4 0 0	4 5 4	4 5 8	4 6 1	4 9 4	5 0 3	5 7 0	5 7 4	5 7 5	5 9 7	6 2 5	6 3 7	6 4 4	6 4 6	6 4 6	6 6 7	6 7 8	6 8 9	6 8 2	6 8 2	6 8 8	6 8 9	6 9 9	6 9 9
Carcass ID Number	2 4 1	1 8 1	1 3 1	1 4 1	2 0 1	1 7 1	2 0 2	2 2 1	2 0 3	1 4 2	1 5 1	2 2 2	2 3 1	1 9 1	1 5 2	1 5 3	1 7 2	1 5 4	2 4 3	1 3 2	2 4 2	1 3 3	1 9 2	1 7 3	1 7 4
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma NOS																								X	
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mucosa, squamous cell carcinoma																		X							
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear										+	+														
Canal, squamous cell carcinoma										X	X														
Eye									+					+	+	+		+	+						
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urethra																						+			
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X		X	X		X				X	X		X	X		X		X			X	X
Mesothelioma benign																									
Mesothelioma malignant																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	6 9 9	6 9 9	7 0 0	7 0 1	7 0 1	7 1 9	7 2 4	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	1 1 8 2	1 8 3	2 3 2	1 4 3	2 2 3	2 0 4	1 9 3	1 3 4	1 3 5	1 4 4	1 4 5	1 5 5	1 7 5	1 8 4	1 8 5	1 9 4	1 9 5	2 0 5	2 2 4	2 2 5	2 3 3	2 3 4	2 3 5	2 4 4	2 4 5	Total Tissues/ Tumors
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma NOS																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																			X							1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mucosa, squamous cell carcinoma																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										2
Canal, squamous cell carcinoma																										2
Eye			+			+					+					+			+	+		+			+	14
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urethra											+						+									3
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X	X	X	X	X	X				X		X	X	X	X			X	X	X					26
Mesothelioma benign													X													1
Mesothelioma malignant					X																					1

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Polysorbate 80

	0 ppm	25,000 ppm	50,000 ppm
Adrenal Medulla: Benign Pheochromocytoma			
Overall rates[a]	21/50 (42%)	16/50 (32%)	28/50 (56%)
Adjusted rates[b]	57.0%	52.5%	86.8%
Terminal rates[c]	14/29 (48%)	6/18 (33%)	14/18 (78%)
First incidence (days)	561	613	570
Life table tests[d]	P=0.006	P=0.496	P=0.005
Logistic regression tests[d]	P=0.030	P=0.256N	P=0.032
Cochran-Armitage test[d]	P=0.095		
Fisher exact test[d]		P=0.204N	P=0.115
Adrenal Medulla: Malignant Pheochromocytoma			
Overall rates	1/50 (2%)	4/50 (8%)	1/50 (2%)
Adjusted rates	3.4%	16.5%	2.0%
Terminal rates	1/29 (3%)	2/18 (11%)	0/18 (0%)
First incidence (days)	729 (T)	644	400
Life table tests	P=0.485	P=0.094	P=0.706
Logistic regression tests	P=0.584N	P=0.154	P=0.690N
Cochran-Armitage test	P=0.601		
Fisher exact test		P=0.181	P=0.753N
Adrenal Medulla: Benign or Malignant Pheochromocytoma			
Overall rates	21/50 (42%)	19/50 (38%)	29/50 (58%)
Adjusted rates	57.0%	61.6%	87.0%
Terminal rates	14/29 (48%)	8/18 (44%)	14/18 (78%)
First incidence (days)	561	613	400
Life table tests	P=0.003	P=0.254	P=0.003
Logistic regression tests	P=0.023	P=0.499N	P=0.027
Cochran-Armitage test	P=0.066		
Fisher exact test		P=0.419N	P=0.081
Pancreatic Islets: Adenoma			
Overall rates	4/50 (8%)	5/48 (10%)	3/48 (6%)
Adjusted rates	13.8%	15.8%	16.7%
Terminal rates	4/29 (14%)	0/18 (0%)	3/18 (17%)
First incidence (days)	729 (T)	616	729 (T)
Life table tests	P=0.499	P=0.323	P=0.560
Logistic regression tests	P=0.506N	P=0.446	P=0.560
Cochran-Armitage test	P=0.451N		
Fisher exact test		P=0.474	P=0.523N
Pancreatic Islets: Adenoma or Carcinoma			
Overall rates	4/50 (8%)	6/48 (13%)	3/48 (6%)
Adjusted rates	13.8%	18.3%	16.7%
Terminal rates	4/29 (14%)	0/18 (0%)	3/18 (17%)
First incidence (days)	729 (T)	616	729 (T)
Life table tests	P=0.497	P=0.219	P=0.560
Logistic regression tests	P=0.501N	P=0.323	P=0.560
Cochran-Armitage test	P=0.455N		
Fisher exact test		P=0.344	P=0.523N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Preputial Gland: Adenoma			
Overall rates	5/48 (10%)	1/50 (2%)	2/49 (4%)
Adjusted rates	15.0%	3.7%	8.6%
Terminal rates	3/28 (11%)	0/18 (0%)	1/18 (6%)
First incidence (days)	561	692	682
Life table tests	P=0.237N	P=0.186N	P=0.355N
Logistic regression tests	P=0.143N	P=0.098N	P=0.231N
Cochran-Armitage test	P=0.126N		
Fisher exact test		P=0.093N	P=0.209N
Preputial Gland: Carcinoma			
Overall rates	5/48 (10%)	6/50 (12%)	5/49 (10%)
Adjusted rates	15.8%	21.4%	18.9%
Terminal rates	3/28 (11%)	2/18 (11%)	1/18 (6%)
First incidence (days)	640	477	682
Life table tests	P=0.358	P=0.307	P=0.419
Logistic regression tests	P=0.542	P=0.521	P=0.570
Cochran-Armitage test	P=0.551N		
Fisher exact test		P=0.529	P=0.617N
Preputial Gland: Adenoma or Carcinoma			
Overall rates	10/48 (21%)	7/50 (14%)	7/49 (14%)
Adjusted rates	29.4%	24.3%	26.2%
Terminal rates	6/28 (21%)	2/18 (11%)	2/18 (11%)
First incidence (days)	561	477	682
Life table tests	P=0.503N	P=0.549N	P=0.569N
Logistic regression tests	P=0.271N	P=0.275N	P=0.352N
Cochran-Armitage test	P=0.232N		
Fisher exact test		P=0.266N	P=0.281N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	30/50 (60%)	23/49 (47%)	26/50 (52%)
Adjusted rates	74.1%	60.5%	78.5%
Terminal rates	19/29 (66%)	6/18 (33%)	12/18 (67%)
First incidence (days)	455	477	454
Life table tests	P=0.257	P=0.521	P=0.232
Logistic regression tests	P=0.286N	P=0.144N	P=0.348N
Cochran-Armitage test	P=0.242N		
Fisher exact test		P=0.135N	P=0.273N
Skin: Keratoacanthoma			
Overall rates	2/50 (4%)	2/50 (4%)	5/50 (10%)
Adjusted rates	6.9%	8.2%	21.0%
Terminal rates	2/29 (7%)	0/18 (0%)	3/18 (17%)
First incidence (days)	729 (T)	667	597
Life table tests	P=0.072	P=0.551	P=0.100
Logistic regression tests	P=0.116	P=0.641	P=0.175
Cochran-Armitage test	P=0.146		
Fisher exact test		P=0.691N	P=0.218

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Polysorbate 80
(continued)

	0 ppm	25,000 ppm	50,000 ppm
Skin (Subcutaneous Tissue): Fibroma			
Overall rates	1/50 (2%)	3/50 (6%)	4/50 (8%)
Adjusted rates	2.7%	11.0%	14.6%
Terminal rates	0/29 (0%)	1/18 (6%)	1/18 (6%)
First incidence (days)	681	616	647
Life table tests	P=0.088	P=0.231	P=0.123
Logistic regression tests	P=0.119	P=0.301	P=0.159
Cochran-Armitage test	P=0.133		
Fisher exact test		P=0.309	P=0.181
Skin (Subcutaneous Tissue): Fibroma or Sarcoma			
Overall rates	1/50 (2%)	3/50 (6%)	5/50 (10%)
Adjusted rates	2.7%	11.0%	16.4%
Terminal rates	0/29 (0%)	1/18 (6%)	1/18 (6%)
First incidence (days)	681	616	458
Life table tests	P=0.046	P=0.231	P=0.070
Logistic regression tests	P=0.073	P=0.301	P=0.114
Cochran-Armitage test	P=0.070		
Fisher exact test		P=0.309	P=0.102
Testes: Adenoma			
Overall rates	39/49 (80%)	35/50 (70%)	41/50 (82%)
Adjusted rates	97.5%	94.2%	97.6%
Terminal rates	28/29 (97%)	16/18 (89%)	17/18 (94%)
First incidence (days)	470	503	494
Life table tests	P=0.006	P=0.072	P=0.005
Logistic regression tests	P=0.132	P=0.224N	P=0.136
Cochran-Armitage test	P=0.431		
Fisher exact test		P=0.193N	P=0.480
Thyroid gland (C-cell): Adenoma			
Overall rates	6/50 (12%)	2/50 (4%)	2/50 (4%)
Adjusted rates	19.9%	11.1%	11.1%
Terminal rates	5/29 (17%)	2/18 (11%)	2/18 (11%)
First incidence (days)	717	729 (T)	729 (T)
Life table tests	P=0.233N	P=0.329N	P=0.332N
Logistic regression tests	P=0.211N	P=0.291N	P=0.300N
Cochran-Armitage test	P=0.080N		
Fisher exact test		P=0.134N	P=0.134N
All Organs: Mononuclear Cell Leukemia			
Overall rates	23/50 (46%)	21/50 (42%)	26/50 (52%)
Adjusted rates	55.8%	56.3%	70.4%
Terminal rates	12/29 (41%)	4/18 (22%)	8/18 (44%)
First incidence (days)	464	503	458
Life table tests	P=0.069	P=0.315	P=0.069
Logistic regression tests	P=0.279	P=0.423N	P=0.315
Cochran-Armitage test	P=0.308		
Fisher exact test		P=0.420N	P=0.345

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
All Organs: Benign Tumors			
Overall rates	48/50 (96%)	47/50 (94%)	47/50 (94%)
Adjusted rates	100.0%	100.0%	100.0%
Terminal rates	29/29 (100%)	18/18 (100%)	18/18(100%)
First incidence (days)	455	477	400
Life table tests	P=0.026	P=0.037	P=0.029
Logistic regression tests	P=0.518	P=0.629N	P=0.580
Cochran-Armitage test	P=0.412N		
Fisher exact test		P=0.500N	P=0.500N
All Organs: Malignant Tumors			
Overall rates	31/50 (62%)	31/50 (62%)	34/50 (68%)
Adjusted rates	71.0%	74.1%	80.2%
Terminal rates	17/29 (59%)	8/18 (44%)	10/18 (56%)
First incidence (days)	455	477	400
Life table tests	P=0.048	P=0.137	P=0.050
Logistic regression tests	P=0.288	P=0.578N	P=0.338
Cochran-Armitage test	P=0.301		
Fisher exact test		P=0.582N	P=0.338
All Organs: Benign or Malignant Tumors			
Overall rates	50/50 (100%)	49/50 (98%)	48/50 (96%)
Adjusted rates	100.0%	100.0%	100.0%
Terminal rates	29/29 (100%)	18/18 (100%)	18/18 (100%)
First incidence (days)	455	477	400
Life table tests	P=0.036	P=0.038	P=0.042
Logistic regression tests	P=0.422N	-[e]	P=0.657N
Cochran-Armitage test	P=0.142N		
Fisher exact test		P=0.500N	P=0.247N

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Value of statistic cannot be computed

TABLE A4
Historical Incidence of Pheochromocytomas of the Adrenal Medulla in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Benign Pheochromocytoma	Malignant Pheochromocytoma	Benign or Malignant Pheochromocytoma
Historical Incidence at Southern Research Insitute			
CI Pigment Red 3	22/50	6/50	24/50
Nitrofurantoin	23/50	3/50	24/50
o-Nitroanisole	7/49	6/49	12/49
Polysorbate 80	21/50	1/50	21/50
Rhodamine 6G	18/50	10/50	23/50
Roxarsone	15/50	5/50	19/50
Total	106/299 (35.5%)	31/299 (10.4%)	123/299 (41.1%)
Standard deviation	12.0%	6.1%	9.2%
Range	14%–46%	2%–20%	24%–48%
Overall Historical Incidence			
Total	284/788 (36.0%)	39/788 (4.9%)	306/788[b] (38.8%)
Standard deviation	9.3%	5.8%	8.4%
Range	14%–47%	0%–20%	22%–48%

[a] Data as of 3 April 1991
[b] Includes one complex pheochromocytoma

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polysorbate 80[a]

	0 ppm	25,000 ppm	50,000 ppm
Disposition Summary			
Animals initially in study	60	60	60
15-month interim evaluation	10	10	10
Early deaths			
Natural death	7	5	7
Moribund	14	27	25
Survivors			
Terminal sacrifice	29	18	18
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large, cecum	(50)	(49)	(48)
Parasite metazoan	4 (8%)	5 (10%)	5 (10%)
Intestine large, colon	(49)	(50)	(49)
Diverticulum		1 (2%)	
Metaplasia, osseous		1 (2%)	
Parasite metazoan	5 (10%)	4 (8%)	3 (6%)
Arteriole, inflammation, chronic			1 (2%)
Intestine large, rectum	(48)	(49)	(49)
Parasite metazoan	3 (6%)	5 (10%)	2 (4%)
Intestine small, ileum	(49)	(47)	(49)
Inflammation, chronic	1 (2%)	1 (2%)	
Intestine small, jejunum	(48)	(50)	(49)
Inflammation, chronic active			1 (2%)
Liver	(50)	(50)	(50)
Angiectasis	4 (8%)		6 (12%)
Basophilic focus	34 (68%)	21 (42%)	25 (50%)
Clear cell focus		1 (2%)	
Congestion	1 (2%)		1 (2%)
Developmental malformation	7 (14%)	9 (18%)	3 (6%)
Focal cellular change	2 (4%)		
Granuloma			1 (2%)
Hematopoietic cell proliferation		1 (2%)	
Necrosis	3 (6%)	8 (16%)	3 (6%)
Vacuolization cytoplasmic	4 (8%)	2 (4%)	2 (4%)
Arteriole, inflammation, chronic			1 (2%)
Bile duct, hyperplasia	49 (98%)	44 (88%)	50 (100%)
Portal, inflammation, chronic	46 (92%)	45 (90%)	45 (90%)
Mesentery	(4)	(10)	(12)
Arteriole inflammation, chronic		1 (10%)	
Artery, inflammation, chronic			1 (8%)
Fat, necrosis	4 (100%)	7 (70%)	8 (67%)
Pancreas	(50)	(50)	(48)
Atrophy		1 (2%)	
Infiltration cellular, lymphocyte			1 (2%)
Inflammation, acute			1 (2%)
Pigmentation			1 (2%)
Acinus, atrophy	25 (50%)	22 (44%)	23 (48%)
Acinus, hyperplasia	1 (2%)	4 (8%)	
Artery, inflammation, chronic		2 (4%)	2 (4%)
Duct, ectasia	1 (2%)		

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Alimentary System (continued)			
Salivary glands	(50)	(50)	(50)
Infiltration cellular, lymphocyte	5 (10%)		1 (2%)
Acinus, atrophy			1 (2%)
Duct, mineralization			1 (2%)
Stomach, forestomach	(50)	(50)	(50)
Edema	1 (2%)		
Hyperkeratosis	1 (2%)		1 (2%)
Inflammation, acute	2 (4%)		1 (2%)
Inflammation, chronic active	1 (2%)	1 (2%)	
Mineralization		4 (8%)	
Ulcer	1 (2%)	1 (2%)	
Epithelium, hyperplasia		1 (2%)	
Stomach, glandular	(49)	(50)	(50)
Dilatation		1 (2%)	
Mineralization	2 (4%)	2 (4%)	1 (2%)
Mucosa, dilatation	30 (61%)	29 (58%)	24 (48%)
Mucosa, erosion		2 (4%)	1 (2%)
Mucosa, ulcer			1 (2%)
Tooth			(4)
Peridontal tissue, hemorrhage			1 (25%)
Peridontal tissue, inflammation, chronic			1 (25%)
Pulp, necrosis			1 (25%)
Cardiovascular System			
Blood vessel	(6)		(1)
Artery, inflammation, chronic	6 (100%)		1 (100%)
Artery, thrombus			1 (100%)
Heart	(50)	(50)	(50)
Cardiomyopathy, chronic	47 (94%)	46 (92%)	45 (90%)
Mineralization	2 (4%)	1 (2%)	1 (2%)
Pigmentation, hemosiderin	1 (2%)		
Artery, inflammation, chronic			1 (2%)
Artery, mineralization			2 (4%)
Atrium left, thrombus		1 (2%)	2 (4%)
Atrium right, thrombus		1 (2%)	
Endocrine System			
Adrenal gland, cortex	(50)	(50)	(50)
Accessory adrenal cortical nodule	2 (4%)		2 (4%)
Cyst			1 (2%)
Degeneration, fatty	26 (52%)	21 (42%)	27 (54%)
Hyperplasia	15 (30%)	13 (26%)	14 (28%)
Hypertrophy			1 (2%)
Infiltration cellular, lymphocyte	1 (2%)		
Necrosis	1 (2%)		1 (2%)
Pigmentation	1 (2%)	1 (2%)	6 (12%)
Adrenal gland, medulla	(50)	(50)	(50)
Cyst			1 (2%)
Hyperplasia	11 (22%)	22 (44%)	12 (24%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Endocrine System (continued)			
Islets, pancreatic	(50)	(48)	(48)
Hyperplasia	1 (2%)	2 (4%)	1 (2%)
Parathyroid gland	(39)	(47)	(45)
Hypertrophy	1 (3%)	1 (2%)	3 (7%)
Pituitary gland	(50)	(49)	(50)
Pars distalis, cyst	3 (6%)	5 (10%)	2 (4%)
Pars distalis, hemorrhage	2 (4%)		
Pars distalis, hyperplasia	10 (20%)	14 (29%)	12 (24%)
Pars distalis, infarct	1 (2%)		
Pars distalis, infiltration cellular, lymphocyte	1 (2%)		
Pars intermedia, angiectasis			1 (2%)
Pars intermedia, cyst		2 (4%)	2 (4%)
Pars intermedia, hyperplasia			1 (2%)
Thyroid gland	(50)	(50)	(50)
Hemorrhage			1 (2%)
Infiltration cellular, lymphocyte			1 (2%)
Ultimobranchial cyst	2 (4%)	1 (2%)	2 (4%)
C-cell, hyperplasia	8 (16%)	12 (24%)	12 (24%)
Follicle, ectasia	6 (12%)	2 (4%)	2 (4%)
Follicle, hyperplasia			1 (2%)
General Body System			
None			
Genital System			
Epididymis	(49)	(50)	(50)
Inflammation, chronic	4 (8%)		
Inflammation, chronic active	1 (2%)		1 (2%)
Preputial gland	(48)	(50)	(49)
Abscess	1 (2%)	1 (2%)	
Inflammation, chronic	19 (40%)	18 (36%)	20 (41%)
Inflammation, chronic active	13 (27%)	21 (42%)	14 (29%)
Duct, ectasia	1 (2%)	4 (8%)	2 (4%)
Prostate	(50)	(50)	(50)
Hemorrhage			1 (2%)
Inflammation, acute	1 (2%)	2 (4%)	1 (2%)
Inflammation, chronic	6 (12%)	2 (4%)	6 (12%)
Inflammation, chronic active	40 (80%)	41 (82%)	32 (64%)
Testes	(49)	(50)	(50)
Atrophy	6 (12%)	7 (14%)	
Granuloma sperm		1 (2%)	
Mineralization			1 (2%)
Necrosis	1 (2%)	1 (2%)	
Artery, inflammation, chronic		3 (6%)	2 (4%)
Interstitial cell, hyperplasia	13 (27%)	15 (30%)	14 (28%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Hematopoietic System			
Blood	(2)	(4)	(1)
Atypical cells		1 (25%)	
Bone marrow	(50)	(50)	(49)
Hemorrhage	1 (2%)	1 (2%)	2 (4%)
Myelofibrosis			1 (2%)
Necrosis	1 (2%)		
Lymph node	(49)	(50)	(50)
Mediastinal, congestion	1 (2%)	4 (8%)	3 (6%)
Mediastinal, hemorrhage	1 (2%)		1 (2%)
Mediastinal, hyperplasia, lymphoid		1 (2%)	
Mediastinal, infiltration cellular, plasma cell	1 (2%)	1 (2%)	1 (2%)
Mediastinal, infiltration cellular, histiocyte		1 (2%)	
Mediastinal, pigmentation		2 (4%)	
Pancreatic, congestion			1 (2%)
Lymph node, mandibular	(49)	(50)	(48)
Congestion	1 (2%)	1 (2%)	1 (2%)
Cyst	4 (8%)	6 (12%)	4 (8%)
Hyperplasia, lymphoid	37 (76%)	31 (62%)	39 (81%)
Inflammation, acute			1 (2%)
Lymph node, mesenteric	(49)	(50)	(49)
Congestion	1 (2%)	3 (6%)	1 (2%)
Cyst		1 (2%)	
Edema			1 (2%)
Hemorrhage			1 (2%)
Hyperplasia, lymphoid			1 (2%)
Infiltration cellular, plasma cell		1 (2%)	
Inflammation, acute		1 (2%)	
Spleen	(50)	(50)	(50)
Fibrosis	6 (12%)	5 (10%)	4 (8%)
Hematopoietic cell proliferation	30 (60%)	31 (62%)	37 (74%)
Hyperplasia, lymphoid		2 (4%)	3 (6%)
Hyperplasia, reticulum cell		1 (2%)	
Necrosis	2 (4%)		
Pigmentation	25 (50%)	26 (52%)	23 (46%)
Thymus	(47)	(40)	(49)
Congestion	1 (2%)	1 (3%)	1 (2%)
Cyst		1 (3%)	2 (4%)
Hyperplasia, lymphoid			1 (2%)
Integumentary System			
Mammary gland	(47)	(39)	(48)
Acinus, ectasia	20 (43%)	15 (38%)	16 (33%)
Acinus, hyperplasia	2 (4%)		
Duct, ectasia	1 (2%)	3 (8%)	2 (4%)
Skin	(49)	(50)	(50)
Abscess	2 (4%)	1 (2%)	
Alopecia		1 (2%)	1 (2%)
Cyst epithelial inclusion		1 (2%)	1 (2%)
Hyperkeratosis			2 (4%)
Inflammation, chronic		2 (4%)	
Inflammation, chronic active		1 (2%)	1 (2%)
Ulcer		1 (2%)	2 (4%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Musculoskeletal System			
Bone	(50)	(50)	(50)
Hyperostosis		1 (2%)	
Cranium, fibrous osteodystrophy	1 (2%)		
Femur, fibrous osteodystrophy	3 (6%)		
Femur, hyperostosis	1 (2%)		
Nervous System			
Brain	(50)	(50)	(50)
Compression	5 (10%)	5 (10%)	6 (12%)
Hemorrhage	2 (4%)	2 (4%)	1 (2%)
Hydrocephalus	1 (2%)		1 (2%)
Necrosis		1 (2%)	
Respiratory System			
Lung	(50)	(50)	(50)
Congestion	4 (8%)	4 (8%)	5 (10%)
Hemorrhage	5 (10%)	4 (8%)	12 (24%)
Alveolar epithelium, hyperplasia	2 (4%)		
Alveolus, infiltration cellular, histiocyte	6 (12%)	4 (8%)	13 (26%)
Alveolus, mineralization			2 (4%)
Arteriole, mineralization	31 (62%)	35 (70%)	34 (68%)
Bronchiole, inflammation, acute			1 (2%)
Interstitium, inflammation, chronic	1 (2%)		1 (2%)
Interstitium, inflammation, chronic active			1 (2%)
Peribronchiolar, inflammation, chronic active			1 (2%)
Nose	(50)	(50)	(50)
Lumen, hemorrhage	9 (18%)	12 (24%)	7 (14%)
Mucosa, inflammation, acute	12 (24%)	7 (14%)	9 (18%)
Mucosa, inflammation, chronic			2 (4%)
Nasolacrimal duct, hemorrhage	3 (6%)		2 (4%)
Nasolacrimal duct, inflammation, acute	14 (28%)	12 (24%)	8 (16%)
Olfactory epithelium, degeneration	32 (64%)	34 (68%)	38 (76%)
Respiratory epithelium, degeneration	7 (14%)	5 (10%)	7 (14%)
Respiratory epithelium, metaplasia, squamous			1 (2%)
Trachea	(50)	(50)	(50)
Inflammation, acute			1 (2%)
Special Senses System			
Ear	(1)	(2)	(2)
Canal, abscess	1 (100%)		
Eye	(10)	(11)	(14)
Cataract	8 (80%)	11 (100%)	12 (86%)
Hemorrhage	3 (30%)	3 (27%)	5 (36%)
Inflammation, chronic			1 (7%)
Anterior chamber, hemorrhage			1 (7%)
Cornea, inflammation, acute			1 (7%)
Posterior chamber, synechia	1 (10%)		2 (14%)
Retina, degeneration	9 (90%)	11 (100%)	12 (86%)
Sclera, mineralization	2 (20%)	1 (9%)	2 (14%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Urinary System			
Kidney	(50)	(50)	(50)
Congestion			1 (2%)
Cyst	2 (4%)		1 (2%)
Infarct			1 (2%)
Necrosis	1 (2%)		
Nephropathy, chronic	50 (100%)	49 (98%)	49 (98%)
Cortex, cyst			1 (2%)
Pelvis, dilatation		1 (2%)	
Renal tubule, mineralization	1 (2%)		
Renal tubule, pigmentation	44 (88%)	48 (96%)	42 (84%)
Urethra	(3)	(2)	(3)
Calculus micro observation only	3 (100%)	2 (100%)	3 (100%)
Urinary bladder	(49)	(50)	(49)
Calculus micro observation only	1 (2%)		1 (2%)
Infiltration cellular, lymphocyte	2 (4%)		
Inflammation, chronic		1 (2%)	

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR FEED STUDY OF POLYSORBATE 80

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Polysorbate 80[a]

	0 ppm	25,000 ppm	50,000 ppm
Disposition Summary			
Animals initially in study	60	60	60
15-month interim evaluation	10	10	10
Early deaths			
Natural death	2	3	4
Moribund	25	22	21
Survivors			
Terminal sacrifice	23	25	25
Animals examined microscopically	50	50	50
Alimentary System			
Esophagus	(49)	(50)	(50)
Squamous cell carcinoma			1 (2%)
Intestine large, cecum	(50)	(48)	(49)
Intestine large, colon	(50)	(49)	(50)
Intestine large, rectum	(50)	(50)	(48)
Leiomyosarcoma	1 (2%)		
Intestine small, duodenum	(50)	(48)	(50)
Adenocarcinoma		1 (2%)	
Intestine small, ileum	(50)	(48)	(49)
Intestine small, jejunum	(50)	(48)	(49)
Liver	(50)	(50)	(50)
Hepatocellular adenoma		1 (2%)	3 (6%)
Histiocytic sarcoma		1 (2%)	
Mesentery	(10)	(4)	(3)
Pancreas	(50)	(47)	(50)
Salivary glands	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)
Cardiovascular System			
Heart	(50)	(50)	(50)
Endocrine System			
Adrenal gland, cortex	(50)	(50)	(50)
Adenoma	1 (2%)	2 (4%)	
Adrenal gland, medulla	(50)	(50)	(50)
Pheochromocytoma NOS	6 (12%)	6 (12%)	7 (14%)
Islets, pancreatic	(50)	(47)	(50)
Adenoma		2 (4%)	
Parathyroid gland	(41)	(41)	(41)
Adenoma			1 (2%)
Pituitary gland	(50)	(50)	(50)
Pars distalis, adenoma	37 (74%)	30 (60%)	36 (72%)
Pars distalis, carcinoma	1 (2%)		
Pars distalis, histiocytic sarcoma		1 (2%)	
Pars intermedia, adenoma	2 (4%)		
Pars intermedia, carcinoma	1 (2%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Endocrine System (continued)			
Thyroid gland	(50)	(50)	(50)
Thymoma malignant, metastatic, thymus			1 (2%)
C-cell, adenoma	5 (10%)	6 (12%)	10 (20%)
C-cell, carcinoma	1 (2%)		
Follicular cell, adenoma	1 (2%)		
Follicular cell, carcinoma	1 (2%)		
General Body System			
None			
Genital System			
Clitoral gland	(48)	(49)	(50)
Adenoma	3 (6%)	3 (6%)	3 (6%)
Carcinoma	7 (15%)	5 (10%)	4 (8%)
Squamous cell carcinoma		1 (2%)	1 (2%)
Ovary	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	
Uterus	(50)	(50)	(50)
Endometrium, polyp stromal	9 (18%)	11 (22%)	12 (24%)
Endometrium, sarcoma stromal			1 (2%)
Vagina	(6)	(9)	(5)
Hematopoietic System			
Blood	(2)	(1)	
Bone marrow	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	
Lymph node	(50)	(50)	(50)
Lymph node, mandibular	(50)	(50)	(50)
Lymph node, mesenteric	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	
Spleen	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	
Thymus	(47)	(46)	(47)
Thymoma malignant			1 (2%)
Integumentary System			
Mammary gland	(50)	(48)	(50)
Adenocarcinoma		2 (4%)	1 (2%)
Adenoma	1 (2%)		
Fibroadenoma	28 (56%)	27 (56%)	26 (52%)
Skin	(50)	(50)	(50)
Squamous cell carcinoma	1 (2%)		
Head, papilloma squamous		1 (2%)	
Subcutaneous tissue, fibroma	2 (4%)	2 (4%)	
Subcutaneous tissue, fibrosarcoma		2 (4%)	
Subcutaneous tissue, lipoma		1 (2%)	

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Polysorbate 80
(continued)

	0 ppm	25,000 ppm	50,000 ppm
Musculoskeletal System			
Skeletal muscle	(1)	(3)	(2)
Lipoma			1 (50%)
Rhabdomyosarcoma	1 (100%)		
Nervous System			
Brain	(50)	(50)	(50)
Carcinoma, deep invasion, metastatic, pituitary gland	2 (4%)		
Oligodendroglioma malignant		2 (4%)	
Respiratory System			
Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)	
Alveolar/bronchiolar carcinoma		1 (2%)	
Nose	(50)	(50)	(50)
Nasolacrimal duct, squamous cell carcinoma			1 (2%)
Trachea	(50)	(50)	(50)
Thymoma malignant, metastatic, thymus			1 (2%)
Special Senses System			
Zymbal's gland		(1)	(2)
Adenoma			1 (50%)
Squamous cell carcinoma			1 (50%)
Urinary System			
Kidney	(50)	(50)	(50)
Urinary bladder	(50)	(50)	(50)
Transitional epithelium, papilloma	1 (2%)		
Systemic Lesions			
Multiple organs[b]	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	
Leukemia mononuclear	26 (52%)	10 (20%)	13 (26%)
Lymphoma malignant mixed	1 (2%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Tumor Summary			
Total animals with primary neoplasms[c]	49	48	48
Total primary neoplasms	138	118	124
Total animals with benign neoplasms	47	44	46
Total benign neoplasms	91	87	93
Total animals with malignant neoplasms	35	21	20
Total malignant neoplasms	41	25	24
Total animals with secondary neoplasms[d]	2		1
Total secondary neoplasms	2		2
Total animals with neoplasms uncertain-benign or malignant	6	6	7
Total uncertain neoplasms	6	6	7

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Number of animals with any tissue examined microscopically
[c] Primary tumors: all tumors except metastatic tumors
[d] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80: 0 ppm

Number of Days on Study	1 3 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 7 7 7 7 9 6 1 4 6 7 7 9 1 1 2 3 3 4 4 4 4 4 6 7 9 0 0 1 1 5 7 4 8 1 3 5 4 7 7 9 0 1 0 4 5 5 6 2 5 9 1 9 0 0
Carcass ID Number	4 4 3 3 4 4 4 4 4 4 3 4 4 4 4 4 4 3 4 3 4 4 3 3 4 3 5 7 7 7 2 7 2 0 5 8 5 4 4 3 2 8 9 0 9 5 3 9 8 4 1 1 1 2 1 1 2 2 1 2 1 3 1 2 2 3 1 1 2 2 4 3 3 2 3
Alimentary System	
Esophagus	M +
Intestine large	+ +
Intestine large, cecum	+ +
Intestine large, colon	+ +
Intestine large, rectum	+ +
Leiomyosarcoma	X
Intestine small	+ +
Intestine small, duodenum	+ +
Intestine small, ileum	+ +
Intestine small, jejunum	+ +
Liver	+ +
Mesentery	+ +
Pancreas	+ +
Salivary glands	+ +
Stomach	+ +
Stomach, forestomach	+ +
Stomach, glandular	+ +
Tooth	+
Cardiovascular System	
Heart	+ +
Endocrine System	
Adrenal gland	+ +
Adrenal gland, cortex	+ +
Adenoma	
Adrenal gland, medulla	+ +
Pheochromocytoma NOS	X X
Islets, pancreatic	+ +
Parathyroid gland	+ + + + + + M + + + + + + + + + + + + M + M + + M
Pituitary gland	+ +
Pars distalis, adenoma	X X X X X X X X X X X X X X
Pars distalis, carcinoma	X
Pars intermedia, adenoma	
Pars intermedia, carcinoma	X
Thyroid gland	+ +
C-cell, adenoma	X X X X
C-cell, carcinoma	
Follicular cell, adenoma	X
Follicular cell, carcinoma	

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80:
0 ppm (continued)

Number of Days on Study	7 1 0	7 1 6	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	4 8 2	3 9 4	3 7 3	3 7 4	3 7 5	3 8 3	3 8 4	3 8 5	3 9 5	4 0 3	4 0 4	4 0 5	4 2 4	4 2 5	4 3 4	4 3 5	4 4 4	4 4 5	4 5 5	4 7 3	4 7 4	4 7 5	4 8 3	4 8 4	4 8 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery																						+				10
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma												X														1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma NOS						X						X							X	X						6
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	M	+	+	+	+	M	+	M	+	+	+	+	+	M	M	+	+	+	+	+	+	+	41
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X	X	X		X		X		X		X	X	X	X	X	X	X		X	X	X	X	X	X	37
Pars distalis, carcinoma																										1
Pars intermedia, adenoma									X											X						2
Pars intermedia, carcinoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma																					X					5
C-cell, carcinoma													X													1
Follicular cell, adenoma																										1
Follicular cell, carcinoma											X															1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80: 0 ppm (continued)

Number of Days on Study	1	3	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7
	9	6	1	4	6	7	7	9	1	1	2	3	3	4	4	4	4	4	6	7	9	0	0	1	1
	5	7	4	8	1	3	5	4	7	7	9	0	1	0	4	5	5	6	2	5	9	1	9	0	0
Carcass ID Number	4	4	3	3	4	4	4	4	4	4	3	4	4	4	4	4	4	3	4	3	4	4	3	3	4
	3	5	7	7	7	2	7	2	0	5	8	5	4	4	3	2	8	9	0	9	5	3	9	8	4
	1	1	1	2	1	1	2	2	1	2	1	3	1	2	2	3	1	1	2	2	4	3	3	2	3
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X																				
Carcinoma										X				X								X	X		
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal		X								X						X		X		X			X		X
Vagina	+	+								+		+	+		+										
Hematopoietic System																									
Blood									+										+						
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma										X															
Fibroadenoma		X					X				X	X	X	X				X	X		X	X		X	X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Subcutaneous tissue, fibroma																X									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+																								
Rhabdomyosarcoma	X																								

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80:
0 ppm (continued)

Number of Days on Study	7 1 0	7 1 6	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	4 8 2	3 9 4	3 7 3	3 7 4	3 7 5	3 8 3	3 8 4	3 8 5	3 9 5	4 0 3	4 0 4	4 0 5	4 2 4	4 2 5	4 3 4	4 3 5	4 4 4	4 4 5	4 5 5	4 7 3	4 7 4	4 7 5	4 8 3	4 8 4	4 8 5	Total Tissues/ Tumors
General Body System																										
None																										
Genital System																										
Clitoral gland	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma												X			X											3
Carcinoma																			X			X	X			7
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal								X								X										9
Vagina																										6
Hematopoietic System																										
Blood																										2
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Fibroadenoma	X	X		X		X	X	X	X	X	X	X		X		X		X	X		X	X				28
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma	X																									1
Subcutaneous tissue, fibroma									X																	2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Rhabdomyosarcoma																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80: 0 ppm (continued)

Number of Days on Study	1 9 5	3 6 7	5 1 4	5 4 8	5 6 1	5 7 3	5 7 5	5 9 4	6 1 7	6 1 7	6 2 9	6 3 0	6 3 1	6 4 0	6 4 4	6 4 5	6 4 5	6 4 6	6 6 2	6 7 5	6 9 9	7 0 1	7 0 9	7 1 0	7 1 0
Carcass ID Number	4 3 1	4 5 1	3 7 1	3 7 2	4 7 1	4 2 1	4 7 2	4 2 2	4 0 1	4 5 2	3 8 1	4 5 3	4 4 1	4 4 2	4 3 2	4 2 3	4 8 1	3 9 1	4 0 2	3 9 2	4 5 4	4 3 3	3 9 3	3 8 2	4 4 3
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, deep invasion, metastatic, pituitary gland										X											X				
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma														X											
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																		+							
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X	X		X		X	X				X		X	X		X	X	X		X	X		X
Lymphoma malignant mixed															X										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80:
0 ppm (continued)

Number of Days on Study	7 1 0	7 1 6	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	4 8 2	3 9 4	3 7 3	3 7 4	3 7 5	3 8 3	3 8 4	3 8 5	3 9 5	4 0 3	4 0 4	4 0 5	4 2 4	4 2 5	4 3 4	4 3 5	4 4 4	4 4 5	4 5 5	4 7 3	4 7 4	4 7 5	4 8 3	4 8 4	4 8 5	Total Tissues/ Tumors
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, deep invasion, metastatic, pituitary gland																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye					+													+			+	+			+	6
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, papilloma											X															1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X	X			X				X			X	X	X	X	X			X					X	26
Lymphoma malignant mixed																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm

Number of Days on Study	2 7 3	3 2 3	4 6 6	4 6 7	4 6 7	5 3 2	5 6 1	6 1 0	6 1 9	6 2 3	6 3 0	6 3 7	6 3 8	6 4 0	6 4 0	6 4 6	6 8 7	7 0 0	7 0 0	7 0 2	7 0 2	7 1 6	7 1 7	7 1 7	7 2 4
Carcass ID Number	6 8 1	7 2 1	6 7 1	6 4 1	6 8 2	6 9 1	6 8 3	7 0 1	6 5 1	6 5 2	6 1 1	6 8 4	6 1 2	6 7 2	6 9 2	7 2 2	6 4 2	6 1 3	6 2 1	6 5 3	6 6 1	7 2 3	6 4 3	6 5 4	6 2 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	M	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma					X																				
Intestine small, ileum	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Histiocytic sarcoma											X														
Mesentery												+													
Pancreas	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth	+																								
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma NOS								X										X							
Islets, pancreatic	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	M	+	+	+	+	M	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X					X	X	X		X	X	X	X		X					X	X	X	X
Pars distalis, histiocytic sarcoma											X														
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																							X		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	6 1 4	6 1 5	6 2 3	6 2 4	6 2 5	6 4 4	6 4 5	6 5 5	6 6 2	6 6 3	6 6 4	6 6 5	6 7 3	6 7 4	6 7 5	6 8 5	6 9 3	6 9 4	6 9 5	7 0 2	7 0 3	7 0 4	7 0 5	7 2 4	7 2 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenocarcinoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma									X																	1
Histiocytic sarcoma																										1
Mesentery											+					+		+								4
Pancreas	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma												X			X											2
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma NOS		X									X							X		X						6
Islets, pancreatic	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma			X																						X	2
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	M	+	+	41
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma		X		X		X	X	X	X	X			X	X	X			X		X	X	X	X	X	X	30
Pars distalis, histiocytic sarcoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma	X					X										X						X	X			6

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm (continued)

Number of Days on Study	2 7 3	3 2 3	4 6 6	4 6 7	4 6 7	5 3 2	5 6 1	6 1 0	6 1 9	6 2 3	6 3 0	6 3 7	6 3 8	6 4 0	6 4 0	6 4 6	6 8 7	6 0 0	7 0 0	7 0 2	7 0 2	7 1 6	7 1 7	7 1 7	7 2 4
Carcass ID Number	6 8 1	7 2 1	6 7 1	6 4 1	6 8 2	6 9 1	6 8 3	7 0 1	6 5 1	6 5 2	6 1 1	6 8 4	6 1 2	6 7 2	6 9 2	6 2 2	7 4 2	6 1 3	6 2 1	6 5 3	6 6 1	6 2 3	6 4 3	6 5 4	6 2 2
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Adenoma																									
Carcinoma											X							X	X						
Squamous cell carcinoma																					X				
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma											X														
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal				X				X																X	
Vagina	+		+	+						+		+				+								+	
Hematopoietic System																									
Blood											+														
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma											X														
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma											X														
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma											X														
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma									X																X
Fibroadenoma						X		X		X		X	X	X		X	X	X		X	X	X		X	X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Head, papilloma squamous																									X
Subcutaneous tissue, fibroma																			X						
Subcutaneous tissue, fibrosarcoma																	X								X
Subcutaneous tissue, lipoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle															+									+	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80:
25,000 ppm (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	6 1 4	6 1 5	6 2 3	6 2 4	6 2 5	6 4 4	6 4 5	6 5 5	6 6 2	6 6 3	6 6 4	6 6 5	6 7 3	6 7 4	6 7 5	6 8 5	6 9 3	6 9 4	6 9 5	7 0 2	7 0 3	7 0 4	7 0 5	7 2 4	7 2 5	Total Tissues/ Tumors
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma		X														X							X			3
Carcinoma			X				X																			5
Squamous cell carcinoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal	X	X	X					X				X			X		X				X					11
Vagina										+						+										9
Hematopoietic System																										
Blood																										1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Thymus	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenocarcinoma																										2
Fibroadenoma	X		X		X	X	X			X				X	X					X	X	X	X	X		27
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Head, papilloma squamous																										1
Subcutaneous tissue, fibroma																							X			2
Subcutaneous tissue, fibrosarcoma																										2
Subcutaneous tissue, lipoma																		X								1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle									+																	3

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm (continued)

Number of Days on Study	2 7 3	3 2 3	4 6 6	4 6 7	4 6 7	5 3 2	5 6 1	6 1 0	6 1 9	6 2 3	6 3 0	6 3 7	6 3 8	6 4 0	6 4 0	6 4 6	6 8 7	7 0 0	7 0 0	7 0 2	7 0 2	7 1 6	7 1 7	7 1 7	7 2 4
Carcass ID Number	6 8 1	7 2 1	6 7 1	6 4 1	6 8 2	6 9 1	6 8 3	7 0 1	6 5 1	6 5 2	6 1 1	6 8 4	6 1 2	6 7 2	6 9 2	7 2 2	6 4 2	6 1 3	6 2 1	6 5 3	6 6 1	7 2 3	6 4 3	6 5 4	6 2 2
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oligodendroglioma malignant		X	X																						
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																X									
Alveolar/bronchiolar carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye			+																	+	+				
Zymbal's gland		+																							
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urethra		+																							
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma											X														
Leukemia mononuclear							X		X	X		X			X		X							X	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	6 1 4	6 1 5	6 2 3	6 2 4	6 2 5	6 4 4	6 4 5	6 5 5	6 6 2	6 6 3	6 6 4	6 6 5	6 7 3	6 7 4	6 7 5	6 8 5	6 9 3	6 9 4	6 9 5	7 0 2	7 0 3	7 0 4	7 0 5	7 2 4	7 2 5	Total Tissues/ Tumors
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Oligodendroglioma malignant																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Alveolar/bronchiolar carcinoma										X																1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye		+		+	+	+			+										+							9
Zymbal's gland																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urethra																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear													X		X	X										10

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm

Number of Days on Study	3 1 6	4 3 2	5 3 5	5 6 7	5 6 8	5 7 1	6 1 0	6 1 0	6 1 2	6 1 2	6 1 9	6 2 3	6 3 3	6 3 4	6 4 0	6 4 4	6 9 4	6 9 4	6 9 8	6 9 9	7 0 0	7 0 2	7 0 8	7 1 6	7 1 7
Carcass ID Number	5 8 1	5 8 2	5 4 1	5 5 1	5 9 1	5 6 1	4 9 1	5 1 1	5 3 1	5 6 2	5 9 2	6 0 1	5 4 2	5 8 3	5 6 3	5 8 4	5 5 2	6 0 2	6 0 3	5 5 3	5 4 3	4 9 2	5 4 4	6 0 4	5 6 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma		X																							
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																							X		
Mesentery																		+			+				
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth												+													
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma NOS																					X		X		X
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Adenoma																									
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X			X			X	X	X	X	X	X	X		X	X	X	X	X			X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma malignant, metastatic, thymus				X																					
C-cell, adenoma										X					X										X
General Body System																									
None																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80:
50,000 ppm (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	4 9 3	4 9 4	4 9 5	5 0 1	5 0 2	5 0 3	5 0 4	5 0 5	5 1 2	5 1 3	5 1 4	5 1 5	5 3 2	5 3 3	5 3 4	5 3 5	5 4 5	5 5 4	5 5 5	5 6 5	5 8 5	5 9 3	5 9 4	5 9 5	6 0 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																										1
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																			X			X				3
Mesentery															+											3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma NOS		X											X			X						X				7
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	M	+	M	+	+	M	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	41
Adenoma																						X				1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma			X	X		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	36
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymoma malignant, metastatic, thymus																										1
C-cell, adenoma	X												X					X	X		X	X		X		10
General Body System																										
None																										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	3 1 6	4 3 2	5 3 5	5 6 7	5 6 8	5 7 1	6 1 0	6 1 0	6 1 2	6 1 2	6 1 9	6 2 3	6 3 3	6 3 4	6 4 0	6 4 4	6 9 4	6 9 4	6 9 8	6 9 9	7 0 0	7 0 2	7 0 8	7 1 6	7 1 7
Carcass ID Number	5 5 8 1	5 8 2	5 4 1	5 5 1	5 9 1	5 6 1	4 9 1	5 1 1	5 3 1	5 6 2	5 9 2	6 0 1	5 4 2	5 8 3	5 6 3	5 8 4	5 5 2	6 0 2	6 0 3	5 5 3	5 4 3	4 9 2	5 4 4	6 0 4	5 6 4
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma												X				X									
Carcinoma							X		X															X	
Squamous cell carcinoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal				X			X								X	X	X	X			X				
Endometrium, sarcoma stromal												X													
Vagina	+	+								+															
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma malignant				X																					
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma									X																
Fibroadenoma							X	X					X	X	X	X	X	X		X			X	X	X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+																	+	+						
Lipoma																		X							
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord																			+						
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasolacrimal duct, squamous cell carcinoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma malignant, metastatic, thymus				X																					

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	4 9 3	4 9 4	4 9 5	5 0 1	5 0 2	5 0 3	5 0 4	5 0 5	5 1 2	5 1 3	5 1 4	5 1 5	5 3 2	5 3 3	5 3 4	5 3 5	5 4 5	5 5 4	5 5 5	5 6 5	5 8 5	5 9 3	5 9 4	5 9 5	6 0 5	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																						X				3
Carcinoma														X												4
Squamous cell carcinoma											X															1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal					X	X						X		X			X									12
Endometrium, sarcoma stromal																										1
Vagina					+													+								5
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	47
Thymoma malignant																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma																										1
Fibroadenoma	X	X	X				X	X	X			X	X	X				X	X			X		X	X	26
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Lipoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nasolacrimal duct, squamous cell carcinoma											X															1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymoma malignant, metastatic, thymus																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	3	4	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7
	1	3	3	6	6	7	1	1	1	1	1	2	3	3	4	4	9	9	9	0	0	0	1	1
	6	2	5	7	8	1	0	0	2	2	9	3	3	4	0	4	4	4	8	9	0	2	8	6	7
Carcass ID Number	5	5	5	5	5	5	4	5	5	5	5	6	5	5	5	5	5	6	6	5	5	4	5	6	5
	8	8	4	5	9	6	9	1	3	6	9	0	4	8	6	8	5	0	0	5	4	9	4	0	6
	1	2	1	1	1	1	1	1	1	2	2	1	2	3	3	4	2	2	3	3	3	2	4	4	4
Special Senses System																									
Eye							+					+					+	+							
Zymbal's gland												+													
Adenoma																									
Squamous cell carcinoma												X													
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X					X							X				X		X		X

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	4 9 3	4 9 4	4 9 5	5 0 1	5 0 2	5 0 3	5 0 4	5 0 5	5 1 2	5 1 3	5 1 4	5 1 5	5 3 2	5 3 3	5 3 4	5 3 5	5 4 5	5 4 4	5 5 5	5 5 5	5 6 5	5 8 5	5 9 3	5 9 4	5 9 5	6 0 5	Total Tissues/ Tumors
Special Senses System																											
Eye	+	+	+															+		+	+						10
Zymbal's gland																	+										2
Adenoma																	X										1
Squamous cell carcinoma																											1
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X		X						X		X	X											X			X	13

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Polysorbate 80

	0 ppm	25,000 ppm	50,000 ppm
Adrenal Medulla: Pheochromocytoma NOS			
Overall rates[a]	6/50 (12%)	6/50 (12%)	7/50 (14%)
Adjusted rates[b]	22.3%	20.4%	24.7%
Terminal rates[c]	4/23 (17%)	4/25 (16%)	4/25 (16%)
First incidence (days)	645	610	700
Life table tests[d]	P=0.491	P=0.566N	P=0.551
Logistic regression tests[d]	P=0.471	P=0.604N	P=0.538
Cochran-Armitage test[d]	P=0.440		
Fisher exact test[d]		P=0.620N	P=0.500
Clitoral Gland: Adenoma			
Overall rates	3/48 (6%)	3/49 (6%)	3/50 (6%)
Adjusted rates	11.1%	12.0%	9.1%
Terminal rates	2/22 (9%)	3/25 (12%)	1/25 (4%)
First incidence (days)	561	729 (T)	623
Life table tests	P=0.544N	P=0.617N	P=0.633N
Logistic regression tests	P=0.552N	P=0.642N	P=0.645N
Cochran-Armitage test	P=0.563N		
Fisher exact test		P=0.651N	P=0.641N
Clitoral Gland: Carcinoma			
Overall rates	7/48 (15%)	5/49 (10%)	4/50 (8%)
Adjusted rates	23.4%	15.7%	11.8%
Terminal rates	3/22 (14%)	2/25 (8%)	1/25 (4%)
First incidence (days)	617	630	610
Life table tests	P=0.190N	P=0.325N	P=0.240N
Logistic regression tests	P=0.179N	P=0.352N	P=0.229N
Cochran-Armitage test	P=0.189N		
Fisher exact test		P=0.365N	P=0.239N
Clitoral Gland: Adenoma or Carcinoma			
Overall rates	10/48 (21%)	8/49 (16%)	7/50 (14%)
Adjusted rates	33.0%	26.7%	20.0%
Terminal rates	5/22 (23%)	5/25 (20%)	2/25 (8%)
First incidence (days)	561	630	610
Life table tests	P=0.217N	P=0.323N	P=0.267N
Logistic regression tests	P=0.207N	P=0.361N	P=0.258N
Cochran-Armitage test	P=0.223N		
Fisher exact test		P=0.379N	P=0.266N
Liver: Hepatocellular Adenoma			
Overall rates	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rates	0.0%	4.0%	11.3%
Terminal rates	0/23 (0%)	1/25 (4%)	2/25 (8%)
First incidence (days)	-[e]	729 (T)	708
Life table tests	P=0.066	P=0.517	P=0.132
Logistic regression tests	P=0.064	P=0.517	P=0.130
Cochran-Armitage test	P=0.060		
Fisher exact test		P=0.500	P=0.121

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Mammary Gland: Fibroadenoma			
Overall rates	28/50 (56%)	27/50 (54%)	26/50 (52%)
Adjusted rates	74.8%	68.4%	69.4%
Terminal rates	14/23 (61%)	13/25 (52%)	14/25 (56%)
First incidence (days)	367	532	610
Life table tests	P=0.311N	P=0.374N	P=0.340N
Logistic regression tests	P=0.336N	P=0.497N	P=0.374N
Cochran-Armitage test	P=0.382N		
Fisher exact test		P=0.500N	P=0.421N
Mammary Gland: Adenoma or Fibroadenoma			
Overall rates	29/50 (58%)	27/50 (54%)	26/50 (52%)
Adjusted rates	75.4%	68.4%	69.4%
Terminal rates	14/23 (61%)	13/25 (52%)	14/25 (56%)
First incidence (days)	367	532	610
Life table tests	P=0.259N	P=0.318N	P=0.286N
Logistic regression tests	P=0.265N	P=0.416N	P=0.300N
Cochran-Armitage test	P=0.308N		
Fisher exact test		P=0.420N	P=0.344N
Mammary Gland: Adenoma, Fibroadenoma, or Adenocarcinoma			
Overall rates	29/50 (58%)	28/50 (56%)	27/50 (54%)
Adjusted rates	75.4%	69.1%	70.2%
Terminal rates	14/23 (61%)	13/25 (52%)	14/25 (56%)
First incidence (days)	367	532	610
Life table tests	P=0.317N	P=0.376N	P=0.345N
Logistic regression tests	P=0.339N	P=0.499N	P=0.377N
Cochran-Armitage test	P=0.381N		
Fisher exact test		P=0.500N	P=0.420N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	37/50 (74%)	30/50 (60%)	36/50 (72%)
Adjusted rates	87.7%	78.1%	89.5%
Terminal rates	18/23 (78%)	17/25 (68%)	21/25 (84%)
First incidence (days)	548	466	535
Life table tests	P=0.355N	P=0.102N	P=0.387N
Logistic regression tests	P=0.403N	P=0.092N	P=0.443N
Cochran-Armitage test	P=0.457N		
Fisher exact test		P=0.101N	P=0.500N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma			
Overall rates	38/50 (76%)	30/50 (60%)	36/50 (72%)
Adjusted rates	88.1%	78.1%	89.5%
Terminal rates	18/23 (78%)	17/25 (68%)	21/25 (84%)
First incidence (days)	548	466	535
Life table tests	P=0.298N	P=0.078N	P=0.329N
Logistic regression tests	P=0.316N	P=0.058N	P=0.348N
Cochran-Armitage test	P=0.372N		
Fisher exact test		P=0.066N	P=0.410N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Polysorbate 80
(continued)

	0 ppm	25,000 ppm	50,000 ppm
Pituitary Gland (Pars Intermedia): Adenoma or Carcinoma			
Overall rates	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rates	10.9%	0.0%	0.0%
Terminal rates	2/23 (9%)	0/25 (0%)	0/25 (0%)
First incidence (days)	617	–	–
Life table tests	P=0.035N	P=0.113N	P=0.115N
Logistic regression tests	P=0.036N	P=0.119N	P=0.117N
Cochran-Armitage test	P=0.037N		
Fisher exact test		P=0.121N	P=0.121N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma			
Overall rates	2/50 (4%)	4/50 (8%)	0/50 (0%)
Adjusted rates	7.1%	13.1%	0.0%
Terminal rates	1/23 (4%)	1/25 (4%)	0/25 (0%)
First incidence (days)	645	687	–
Life table tests	P=0.205N	P=0.383	P=0.234N
Logistic regression tests	P=0.212N	P=0.346	P=0.234N
Cochran-Armitage test	P=0.222N		
Fisher exact test		P=0.339	P=0.247N
Thyroid Gland (C-cell): Adenoma			
Overall rates	5/50 (10%)	6/50 (12%)	10/50 (20%)
Adjusted rates	14.3%	22.9%	34.3%
Terminal rates	1/23 (4%)	5/25 (20%)	7/25 (28%)
First incidence (days)	617	717	612
Life table tests	P=0.121	P=0.551	P=0.162
Logistic regression tests	P=0.105	P=0.509	P=0.139
Cochran-Armitage test	P=0.097		
Fisher exact test		P=0.500	P=0.131
Thyroid Gland (C-cell): Adenoma or Carcinoma			
Overall rates	6/50 (12%)	6/50 (12%)	10/50 (20%)
Adjusted rates	18.1%	22.9%	34.3%
Terminal rates	2/23 (9%)	5/25 (20%)	7/25 (28%)
First incidence (days)	617	717	612
Life table tests	P=0.194	P=0.566N	P=0.245
Logistic regression tests	P=0.174	P=0.610N	P=0.219
Cochran-Armitage test	P=0.161		
Fisher exact test		P=0.620N	P=0.207
Uterus: Stromal Polyp			
Overall rates	9/50 (18%)	11/50 (22%)	12/50 (24%)
Adjusted rates	26.1%	37.3%	34.3%
Terminal rates	2/23 (9%)	8/25 (32%)	5/25 (20%)
First incidence (days)	367	467	567
Life table tests	P=0.330	P=0.466	P=0.365
Logistic regression tests	P=0.276	P=0.402	P=0.308
Cochran-Armitage test	P=0.271		
Fisher exact test		P=0.402	P=0.312

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Uterus: Stromal Polyp or Stromal Sarcoma			
Overall rates	9/50 (18%)	11/50 (22%)	13/50 (26%)
Adjusted rates	26.1%	37.3%	35.9%
Terminal rates	2/23 (9%)	8/25 (32%)	5/25 (20%)
First incidence (days)	367	467	567
Life table tests	P=0.255	P=0.466	P=0.289
Logistic regression tests	P=0.203	P=0.402	P=0.229
Cochran-Armitage test	P=0.199		
Fisher exact test		P=0.402	P=0.235
All Organs: Mononuclear Cell Leukemia			
Overall rates	26/50 (52%)	10/50 (20%)	13/50 (26%)
Adjusted rates	65.1%	27.3%	40.2%
Terminal rates	10/23 (43%)	3/25 (12%)	7/25 (28%)
First incidence (days)	514	561	568
Life table tests	P=0.007N	P=0.003N	P=0.012N
Logistic regression tests	P=0.003N	P<0.001N	P=0.006N
Cochran-Armitage test	P=0.004N		
Fisher exact test		P<0.001N	P=0.007N
All Organs: Benign Tumors			
Overall rates	47/50 (94%)	44/50 (88%)	46/50 (92%)
Adjusted rates	97.9%	97.8%	100.0%
Terminal rates	22/23 (96%)	24/25 (96%)	25/25(100%)
First incidence (days)	367	466	535
Life table tests	P=0.331N	P=0.233N	P=0.366N
Logistic regression tests	P=0.319N	P=0.259N	P=0.396N
Cochran-Armitage test	P=0.429N		
Fisher exact test		P=0.243N	P=0.500N
All Organs: Malignant Tumors			
Overall rates	35/50 (70%)	21/50 (42%)	20/50 (40%)
Adjusted rates	78.7%	49.8%	51.5%
Terminal rates	14/23 (61%)	6/25 (24%)	8/25 (32%)
First incidence (days)	195	323	432
Life table tests	P=0.009N	P=0.018N	P=0.012N
Logistic regression tests	P=0.002N	P=0.004N	P=0.003N
Cochran-Armitage test	P=0.002N		
Fisher exact test		P=0.004N	P=0.002N
All Organs: Benign or Malignant Tumors			
Overall rates	49/50 (98%)	48/50 (96%)	48/50 (96%)
Adjusted rates	98.0%	98.0%	100.0%
Terminal rates	22/23 (96%)	24/25 (96%)	25/25 (100%)
First incidence (days)	195	323	432
Life table tests	P=0.339N	P=0.344N	P=0.370N
Logistic regression tests	P=0.324N	P=0.500N	P=0.454N
Cochran-Armitage test	P=0.390N		
Fisher exact test		P=0.500N	P=0.500N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Polysorbate 80
(continued)

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Not applicable; no tumors in animal group

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Polysorbate 80[a]

	0 ppm	25,000 ppm	50,000 ppm
Disposition Summary			
Animals initially in study	60	60	60
15-month interim evaluation	10	10	10
Early deaths			
Natural death	2	3	4
Moribund	25	22	21
Survivors			
Terminal sacrifice	23	25	25
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large, cecum	(50)	(48)	(49)
Inflammation, acute	1 (2%)		
Mineralization			1 (2%)
Parasite metazoan	3 (6%)	5 (10%)	2 (4%)
Ulcer		1 (2%)	
Intestine large, colon	(50)	(49)	(50)
Mineralization			1 (2%)
Parasite metazoan	5 (10%)	5 (10%)	7 (14%)
Intestine large, rectum	(50)	(50)	(48)
Parasite metazoan	5 (10%)	11 (22%)	7 (15%)
Liver	(50)	(50)	(50)
Angiectasis	3 (6%)		1 (2%)
Basophilic focus	42 (84%)	37 (74%)	43 (86%)
Developmental malformation	5 (10%)	4 (8%)	10 (20%)
Fatty change	6 (12%)	6 (12%)	8 (16%)
Granuloma	5 (10%)	11 (22%)	8 (16%)
Hematopoietic cell proliferation	4 (8%)	10 (20%)	2 (4%)
Mineralization			1 (2%)
Necrosis	2 (4%)		
Bile duct, hyperplasia	23 (46%)	24 (48%)	28 (56%)
Hepatocyte, hyperplasia	2 (4%)	1 (2%)	
Portal, inflammation, chronic	15 (30%)	21 (42%)	16 (32%)
Mesentery	(10)	(4)	(3)
Fat, inflammation, acute	1 (10%)		
Fat, necrosis	7 (70%)	4 (100%)	3 (100%)
Pancreas	(50)	(47)	(50)
Acinus, atrophy	19 (38%)	15 (32%)	20 (40%)
Acinus, hyperplasia	1 (2%)		1 (2%)
Artery, inflammation, chronic		1 (2%)	
Salivary glands	(50)	(50)	(50)
Inflammation, acute	2 (4%)		
Acinus, atrophy	1 (2%)		
Acinus, hyperplasia	1 (2%)		
Duct, ectasia	1 (2%)		
Duct, hyperplasia			1 (2%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Alimentary System (continued)			
Stomach, forestomach	(50)	(50)	(50)
Edema		1 (2%)	
Hyperkeratosis	1 (2%)	1 (2%)	
Hyperplasia, squamous		1 (2%)	
Inflammation, acute	2 (4%)		
Inflammation, chronic	1 (2%)	2 (4%)	2 (4%)
Mineralization	1 (2%)	3 (6%)	2 (4%)
Ulcer	1 (2%)	3 (6%)	
Stomach, glandular	(50)	(50)	(50)
Inflammation, acute	1 (2%)		
Inflammation, chronic active			1 (2%)
Mineralization	3 (6%)	6 (12%)	8 (16%)
Mucosa, dilatation	41 (82%)	39 (78%)	31 (62%)
Mucosa, ulcer			1 (2%)
Tooth	(1)	(1)	(1)
Peridontal tissue, hemorrhage	1 (100%)		
Cardiovascular System			
Heart	(50)	(50)	(50)
Cardiomyopathy, chronic	44 (88%)	40 (80%)	45 (90%)
Hemorrhage	1 (2%)		
Mineralization			1 (2%)
Coronary artery, mineralization	1 (2%)		1 (2%)
Endocrine System			
Adrenal gland, cortex	(50)	(50)	(50)
Accessory adrenal cortical nodule	1 (2%)		
Angiectasis	1 (2%)	4 (8%)	
Cyst	1 (2%)		
Degeneration, fatty	26 (52%)	22 (44%)	23 (46%)
Hematopoietic cell proliferation	1 (2%)		
Hemorrhage		1 (2%)	
Hyperplasia	7 (14%)	16 (32%)	9 (18%)
Hypertrophy	4 (8%)		4 (8%)
Mineralization			1 (2%)
Pigmentation	28 (56%)	31 (62%)	32 (64%)
Adrenal gland, medulla	(50)	(50)	(50)
Hematopoietic cell proliferation	1 (2%)		
Hyperplasia	8 (16%)	4 (8%)	6 (12%)
Islets, pancreatic	(50)	(47)	(50)
Hyperplasia			1 (2%)
Parathyroid gland	(41)	(41)	(41)
Hyperplasia			1 (2%)
Pituitary gland	(50)	(50)	(50)
Pars distalis, angiectasis		4 (8%)	1 (2%)
Pars distalis, cyst	10 (20%)	13 (26%)	15 (30%)
Pars distalis, hyperplasia	5 (10%)	13 (26%)	7 (14%)
Pars distalis, metaplasia, osseous	1 (2%)		
Pars intermedia, cyst	1 (2%)	1 (2%)	2 (4%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Endocrine System (continued)			
Thyroid gland	(50)	(50)	(50)
Ultimobranchial cyst		1 (2%)	2 (4%)
C-cell, hyperplasia	33 (66%)	23 (46%)	30 (60%)
Follicle, ectasia		1 (2%)	
General Body System			
None			
Genital System			
Clitoral gland	(48)	(49)	(50)
Abscess	1 (2%)	1 (2%)	1 (2%)
Cyst			1 (2%)
Inflammation, chronic	10 (21%)	14 (29%)	9 (18%)
Inflammation, chronic active	6 (13%)	4 (8%)	3 (6%)
Duct, ectasia	5 (10%)	2 (4%)	1 (2%)
Duct, hyperplasia			1 (2%)
Ovary	(50)	(50)	(50)
Hyperplasia, adenomatous			1 (2%)
Follicle, cyst			1 (2%)
Periovarian tissue, cyst	1 (2%)		
Uterus	(50)	(50)	(50)
Ectasia	3 (6%)	3 (6%)	2 (4%)
Hemorrhage			3 (6%)
Inflammation, acute			1 (2%)
Cervix, epithelium, cyst		1 (2%)	
Endometrium, hyperplasia, cystic	9 (18%)	10 (20%)	13 (26%)
Vagina	(6)	(9)	(5)
Inflammation, acute			1 (20%)
Hematopoietic System			
Bone marrow	(50)	(50)	(50)
Hemorrhage			1 (2%)
Hyperplasia, reticulum cell		1 (2%)	
Myelofibrosis	1 (2%)		1 (2%)
Lymph node	(50)	(50)	(50)
Inguinal, hyperplasia, lymphoid	1 (2%)		
Mediastinal, congestion	3 (6%)	11 (22%)	5 (10%)
Mediastinal, hyperplasia, lymphoid	4 (8%)	3 (6%)	2 (4%)
Mediastinal, hyperplasia, reticulum cell	1 (2%)	1 (2%)	
Mediastinal, pigmentation			1 (2%)
Pancreatic, congestion	1 (2%)		
Lymph node, mandibular	(50)	(50)	(50)
Congestion			2 (4%)
Cyst			1 (2%)
Hyperplasia, lymphoid	31 (62%)	23 (46%)	25 (50%)
Pigmentation		1 (2%)	

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Hematopoietic System (continued)			
Lymph node, mesenteric	(50)	(50)	(50)
Congestion	2 (4%)	2 (4%)	
Hyperplasia, lymphoid	16 (32%)	11 (22%)	12 (24%)
Hyperplasia, reticulum cell	1 (2%)	1 (2%)	
Spleen	(50)	(50)	(50)
Fibrosis		1 (2%)	
Hematopoietic cell proliferation	44 (88%)	34 (68%)	39 (78%)
Hyperplasia, histiocytic, lymphoid	1 (2%)		1 (2%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)	
Infarct			1 (2%)
Necrosis		1 (2%)	
Pigmentation	37 (74%)	35 (70%)	42 (84%)
Capsule, fibrosis	1 (2%)		
Capsule, inflammation, chronic			1 (2%)
Thymus	(47)	(46)	(47)
Congestion		1 (2%)	3 (6%)
Cyst	2 (4%)	4 (9%)	5 (11%)
Hemorrhage	1 (2%)		
Hyperplasia, lymphoid	3 (6%)	1 (2%)	5 (11%)
Integumentary System			
Mammary gland	(50)	(48)	(50)
Inflammation, acute			1 (2%)
Acinus, ectasia	45 (90%)	28 (58%)	35 (70%)
Acinus, hyperplasia	4 (8%)	3 (6%)	1 (2%)
Duct, ectasia	12 (24%)	20 (42%)	21 (42%)
Skin	(50)	(50)	(50)
Alopecia		1 (2%)	
Cyst epithelial inclusion	3 (6%)	1 (2%)	1 (2%)
Inflammation, chronic active	2 (4%)		
Inflammation, suppurative	2 (4%)		
Musculoskeletal System			
Bone	(50)	(50)	(50)
Cranium, fibrous osteodystrophy	1 (2%)		
Cranium, inflammation, chronic			1 (2%)
Cranium, osteopetrosis	5 (10%)	5 (10%)	3 (6%)
Femur, fibrous osteodystrophy	1 (2%)		
Femur, osteopetrosis	1 (2%)		
Rib, hyperplasia	1 (2%)		
Skeletal muscle	(1)	(3)	(2)
Inflammation, chronic		1 (33%)	
Nervous System			
Brain	(50)	(50)	(50)
Compression	19 (38%)	13 (26%)	8 (16%)
Hemorrhage	1 (2%)		2 (4%)
Hydrocephalus		1 (2%)	

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Respiratory System			
Lung	(50)	(50)	(50)
Congestion	1 (2%)	3 (6%)	9 (18%)
Hemorrhage	13 (26%)	5 (10%)	11 (22%)
Alveolar epithelium, hyperplasia	1 (2%)	3 (6%)	1 (2%)
Alveolus, infiltration cellular, histiocyte	28 (56%)	14 (28%)	20 (40%)
Arteriole, mineralization	27 (54%)	25 (50%)	34 (68%)
Interstitium, inflammation, chronic	1 (2%)	2 (4%)	2 (4%)
Mediastinum, inflammation, acute		1 (2%)	
Peribronchiolar, inflammation, acute			1 (2%)
Peribronchiolar, inflammation, chronic active		1 (2%)	
Nose	(50)	(50)	(50)
Lumen, hemorrhage	22 (44%)	17 (34%)	22 (44%)
Lumen, inflammation, acute		1 (2%)	
Mucosa, cyst	1 (2%)		
Mucosa, inflammation, acute	3 (6%)	4 (8%)	3 (6%)
Nasolacrimal duct, hemorrhage	1 (2%)		
Nasolacrimal duct, inflammation, acute	14 (28%)	9 (18%)	9 (18%)
Olfactory epithelium, degeneration	46 (92%)	44 (88%)	44 (88%)
Respiratory epithelium, degeneration	30 (60%)	22 (44%)	28 (56%)
Vomeronasal organ, inflammation, acute	1 (2%)		
Special Senses System			
Eye	(6)	(9)	(10)
Cataract	5 (83%)	8 (89%)	10 (100%)
Hemorrhage	1 (17%)	2 (22%)	4 (40%)
Synechia	1 (17%)		3 (30%)
Retina, degeneration	6 (100%)	9 (100%)	10 (100%)
Sclera, mineralization		1 (11%)	1 (10%)
Urinary System			
Kidney	(50)	(50)	(50)
Congestion		1 (2%)	
Cyst			3 (6%)
Inflammation, acute		1 (2%)	
Mineralization		1 (2%)	1 (2%)
Nephropathy, chronic	48 (96%)	43 (86%)	48 (96%)
Pelvis, inflammation, acute			2 (4%)
Pelvis, inflammation, chronic		1 (2%)	
Pelvis, necrosis			1 (2%)
Renal tubule, pigmentation	47 (94%)	47 (94%)	45 (90%)
Urinary bladder	(50)	(50)	(50)
Calculus gross observation		2 (4%)	
Hemorrhage			2 (4%)
Inflammation, chronic		2 (4%)	
Mineralization	1 (2%)		

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR FEED STUDY OF POLYSORBATE 80

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Polysorbate 80[a]

	0 ppm	25,000 ppm	50,000 ppm
Disposition Summary			
Animals initially in study	60	60	60
15-month interim evaluation	7	7	10
Early deaths			
Natural death	9	8	9
Moribund	9	11	9
Survivors			
Terminal sacrifice	32	34	32
Died during last week of study	1		
Missing	1		
Missexed	1		
Animals examined microscopically	49[b]	50[b]	50
Alimentary System			
Intestine large, cecum	(47)	(16)	(50)
Intestine small, duodenum	(46)	(16)	(49)
Intestine small, ileum	(46)	(15)	(48)
Intestine small, jejunum	(46)	(16)	(49)
Liver	(49)	(23)	(50)
Hemangiosarcoma		1 (4%)	
Hepatocellular carcinoma	11 (22%)	7 (30%)	5 (10%)
Hepatocellular adenoma	5 (10%)	7 (30%)	6 (12%)
Neoplasm NOS	1 (2%)		
Mesentery	(1)	(5)	(3)
Squamous cell carcinoma, metastatic, stomach		1 (20%)	
Pancreas	(48)	(17)	(50)
Salivary glands	(49)	(16)	(49)
Stomach, forestomach	(48)	(50)	(50)
Papilloma squamous	1 (2%)		
Squamous cell carcinoma		1 (2%)	
Stomach, glandular	(48)	(50)	(50)
Cardiovascular System			
Heart	(49)	(16)	(50)
Endocrine System			
Adrenal gland, cortex	(49)	(16)	(50)
Adenoma			2 (4%)
Adrenal gland, medulla	(49)	(16)	(50)
Pheochromocytoma NOS	1 (2%)	1 (6%)	2 (4%)
Islets, pancreatic	(48)	(17)	(50)
Pituitary gland	(47)	(15)	(49)
Pars intermedia, adenoma		1 (7%)	
Thyroid gland	(49)	(16)	(49)
Follicle, adenoma	1 (2%)		
General Body System			
None			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Polysorbate 80
(continued)

	0 ppm	25,000 ppm	50,000 ppm
Genital System			
Epididymis	(49)	(16)	(50)
Preputial gland	(13)	(14)	(11)
Squamous cell carcinoma		2 (14%)	
Prostate	(49)	(17)	(50)
Seminal vesicle	(49)	(16)	(49)
Testes	(49)	(17)	(50)
Interstitial cell, adenoma			1 (2%)
Hematopoietic System			
Bone marrow	(49)	(17)	(50)
Lymph node	(49)	(28)	(50)
Lymph node, mandibular	(49)	(14)	(49)
Lymph node, mesenteric	(48)	(28)	(46)
Spleen	(48)	(21)	(49)
Hemangioma			1 (2%)
Thymus	(47)	(16)	(49)
Integumentary System			
Skin	(49)	(42)	(50)
Papilloma squamous		1 (2%)	
Squamous cell carcinoma	1 (2%)	1 (2%)	
Subcutaneous tissue, fibroma	5 (10%)	5 (12%)	3 (6%)
Subcutaneous tissue, fibrosarcoma	4 (8%)	5 (12%)	5 (10%)
Subcutaneous tissue, fibrous histiocytoma	1 (2%)	3 (7%)	2 (4%)
Subcutaneous tissue, hemangioma		1 (2%)	1 (2%)
Subcutaneous tissue, neurofibrosarcoma	1 (2%)		
Subcutaneous tissue, sarcoma		1 (2%)	2 (4%)
Musculoskeletal System			
Skeletal muscle		(1)	(2)
Sarcoma			1 (50%)
Nervous System			
Brain	(49)	(16)	(50)
Respiratory System			
Lung	(49)	(21)	(50)
Alveolar/bronchiolar adenoma	5 (10%)	5 (24%)	5 (10%)
Alveolar/bronchiolar carcinoma	1 (2%)	1 (5%)	
Hepatocellular carcinoma, metastatic, liver	1 (2%)	2 (10%)	2 (4%)
Special Senses System			
Harderian gland		(3)	(1)
Adenoma		3 (100%)	1 (100%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Urinary System			
Kidney	(49)	(16)	(50)
Urinary bladder	(47)	(16)	(50)
Systemic Lesions			
Multiple organs[c]	(49)	(50)	(50)
Leukemia granulocytic			1 (2%)
Lymphoma malignant histiocytic			1 (2%)
Lymphoma malignant lymphocytic		2 (4%)	
Lymphoma malignant mixed	3 (6%)	2 (4%)	5 (10%)
Mesothelioma malignant		1 (2%)	
Tumor Summary			
Total animals with primary neoplasms[d]	32	34	31
Total primary neoplasms	41	51	44
Total animals with benign neoplasms	15	20	15
Total benign neoplasms	17	23	20
Total animals with malignant neoplasms	21	23	17
Total malignant neoplasms	22	27	22
Total animals with secondary neoplasms[e]	1	3	2
Total secondary neoplasms	1	3	2
Total animals with neoplasms uncertain-benign or malignant	2	1	2
Total uncertain neoplasms	2	1	2

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Does not include three animals that died before the interim sacrifice
[c] Number of animals with any tissue examined microscopically
[d] Primary tumors: all tumors except metastatic tumors
[e] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80: 0 ppm

Number of Days on Study	0 9 0	2 6 0	4 3 6	4 4 0	4 7 2	4 9 2	5 3 8	5 7 5	5 8 0	6 3 4	6 3 7	6 3 9	6 4 0	6 5 4	7 0 7	7 1 5	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 4 1	0 3 2	0 2 3	0 6 1	0 8 2	1 2 1	0 2 4	0 6 3	0 6 2	0 4 3	0 1 3	0 6 4	0 2 5	0 1 2	0 7 2	0 3 3	0 1 1	0 1 4	0 1 5	0 2 1	0 2 2	0 3 1	0 3 4	0 3 5	0 4 2
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+
Intestine large	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	A	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma													X		X				X			X	X	X
Hepatocellular adenoma															X									
Neoplasm NOS				X																				
Mesentery														+										
Pancreas	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous															X									
Stomach, glandular	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																+								
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma NOS																								
Islets, pancreatic	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	M	M	M	M	+	+	M	M	M	M	+	M	+	+	+	M	M	M	M
Pituitary gland	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicle, adenoma																					X			
General Body System																								
None																								

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

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80:
0 ppm (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 4 4	0 4 5	0 4 5	0 4 7 1	0 4 7 4	0 4 7 5	0 4 8 1	0 4 8 3	0 4 8 4	0 4 8 5	0 4 9 1	0 4 9 2	0 4 9 3	0 4 9 4	0 4 9 5	0 5 1 1	1 5 1 2	1 5 1 3	1 5 1 4	1 5 1 5	1 5 2 2	1 5 2 3	1 5 2 4	1 5 2 5	Total Tissues/ Tumors
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	44
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma							X				X			X				X			X				11
Hepatocellular adenoma	X		X							X													X		5
Neoplasm NOS																									1
Mesentery																									1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Papilloma squamous																									1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tooth																									1
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma NOS					X																				1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	M	M	M	+	+	+	+	+	M	M	+	M	M	M	M	+	M	M	+	+	+	+	+	25
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicle, adenoma																									1
General Body System																									
None																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80: 0 ppm (continued)

Number of Days on Study	0 9 0	2 6 0	4 3 6	4 4 0	4 7 2	4 9 2	5 3 8	5 7 5	5 8 0	6 3 4	6 3 7	6 3 9	6 4 0	6 5 4	7 0 7	7 1 5	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 4 1	0 3 2	0 2 3	0 6 1	0 8 2	1 2 1	0 2 4	0 6 3	0 6 2	0 4 3	0 1 3	0 6 4	0 2 5	0 1 2	0 7 2	0 3 3	0 1 1	0 1 4	0 1 5	0 2 1	0 2 2	0 3 1	0 3 4	0 3 5	0 4 2
Genital System																								
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis			+		+																			
Preputial gland	+		+							+	+	+	+											
Prostate	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																								
Blood									+															
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																								
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																								
Subcutaneous tissue, fibroma							X				X								X					
Subcutaneous tissue, fibrosarcoma									X		X	X				X								
Subcutaneous tissue, fibrous histiocytoma														X										
Subcutaneous tissue, neurofibrosarcoma																								
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																			X					
Alveolar/bronchiolar carcinoma																							X	
Hepatocellular carcinoma, metastatic, liver													X											
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80:
0 ppm (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 4 4	0 4 5	0 5 5	0 6 1	0 7 4	0 7 5	0 8 1	0 8 3	0 8 4	0 8 5	0 9 1	0 9 2	0 9 3	0 9 4	0 9 5	1 1 1	1 1 2	1 1 3	1 1 4	1 1 5	1 2 2	1 2 3	1 2 4	1 2 5	Total Tissues/ Tumors
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Penis																									2
Preputial gland		+			+		+			+	+						+	+							13
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hematopoietic System																									
Blood																									1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	47
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell carcinoma										X															1
Subcutaneous tissue, fibroma		X								X															5
Subcutaneous tissue, fibrosarcoma																									4
Subcutaneous tissue, fibrous histiocytoma																									1
Subcutaneous tissue, neurofibrosarcoma									X																1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma							X						X		X					X					5
Alveolar/bronchiolar carcinoma																									1
Hepatocellular carcinoma, metastatic, liver																									1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80: 0 ppm (continued)

Number of Days on Study	0 9 0	2 6 0	4 3 6	4 4 0	4 7 2	4 9 2	5 3 8	5 7 5	5 8 0	6 3 4	6 3 7	6 3 9	6 4 0	6 5 4	7 0 7	7 1 5	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 4 1	0 3 2	0 2 3	0 6 1	0 8 2	1 2 1	0 2 4	0 6 3	0 6 2	0 4 3	0 1 3	0 6 4	0 2 5	0 1 2	0 7 2	0 3 3	0 1 1	0 1 4	0 1 5	0 2 1	0 2 2	0 3 1	0 3 4	0 3 5	0 4 2
Special Senses System																									
Eye																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urethra						+														+					
Urinary bladder	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed								X																	

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80:
0 ppm (continued)

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	04404	04405	04605	04701	04704	04705	04801	04803	04804	04805	04901	04902	04903	04904	04905	04911	04912	04913	04914	04915	04922	04923	04924	04925	Total Tissues/ Tumors
Special Senses System																									
Eye																						+			1
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urethra			+	+	+							+								+		+		+	9
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant mixed												X											X		3

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm

Number of Days on Study	5 3 3	5 4 1	5 8 1	5 9 6	5 9 8	6 3 4	6 4 6	6 5 1	6 7 2	6 7 8	7 0 5	7 0 6	7 0 7	7 0 8	7 0 8	7 0 9	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	2 9 1	2 5 1	3 2 1	3 4 1	2 8 1	2 6 1	2 6 2	2 8 2	2 8 3	2 5 2	2 8 4	3 0 1	3 4 2	3 1 1	3 4 3	3 9 2	5 5 3	5 5 4	5 5 5	6 6 3	6 6 4	6 6 5	8 8 5	9 9 3	9 9 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+									
Gallbladder	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Intestine small, ileum	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+									
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+						
Hemangiosarcoma											X														
Hepatocellular carcinoma	X	X						X						X											
Hepatocellular adenoma	X		X			X										X			X						
Mesentery					+	+									+						+				
Squamous cell carcinoma, metastatic, stomach						X																			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma						X																			
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Pheochromocytoma NOS								X																	
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Parathyroid gland	+	M	M	M	+	M	+	+	+	M	M	M	M	M	+	+									
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+									
Pars intermedia, adenoma								X																	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	2 9 5	3 0 2	3 0 3	3 0 4	3 0 5	3 1 2	3 1 3	3 1 4	3 1 5	3 2 2	3 2 3	3 2 4	3 2 5	3 3 1	3 3 2	3 3 3	3 3 4	3 3 5	3 4 4	4 4 5	6 6 1	6 6 2	6 6 3	6 6 4	6 6 5	Total Tissues/ Tumors
Alimentary System																									
Esophagus																									15
Gallbladder																									15
Intestine large																									16
Intestine large, cecum																									16
Intestine large, colon																									16
Intestine large, rectum																									16
Intestine small																									16
Intestine small, duodenum																									16
Intestine small, ileum																									15
Intestine small, jejunum																									16
Liver		+			+	+		+	+										+						23
Hemangiosarcoma																									1
Hepatocellular carcinoma					X	X													X						7
Hepatocellular adenoma		X							X																7
Mesentery							+																		5
Squamous cell carcinoma, metastatic, stomach																									1
Pancreas																						+			17
Salivary glands																									16
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																									1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																									
Heart																									16
Endocrine System																									
Adrenal gland																									16
Adrenal gland, cortex																									16
Adrenal gland, medulla																									16
Pheochromocytoma NOS																									1
Islets, pancreatic																						+			17
Parathyroid gland																									7
Pituitary gland																									15
Pars intermedia, adenoma																									1
Thyroid gland									+																16

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm (continued)

Number of Days on Study	5 3 3	5 4 1	5 8 1	5 9 6	5 9 8	6 3 4	6 4 6	6 5 1	6 7 2	6 7 8	7 0 5	7 0 6	7 0 7	7 0 8	7 0 8	7 0 9	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	2 9 1	2 5 1	3 2 1	3 4 1	2 8 1	2 6 1	2 6 2	2 8 2	2 8 3	2 5 2	2 8 4	3 0 1	3 4 2	3 1 1	3 4 3	3 9 2	5 5 3	5 5 4	5 5 5	6 6 3	6 6 4	6 6 5	8 5 3	9 3 4	9 9 4
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Preputial gland	M		+					+						+										+	
Squamous cell carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+				+	
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+									
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+				+	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	
Papilloma squamous																									
Squamous cell carcinoma																									
Subcutaneous tissue, fibroma																X								X	
Subcutaneous tissue, fibrosarcoma			X						X							X									
Subcutaneous tissue, fibrous histiocytoma				X											X										
Subcutaneous tissue, hemangioma													X												
Subcutaneous tissue, sarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Skeletal muscle					+																				
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	2 9 5	3 0 2	3 0 3	3 0 4	3 0 5	3 1 2	3 1 3	3 1 4	3 1 5	3 2 2	3 2 3	3 2 4	3 2 5	3 3 1	3 3 2	3 3 3	3 3 4	3 3 5	3 4 4	3 4 5	3 6 1	3 6 2	3 6 3	3 6 4	3 6 5	Total Tissues/ Tumors
General Body System																										
None																										
Genital System																										
Epididymis																										16
Preputial gland	+	+		+					+		+		+	+				+				+			+	14
Squamous cell carcinoma	X										X															2
Prostate														+												17
Seminal vesicle																										16
Testes																						+				17
Hematopoietic System																										
Bone marrow					+																					17
Lymph node	+	+	+			+			+			+	+									+	+			28
Lymph node, mandibular																										14
Lymph node, mesenteric	+	+	+			+			+			+	+									+	+			28
Spleen			+		+						+					+	+									21
Thymus												+														16
Integumentary System																										
Mammary gland																										
Skin			+	+	+	+	+		+	+	+			+	+	+	+	+	+		+	+	+	+	+	+	42
Papilloma squamous																									X	1
Squamous cell carcinoma							X																			1
Subcutaneous tissue, fibroma									X							X							X			5
Subcutaneous tissue, fibrosarcoma					X				X																	5
Subcutaneous tissue, fibrous histiocytoma						X																				3
Subcutaneous tissue, hemangioma																										1
Subcutaneous tissue, sarcoma			X																							1
Musculoskeletal System																										
Bone																										16
Skeletal muscle																										1
Nervous System																										
Brain																										16

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm (continued)

Number of Days on Study	5 3 3	5 4 1	5 8 1	5 9 6	5 9 8	6 3 4	6 4 6	6 5 1	6 7 2	6 7 8	7 0 5	7 0 6	7 0 7	7 0 8	7 0 8	7 0 9	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	2 9 1	2 5 1	3 2 1	3 4 1	2 8 1	2 6 1	2 6 2	2 8 2	2 8 3	2 5 2	2 8 4	3 0 1	3 4 2	3 1 1	3 4 2	3 9 2	2 2 3	2 5 4	2 5 5	2 5 3	2 6 4	2 6 5	2 8 5	2 9 3	2 9 4
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+					
Alveolar/bronchiolar adenoma					X							X													
Alveolar/bronchiolar carcinoma																									
Hepatocellular carcinoma, metastatic, liver	X	X																							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Special Senses System																									
Eye																									
Harderian gland																									
Adenoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Urethra	+		+		+									+	+										
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic										X															
Lymphoma malignant mixed																									
Mesothelioma malignant					X																				

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	2 9 5	3 0 2	3 0 3	3 0 4	3 0 5	3 1 2	3 1 3	3 1 4	3 1 5	3 2 2	3 2 3	3 2 4	3 2 5	3 3 1	3 3 2	3 3 3	3 3 4	3 3 5	3 4 4	3 4 5	3 6 1	3 6 2	3 6 3	3 6 4	3 6 5	Total Tissues/ Tumors
Respiratory System																										
Lung							+										+	+						+	21
Alveolar/bronchiolar adenoma							X										X							X	5
Alveolar/bronchiolar carcinoma																		X							1
Hepatocellular carcinoma, metastatic, liver																									2
Nose																									16
Trachea																									16
Special Senses System																										
Eye			+						+																2
Harderian gland											+						+					+			3
Adenoma											X						X					X			3
Urinary System																										
Kidney																									16
Urethra																									5
Urinary bladder																									16
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic	X																									2
Lymphoma malignant mixed					X						X															2
Mesothelioma malignant																										1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm

Number of Days on Study	1 4 7	1 5 0	1 7 0	2 8 0	5 6 1	5 7 0	6 0 4	6 0 8	6 1 3	6 1 4	6 3 7	6 4 4	6 4 5	6 7 2	7 0 8	7 0 9	7 1 5	7 1 7	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1
Carcass ID Number	2 4 1	2 4 2	2 4 3	2 3 1	1 5 3	1 4 4	2 2 1	1 9 1	2 0 1	2 2 2	1 4 5	2 3 3	2 3 2	1 9 2	2 3 4	2 0 2	1 4 3	1 9 3	1 3 1	1 3 2	1 3 3	1 3 4	1 4 5	1 4 1	1 4 2
Alimentary System																								
Esophagus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma								X	X									X						X
Hepatocellular adenoma																			X		X			
Mesentery												+	+	+										
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth												+												
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma NOS					X														X					
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	+	M	M	M	M	M	M	M	+	+	M	M	M	M	M	+	+	M	+	+	M	M
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																								
None																								

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	1 5 1	1 5 2	1 5 4	1 5 5	1 7 1	1 7 2	1 7 3	1 7 4	1 7 5	1 8 1	1 8 2	1 8 3	1 8 4	1 8 5	1 9 4	1 9 5	2 0 3	2 0 4	2 0 5	2 2 3	2 2 4	2 2 5	2 3 5	2 4 4	2 4 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																	X									5
Hepatocellular adenoma									X						X	X				X						6
Mesentery																										3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma				X					X																	2
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma NOS																										2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	M	M	M	M	+	+	+	+	+	M	M	M	+	M	M	+	M	M	M	+	M	M	+	+	M	19
Pituitary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
General Body System																										
None																										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	1 4 7	1 5 0	1 7 0	2 8 0	5 6 1	5 7 0	6 0 4	6 0 8	6 1 3	6 1 4	6 3 7	6 4 4	6 4 5	6 7 2	7 0 8	7 0 9	7 1 5	7 1 7	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1
Carcass ID Number	2 4 1	2 4 2	2 4 3	2 3 1	1 5 3	1 4 4	2 2 1	1 9 1	2 0 1	2 2 2	1 4 5	2 3 3	2 3 2	1 9 2	2 3 4	2 0 2	1 4 3	1 9 3	1 3 1	1 3 2	1 3 3	1 3 4	1 3 5	1 4 1	1 4 2
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis		+	+									+													
Preputial gland												+						+		+					+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	M	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																						X			
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, fibrosarcoma					X	X								X	X				X						
Subcutaneous tissue, fibrous histiocytoma											X						X								
Subcutaneous tissue, hemangioma																					X				
Subcutaneous tissue, sarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle														+					+						
Sarcoma																			X						
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma							X															X			
Hepatocellular carcinoma, metastatic, liver																		X							X
Nose	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	1 5 1	1 5 2	1 5 4	1 5 5	1 5 7 1	1 5 7 2	1 5 7 3	1 5 7 4	1 5 7 5	1 5 8 1	1 5 8 2	1 5 8 3	1 5 8 4	1 5 8 5	1 5 9 4	1 5 9 5	2 0 0 3	2 0 0 4	2 0 0 5	2 2 3	2 2 4	2 2 5	2 3 5	2 4 4	2 4 5	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Penis																										3
Preputial gland									+		+	+	+		+				+	+						11
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma	X																									1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	49
Hemangioma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	+	3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma											X											X	X			3
Subcutaneous tissue, fibrosarcoma																										5
Subcutaneous tissue, fibrous histiocytoma																										2
Subcutaneous tissue, hemangioma																										1
Subcutaneous tissue, sarcoma				X		X																				2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Sarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma				X				X						X												5
Hepatocellular carcinoma, metastatic, liver																										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	1 4 7	1 5 0	1 7 0	2 8 0	5 6 1	5 7 0	6 0 4	6 0 8	6 1 3	6 1 4	6 3 7	6 4 4	6 4 5	6 7 2	7 0 8	7 0 9	7 1 5	7 1 7	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1
Carcass ID Number	2 4 1	2 4 2	2 4 3	2 3 1	1 5 3	1 4 4	2 2 1	1 9 1	2 0 1	2 2 2	1 4 5	2 3 3	2 3 2	1 9 2	2 3 4	2 0 2	1 4 3	1 9 3	1 3 1	1 3 2	1 3 3	1 3 4	1 3 5	1 4 1	1 4 2
Special Senses System																									
Eye																				+					
Harderian gland																			+						
Adenoma																			X						
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urethra	+					+							+	+	+	+							+	+	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia granulocytic						X																			
Lymphoma malignant histiocytic								X																	
Lymphoma malignant mixed																X		X							X

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	1 5 1	1 5 2	1 5 4	1 5 5	1 5 7 1	1 7 2	1 7 3	1 7 4	1 7 5	1 8 1	1 8 2	1 8 3	1 8 4	1 8 5	1 9 4	1 9 5	2 0 3	2 0 4	2 0 5	2 2 3	2 2 4	2 2 5	2 3 5	2 4 4	2 4 5	Total Tissues/ Tumors
Special Senses System																										
Eye												+														2
Harderian gland																										1
Adenoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urethra							+		+			+					+			+	+			+		15
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia granulocytic																										1
Lymphoma malignant histiocytic																										1
Lymphoma malignant mixed										X									X							5

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Polysorbate 80

	0 ppm	25,000 ppm	50,000 ppm
Harderian Gland: Adenoma			
Overall rates[a]	0/49 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rates[b]	0.0%	8.8%	3.1%
Terminal rates[c]	0/33 (0%)	3/34 (9%)	1/32 (3%)
First incidence (days)	–[e]	729 (T)	729 (T)
Life table tests[d]	P=0.367	P=0.126	P=0.494
Logistic regression tests[d]	P=0.367	P=0.126	P=0.494
Cochran-Armitage test[d]	P=0.384		
Fisher exact test[d]		P=0.125	P=0.505
Liver: Hepatocellular Adenoma			
Overall rates	5/49 (10%)	7/23 (30%)[f]	6/50 (12%)
Adjusted rates	14.6%		18.8%
Terminal rates	4/33 (12%)		6/32 (19%)
First incidence (days)	707		729 (T)
Life table tests			P=0.482
Logistic regression tests			P=0.509
Cochran-Armitage test			
Fisher exact test			P=0.514
Liver: Hepatocellular Carcinoma			
Overall rates	11/49 (22%)	7/23 (30%)[f]	5/50 (10%)
Adjusted rates	31.3%		13.3%
Terminal rates	9/33 (27%)		2/32 (6%)
First incidence (days)	640		608
Life table tests			P=0.096N
Logistic regression tests			P=0.075N
Cochran-Armitage test			
Fisher exact test			P=0.079N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rates	15/49 (31%)	13/23 (57%)[f]	11/50 (22%)
Adjusted rates	42.7%		30.7%
Terminal rates	13/33 (39%)		8/32 (25%)
First incidence (days)	640		608
Life table tests			P=0.264N
Logistic regression tests			P=0.208N
Cochran-Armitage test			
Fisher exact test			P=0.228N
Lung: Alveolar/bronchiolar Adenoma			
Overall rates	5/49 (10%)	5/21 (24%)[f]	5/50 (10%)
Adjusted rates	15.2%		14.5%
Terminal rates	5/33 (15%)		4/32 (13%)
First incidence (days)	729 (T)		604
Life table tests			P=0.620
Logistic regression tests			P=0.611N
Cochran-Armitage test			
Fisher exact test			P=0.617N

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rates	6/49 (12%)	6/21 (29%)[f]	5/50 (10%)
Adjusted rates	18.2%		14.5%
Terminal rates	6/33 (18%)		4/32 (13%)
First incidence (days)	729 (T)		604
Life table tests			P=0.514N
Logistic regression tests			P=0.477N
Cochran-Armitage test			
Fisher exact test			P=0.486N
Skin (Subcutaneous Tissue): Fibroma			
Overall rates	5/49 (10%)	5/50 (10%)	3/50 (6%)
Adjusted rates	13.5%	14.3%	9.4%
Terminal rates	3/33 (9%)	4/34 (12%)	3/32 (9%)
First incidence (days)	538	709	729 (T)
Life table tests	P=0.305N	P=0.598N	P=0.364N
Logistic regression tests	P=0.280N	P=0.584N	P=0.344N
Cochran-Armitage test	P=0.286N		
Fisher exact test		P=0.617N	P=0.346N
Skin (Subcutaneous Tissue): Fibrosarcoma			
Overall rates	4/49 (8%)	5/50 (10%)	5/50 (10%)
Adjusted rates	10.2%	12.6%	12.3%
Terminal rates	0/33 (0%)	2/34 (6%)	1/32 (3%)
First incidence (days)	580	581	561
Life table tests	P=0.454	P=0.558	P=0.524
Logistic regression tests	P=0.448	P=0.467	P=0.515
Cochran-Armitage test	P=0.444		
Fisher exact test		P=0.513	P=0.513
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma			
Overall rates	8/49 (16%)	8/50 (16%)	8/50 (16%)
Adjusted rates	20.2%	20.8%	20.8%
Terminal rates	3/33 (9%)	5/34 (15%)	4/32 (13%)
First incidence (days)	538	581	561
Life table tests	P=0.543N	P=0.542N	P=0.589N
Logistic regression tests	P=0.538N	P=0.601N	P=0.591N
Cochran-Armitage test	P=0.537N		
Fisher exact test		P=0.590N	P=0.590N
Skin (Subcutaneous Tissue): Fibrous Histiocytoma			
Overall rates	1/49 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rates	2.8%	7.6%	5.4%
Terminal rates	0/33 (0%)	1/34 (3%)	0/32 (0%)
First incidence (days)	654	596	637
Life table tests	P=0.411	P=0.347	P=0.506
Logistic regression tests	P=0.408	P=0.290	P=0.507
Cochran-Armitage test	P=0.407		
Fisher exact test		P=0.316	P=0.508

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Polysorbate 80
(continued)

	0 ppm	25,000 ppm	50,000 ppm
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma			
Overall rates	4/49 (8%)	6/50 (12%)	7/50 (14%)
Adjusted rates	10.2%	15.3%	18.0%
Terminal rates	0/33 (0%)	3/34 (9%)	3/32 (9%)
First incidence (days)	580	581	561
Life table tests	P=0.235	P=0.431	P=0.288
Logistic regression tests	P=0.226	P=0.349	P=0.273
Cochran-Armitage test	P=0.225		
Fisher exact test		P=0.383	P=0.274
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma or Sarcoma			
Overall rates	8/49 (16%)	9/50 (18%)	10/50 (20%)
Adjusted rates	20.2%	23.5%	26.5%
Terminal rates	3/33 (9%)	6/34 (18%)	6/32 (19%)
First incidence (days)	538	581	561
Life table tests	P=0.360	P=0.561	P=0.413
Logistic regression tests	P=0.364	P=0.519	P=0.415
Cochran-Armitage test	P=0.365		
Fisher exact test		P=0.518	P=0.416
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, or Mixed)			
Overall rates	3/49 (6%)	4/50 (8%)	6/50 (12%)
Adjusted rates	8.3%	11.0%	16.6%
Terminal rates	2/33 (6%)	3/34 (9%)	3/32 (9%)
First incidence (days)	575	678	608
Life table tests	P=0.188	P=0.534	P=0.248
Logistic regression tests	P=0.193	P=0.542	P=0.253
Cochran-Armitage test	P=0.195		
Fisher exact test		P=0.511	P=0.254
All Organs: Benign Tumors			
Overall rates	15/49 (31%)	20/50 (40%)	15/50 (30%)
Adjusted rates	41.2%	46.5%	45.0%
Terminal rates	12/33 (36%)	12/34 (35%)	14/32 (44%)
First incidence (days)	538	533	604
Life table tests	P=0.525	P=0.281	P=0.563
Logistic regression tests	P=0.506N	P=0.288	P=0.543N
Cochran-Armitage test	P=0.514N		
Fisher exact test		P=0.222	P=0.560N
All Organs: Malignant Tumors			
Overall rates	21/49 (43%)	23/50 (46%)	17/50 (34%)
Adjusted rates	51.2%	48.2%	39.9%
Terminal rates	13/33 (39%)	10/34 (29%)	7/32 (22%)
First incidence (days)	575	533	561
Life table tests	P=0.264N	P=0.546	P=0.295N
Logistic regression tests	P=0.213N	P=0.474	P=0.240N
Cochran-Armitage test	P=0.212N		
Fisher exact test		P=0.455	P=0.242N

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Polysorbate 80
(continued)

	0 ppm	25,000 ppm	50,000 ppm
All Organs: Benign or Malignant Tumors			
Overall rates	32/49 (65%)	34/50 (68%)	31/50 (62%)
Adjusted rates	74.3%	69.3%	71.8%
Terminal rates	22/33 (67%)	19/34 (56%)	20/32 (63%)
First incidence (days)	440	533	561
Life table tests	P=0.473N	P=0.551N	P=0.513N
Logistic regression tests	P=0.409N	P=0.570	P=0.445N
Cochran-Armitage test	P=0.404N		
Fisher exact test		P=0.472	P=0.447N

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Not applicable; no tumors in animal group
[f] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus statistical comparisons with the controls are not appropriate.

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polysorbate 80[a]

	0 ppm	25,000 ppm	50,000 ppm
Disposition Summary			
Animals initially in study	60	60	60
15-month interim evaluation	7	7	10
Early deaths			
Natural death	9	8	9
Moribund	9	11	9
Survivors			
Terminal sacrifice	32	34	32
Died during last week of study	1		
Missing	1		
Missexed	1		
Animals examined microscopically	49[b]	50[b]	50
Alimentary System			
Gallbladder	(44)	(15)	(49)
Ectasia		1 (7%)	
Infiltration cellular, lymphocyte	15 (34%)	2 (13%)	18 (37%)
Epithelium, degeneration			1 (2%)
Intestine large, cecum	(47)	(16)	(50)
Serosa, inflammation, chronic active			1 (2%)
Intestine small, duodenum	(46)	(16)	(49)
Serosa, inflammation, chronic active			1 (2%)
Intestine small, ileum	(46)	(15)	(48)
Inflammation, acute	1 (2%)		
Intestine small, jejunum	(46)	(16)	(49)
Hyperplasia, lymphoid			1 (2%)
Serosa, inflammation, chronic active			1 (2%)
Liver	(49)	(23)	(50)
Angiectasis			1 (2%)
Cytomegaly	1 (2%)		
Developmental malformation		1 (4%)	
Fatty change	3 (6%)		
Focal cellular change	2 (4%)	1 (4%)	2 (4%)
Hematopoietic cell proliferation	9 (18%)		7 (14%)
Infarct			1 (2%)
Infiltration cellular, lymphocyte	12 (24%)	5 (22%)	7 (14%)
Necrosis	3 (6%)	3 (13%)	4 (8%)
Vacuolation cytoplasmic	1 (2%)		
Bile duct, cyst		1 (4%)	1 (2%)
Hepatocyte, hypertrophy			1 (2%)
Portal, inflammation, chronic	2 (4%)		
Mesentery	(1)	(5)	(3)
Hemorrhage			1 (33%)
Inflammation, chronic			1 (33%)
Inflammation, chronic active			1 (33%)
Fat, necrosis	1 (100%)	3 (60%)	
Pancreas	(48)	(17)	(50)
Infiltration cellular	1 (2%)		
Infiltration cellular, lymphocyte	7 (15%)	3 (18%)	10 (20%)
Acinus, atrophy		2 (12%)	1 (2%)
Acinus, hyperplasia	1 (2%)		
Duct, cyst		1 (6%)	2 (4%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Alimentary System (continued)			
Salivary glands	(49)	(16)	(49)
Infiltration cellular, lymphocyte	38 (78%)	9 (56%)	42 (86%)
Acinus, atrophy	1 (2%)		
Stomach, forestomach	(48)	(50)	(50)
Cyst epithelial inclusion		1 (2%)	
Erosion			1 (2%)
Hyperkeratosis		1 (2%)	
Hyperplasia, squamous	3 (6%)	4 (8%)	19 (38%)
Infiltration cellular, lymphocyte	3 (6%)		6 (12%)
Inflammation, acute		3 (6%)	5 (10%)
Inflammation, chronic active		1 (2%)	7 (14%)
Mineralization	1 (2%)	2 (4%)	6 (12%)
Ulcer	1 (2%)		1 (2%)
Epithelium, hyperplasia	1 (2%)		
Stomach, glandular	(48)	(50)	(50)
Erosion			1 (2%)
Infiltration cellular, lymphocyte	13 (27%)	10 (20%)	22 (44%)
Inflammation, acute	5 (10%)	2 (4%)	2 (4%)
Inflammation, chronic active	1 (2%)		1 (2%)
Mineralization	2 (4%)	2 (4%)	9 (18%)
Mucosa, dilatation		1 (2%)	
Mucosa, ectasia	4 (8%)	1 (2%)	3 (6%)
Mucosa, erosion	3 (6%)		
Mucosa, inflammation, acute			1 (2%)
Mucosa, mineralization		1 (2%)	
Mucosa, ulcer			1 (2%)
Tooth	(1)		(1)
Pulp, inflammation, chronic			1 (100%)
Pulp, inflammation, chronic active	1 (100%)		
Cardiovascular System			
Heart	(49)	(16)	(50)
Infiltration cellular, lymphocyte	6 (12%)		7 (14%)
Inflammation, acute			1 (2%)
Inflammation, chronic			1 (2%)
Mineralization		1 (6%)	
Endocrine System			
Adrenal gland, cortex	(49)	(16)	(50)
Cyst	1 (2%)		
Hyperplasia			1 (2%)
Hypertrophy	7 (14%)		11 (22%)
Spindle cell, hyperplasia	48 (98%)	14 (88%)	46 (92%)
Spindle cell, hyperplasia, lymphoid		1 (6%)	1 (2%)
Adrenal gland, medulla	(49)	(16)	(50)
Hyperplasia	2 (4%)	1 (6%)	2 (4%)
Islets, pancreatic	(48)	(17)	(50)
Infiltration cellular, lymphocyte			1 (2%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Endocrine System (continued)			
Pituitary gland	(47)	(15)	(49)
Pars distalis, cyst			3 (6%)
Pars distalis, hyperplasia	2 (4%)		4 (8%)
Pars intermedia, cyst	1 (2%)		1 (2%)
Thyroid gland	(49)	(16)	(49)
Infiltration cellular, lymphocyte	1 (2%)	1 (6%)	4 (8%)
Ultimobranchial cyst	1 (2%)		
C-cell, hyperplasia	1 (2%)		1 (2%)
Follicle, ectasia	1 (2%)	1 (6%)	1 (2%)
Follicle, hyperplasia	4 (8%)		2 (4%)
Follicle, hyperplasia, cystic		1 (6%)	
General Body System			
None			
Genital System			
Epididymis	(49)	(16)	(50)
Infiltration cellular, lymphocyte	19 (39%)	4 (25%)	25 (50%)
Serosa, inflammation, chronic active			1 (2%)
Penis	(2)		(3)
Inflammation, acute	2 (100%)		2 (67%)
Preputial gland	(13)	(14)	(11)
Abscess	4 (31%)	5 (36%)	1 (9%)
Infiltration cellular, lymphocyte		3 (21%)	1 (9%)
Inflammation, chronic	3 (23%)	1 (7%)	4 (36%)
Inflammation, chronic active	4 (31%)	3 (21%)	5 (45%)
Duct, cyst	1 (8%)	2 (14%)	1 (9%)
Duct, ectasia			1 (9%)
Prostate	(49)	(17)	(50)
Ectasia	1 (2%)		
Infiltration cellular, lymphocyte	1 (2%)		
Inflammation, acute			1 (2%)
Inflammation, chronic	1 (2%)		
Inflammation, chronic active	1 (2%)		
Seminal vesicle	(49)	(16)	(49)
Ectasia	1 (2%)		1 (2%)
Infiltration cellular, lymphocyte	2 (4%)		4 (8%)
Inflammation, chronic			3 (6%)
Serosa, inflammation, chronic active			1 (2%)
Testes	(49)	(17)	(50)
Atrophy			2 (4%)
Granuloma sperm			1 (2%)
Mineralization	1 (2%)	3 (18%)	2 (4%)
Necrosis		1 (6%)	
Spermatogenic arrest			1 (2%)
Interstitial cell, hyperplasia	16 (33%)		9 (18%)
Seminiferous tubule, mineralization	1 (2%)		

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Hematopoietic System			
Blood	(1)		
Atypical cells	1 (100%)		
Bone marrow	(49)	(17)	(50)
Angiectasis			1 (2%)
Hyperplasia, reticulum cell			1 (2%)
Myelofibrosis	2 (4%)		
Myeloid cell, hyperplasia	35 (71%)	12 (71%)	36 (72%)
Lymph node	(49)	(28)	(50)
Iliac, hyperplasia, lymphoid			1 (2%)
Inguinal, hyperplasia, lymphoid	1 (2%)	3 (11%)	
Mediastinal, congestion		1 (4%)	
Mediastinal, hyperplasia, lymphoid		2 (7%)	1 (2%)
Renal, hyperplasia, lymphoid			1 (2%)
Lymph node, mandibula	(49)	(14)	(49)
Hyperplasia			1 (2%)
Hyperplasia, lymphoid	40 (82%)	4 (29%)	37 (76%)
Pigmentation	10 (20%)	1 (7%)	9 (18%)
Lymph node, mesenteric	(48)	(28)	(46)
Angiectasis		2 (7%)	3 (7%)
Congestion	12 (25%)	11 (39%)	10 (22%)
Hemorrhage	1 (2%)	2 (7%	
Hyperplasia	1 (2%)		
Hyperplasia, lymphoid	33 (69%)	15 (54%)	35 (76%)
Inflammation, acute	5 (10%)	1 (4%)	3 (7%)
Inflammation, chronic		1 (4%)	
Spleen	(48)	(21)	(49)
Angiectasis	1 (2%)		
Atrophy			2 (4%)
Hematopoietic cell proliferation	26 (54%)	13 (62%)	35 (71%)
Hyperplasia, glandular			1 (2%)
Hyperplasia, lymphoid	7 (15%)	2 (10%)	10 (20%)
Pigmentation	11 (23%)	2 (10%)	9 (18%)
Capsule, inflammation, chronic active			1 (2%)
Thymus	(47)	(16)	(49)
Cyst	8 (17%)	1 (6%)	9 (18%)
Hyperplasia, lymphoid		1 (6%)	
Integumentary System			
Skin	(49)	(42)	(50)
Alopecia		1 (2%)	
Angiectasis		1 (2%)	
Erosion	1 (2%)	1 (2%)	1 (2%)
Fibrosis, focal			1 (2%)
Hyperplasia		1 (2%)	
Inflammation, chronic	18 (37%)	15 (36%)	24 (48%)
Inflammation, chronic active	4 (8%)	1 (2%)	3 (6%)
Metaplasia, osseous	1 (2%)		
Ulcer	8 (16%)	6 (14%)	5 (10%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Integumentary System (continued)			
Skin (continued)			
Epithelium, hyperplasia			1 (2%)
Prepuce, inflammation, acute			1 (2%)
Prepuce, inflammation, chronic		1 (2%)	
Prepuce, ulcer			1 (2%)
Subcutaneous tissue, abscess	1 (2%)		
Subcutaneous tissue, inflammation, chronic active			1 (2%)
Musculoskeletal System			
Skeletal muscle		(1)	(2)
Abscess			1 (50%)
Nervous System			
Brain	(49)	(16)	(50)
Mineralization	40 (82%)	9 (56%)	29 (58%)
Respiratory System			
Lung	(49)	(21)	(50)
Congestion	4 (8%)	1 (5%)	4 (8%)
Hemorrhage	5 (10%)	1 (5%)	4 (8%)
Infiltration cellular, lymphocyte	42 (86%)	12 (57%)	43 (86%)
Infiltration cellular, histiocyte	1 (2%)		
Alveolar epithelium, hyperplasia	4 (8%)		2 (4%)
Alveolus, infiltration cellular, histiocyte	6 (12%)		2 (4%)
Alveolus, inflammation, acute	1 (2%)		
Bronchiole, inflammation, acute	4 (8%)		
Bronchiole, inflammation, chronic active	1 (2%)		
Interstitium, inflammation, acute	1 (2%)		
Nose	(49)	(16)	(49)
Lumen, hemorrhage	24 (49%)	5 (31%)	29 (59%)
Mucosa, inflammation, acute	1 (2%)		3 (6%)
Nasolacrimal duct, hemorrhage	3 (6%)		1 (2%)
Nasolacrimal duct, inflammation, acute	1 (2%)	4 (25%)	1 (2%)
Olfactory epithelium, degeneration	26 (53%)	6 (38%)	23 (47%)
Respiratory epithelium, degeneration	1 (2%)		
Special Senses System			
Eye	(1)	(2)	(2)
Atrophy			1 (50%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Urinary System			
Kidney	(49)	(16)	(50)
Congestion		1 (6%)	
Hemorrhage		1 (6%)	
Infiltration cellular, lymphocyte	43 (88%)	12 (75%)	46 (92%)
Inflammation, chronic		2 (13%)	
Metaplasia, osseous	3 (6%)		1 (2%)
Glomerulus, ectasia	1 (2%)		
Glomerulus, renal tubule, mineralization		1 (6%)	
Pelvis, inflammation, acute			2 (4%)
Renal tubule, casts protein	4 (8%)		4 (8%)
Renal tubule, ectasia	8 (16%)	1 (6%)	7 (14%)
Renal tubule, mineralization	12 (24%)	1 (6%)	5 (10%)
Renal tubule, necrosis	14 (29%)	1 (6%)	23 (46%)
Renal tubule, pigmentation	1 (2%)		
Renal tubule, regeneration	33 (67%)	3 (19%)	36 (72%)
Urethra	(9)	(5)	(15)
Calculus micro observation only	9 (100%)	5 (100%)	14 (93%)
Infiltration cellular, lymphocyte			1 (7%)
Urinary bladder	(47)	(16)	(50)
Ectasia		2 (13%)	
Infiltration cellular, lymphocyte	20 (43%)	4 (25%)	25 (50%)
Inflammation, chronic			2 (4%)

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Does not include three animals that died before the interim sacrifice

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR FEED STUDY OF POLYSORBATE 80

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Polysorbate 80[a]

	0 ppm	25,000 ppm	50,000 ppm
Disposition Summary			
Animals initially in study	60	60	60
15-month interim evaluation	9	10	10
Early deaths			
Natural death	13	15	15
Moribund	8	7	9
Survivors			
Terminal sacrifice	30	28	26
Animals examined microscopically	50[b]	50	50
Alimentary System			
Esophagus	(49)	(22)	(49)
Gallbladder	(46)	(18)	(47)
Intestine large, rectum	(48)	(17)	(47)
Leiomyoma			1 (2%)
Intestine small, ileum	(47)	(18)	(45)
Intestine small, jejunum	(48)	(19)	(47)
Fibrosarcoma, metastatic, mesentery			1 (2%)
Liver	(50)	(25)	(49)
Hepatocellular carcinoma	1 (2%)	1 (4%)	
Hepatocellular adenoma	2 (4%)	1 (4%)	2 (4%)
Mesentery	(10)	(5)	(13)
Fibrosarcoma			1 (8%)
Fibrosarcoma, metastatic, skin	1 (10%)		
Pancreas	(47)	(22)	(48)
Fibrosarcoma, metastatic, mesentery			1 (2%)
Salivary glands	(46)	(18)	(46)
Stomach, forestomach	(49)	(50)	(49)
Glandular, fibrosarcoma, metastatic, mesentery			1 (2%)
Stomach, glandular	(49)	(49)	(48)
Tooth			(1)
Cardiovascular System			
Heart	(49)	(22)	(50)
Endocrine System			
Adrenal gland, cortex	(50)	(22)	(50)
Adrenal gland, medulla	(50)	(22)	(50)
Pheochromocytoma NOS			2 (4%)
Islets, pancreatic	(47)	(21)	(48)
Pituitary gland	(46)	(20)	(48)
Pars distalis, adenoma	11 (24%)	2 (10%)	15 (31%)
Thyroid gland	(49)	(19)	(46)
Follicle, adenoma	1 (2%)		1 (2%)
General Body System			
None			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Polysorbate 80
(continued)

	0 ppm	25,000 ppm	50,000 ppm
Genital System			
Ovary	(48)	(36)	(50)
Adenoma	1 (2%)		
Granulosa cell tumor benign		1 (3%)	
Luteoma			1 (2%)
Uterus	(50)	(42)	(50)
Leiomyoma			1 (2%)
Endometrium, polyp stromal	3 (6%)		
Endometrium, sarcoma stromal	1 (2%)	1 (2%)	
Hematopoietic System			
Bone marrow	(50)	(22)	(50)
Hemangiosarcoma, metastatic, spleen			1 (2%)
Lymph node	(49)	(26)	(49)
Lymph node, mandibular	(46)	(16)	(46)
Lymph node, mesenteric	(48)	(23)	(48)
Spleen	(49)	(25)	(49)
Hemangiosarcoma		1 (4%)	1 (2%)
Thymus	(48)	(20)	(48)
Integumentary System			
Mammary gland	(49)	(19)	(48)
Adenocarcinoma			1 (2%)
Skin	(50)	(38)	(50)
Sebaceous gland, carcinoma			1 (2%)
Subcutaneous tissue, fibrosarcoma	4 (8%)		2 (4%)
Subcutaneous tissue, hemangioma			1 (2%)
Musculoskeletal System			
Bone	(50)	(22)	(50)
Sternum, osteosarcoma	1 (2%)		
Skeletal muscle			(2)
Hemangiosarcoma, metastatic, spleen			1 (50%)
Nervous System			
Brain	(50)	(22)	(50)
Spinal cord	(1)		(1)
Respiratory System			
Lung	(50)	(22)	(50)
Alveolar/bronchiolar adenoma	3 (6%)	1 (5%)	2 (4%)
Alveolar/bronchiolar carcinoma		1 (5%)	1 (2%)
Nose	(49)	(22)	(50)
Trachea	(49)	(21)	(47)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Special Senses System			
Eye		(1)	(1)
Harderian gland			(1)
Urinary System			
Kidney	(50)	(23)	(50)
Urinary bladder	(49)	(18)	(48)
Hemangioma	1 (2%)		
Systemic Lesions			
Multiple organs[c]	(50)	(50)	(50)
Lymphoma malignant lymphocytic	5 (10%)	3 (6%)	3 (6%)
Lymphoma malignant mixed	12 (24%)	6 (12%)	8 (16%)
Lymphoma malignant undifferentiated cell	1 (2%)		
Tumor Summary			
Total animals with primary neoplasms[d]	30	16	29
Total primary neoplasms	47	18	44
Total animals with benign neoplasms	17	5	19
Total benign neoplasms	22	5	24
Total animals with malignant neoplasms	22	11	17
Total malignant neoplasms	25	13	18
Total animals with secondary neoplasms[e]	1		2
Total secondary neoplasms	1		5
Total animals with neoplasms uncertain-benign or malignant			2
Total uncertain neoplasms			2

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Does not include one animal that died before the interim sacrifice
[c] Number of animals with any tissue examined microscopically
[d] Primary tumors: all tumors except metastatic tumors
[e] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80: 0 ppm

Number of Days on Study	3 9 1	5 5 1	5 5 1	5 8 1	5 8 2	5 8 2	6 0 1	6 1 1	6 3 1	6 3 2	6 3 2	6 5 2	6 8 3	6 9 3	7 0 3	7 0 4	7 0 2	7 0 1	7 0 2	7 0 4	7 2 3	7 2 4	7 2 5	7 2 2	7 2 3
Carcass ID Number	4 2 1	4 5 1	4 4 1	3 8 1	4 4 2	4 5 2	4 0 1	3 9 1	3 7 1	3 7 2	4 2 2	4 2 3	3 9 2	3 9 3	4 4 3	4 2 4	4 0 2	4 8 1	4 8 2	4 4 4	3 7 3	3 7 4	3 7 5	4 8 2	3 8 3
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Intestine large	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Hepatocellular adenoma																									
Mesentery		+	+		+				+	+	+				+			+						+	
Fibrosarcoma, metastatic, skin											X														
Pancreas	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	M	M	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	M	M	+	+	M	M	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																				X					
Thyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicle, adenoma																									
General Body System																									
None																									

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

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80:
0 ppm (continued)

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	38 4	38 5	39 4	39 5	40 3	40 4	40 5	42 5	43 1	43 2	43 3	43 4	43 5	44 5	45 3	45 4	45 5	47 1	47 2	47 3	47 4	47 5	48 3	48 4	48 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma								X																		1
Hepatocellular adenoma	X																	X								2
Mesentery							+																			10
Fibrosarcoma, metastatic, skin																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	47
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	47
Parathyroid gland	+	M	+	M	+	M	+	M	M	M	M	M	M	+	M	M	M	+	M	M	+	+	M	M	M	26
Pituitary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	M	+	+	+	+	46
Pars distalis, adenoma	X	X		X				X		X			X					X				X		X	X	11
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicle, adenoma																						X				1
General Body System																										
None																										

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80: 0 ppm (continued)

Number of Days on Study	3	5	5	5	5	5	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	
	9	5	5	8	8	8	0	1	3	3	3	5	8	9	0	0	0	0	0	0	2	2	2	2	2
	1	7	9	0	6	7	9	4	6	6	7	3	3	6	0	6	7	7	7	8	9	9	9	9	9
Carcass ID Number	4	5	4	4	3	4	4	4	3	3	3	4	4	3	3	4	4	4	4	4	4	3	3	3	3
	1	1	4	8	4	5	0	9	7	7	2	2	9	9	4	2	0	8	8	4	7	7	7	8	8
	1	1	1	1	2	2	1	1	1	2	2	3	2	3	3	4	2	1	2	4	3	4	5	2	3
Genital System																									
Ovary	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma														X											
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal																									
Endometrium, sarcoma stromal																			X						
Hematopoietic System																									
Blood												+													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	M	M	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma											X	X	X							X					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sternum, osteosarcoma							X																		
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord							+																		
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Nose	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																+									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80:
0 ppm (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	3 8 4	3 8 5	3 9 4	3 9 5	4 0 3	4 0 4	4 0 5	4 2 5	4 3 1	4 3 2	4 3 3	4 3 4	4 3 5	4 4 5	4 5 3	4 5 4	4 5 5	4 7 1	4 7 2	4 7 3	4 7 4	4 7 5	4 8 3	4 8 4	4 8 5	Total Tissues/ Tumors
Genital System																										
Ovary	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal			X																		X				X	3
Endometrium, sarcoma stromal																										1
Hematopoietic System																										
Blood																										1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										4
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sternum, osteosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma					X										X		X									3
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Ear																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80: 0 ppm (continued)

Number of Days on Study	3	5	5	5	5	5	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7
	9	5	5	8	8	8	0	1	3	3	3	5	8	9	0	0	0	0	0	0	2	2	2	2	2
	1	7	9	0	6	7	9	4	6	6	7	3	3	6	0	6	7	7	7	8	9	9	9	9	9
Carcass ID Number	4	4	4	3	4	4	4	3	3	3	4	4	3	3	4	4	4	4	4	4	3	3	3	3	3
	2	5	4	8	4	5	0	9	7	7	2	2	9	9	4	2	0	8	8	4	7	7	7	8	8
	1	1	1	1	2	2	1	1	1	2	2	3	2	3	3	4	2	1	2	4	3	4	5	2	3
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																			X			X			
Lymphoma malignant mixed												X		X	X					X					
Lymphoma malignant undifferentiated cell type																	X								

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80:
0 ppm (continued)

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	38 4	38 5	39 4	39 5	40 3	40 4	40 5	42 5	43 1	43 2	43 3	43 4	43 5	44 5	45 3	45 4	45 5	47 1	47 2	47 3	47 4	47 5	48 3	48 4	48 5	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangioma										X																1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic		X					X			X																5
Lymphoma malignant mixed	X		X	X		X					X	X					X		X							12
Lymphoma malignant undifferentiated cell type																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm

Number of Days on Study	1 1 2 4 4 4 4 5 5 5 5 6 6 6 6 6 6 7 7 7 7 7 7 7 1 9 4 3 5 9 9 2 8 8 8 1 2 3 3 3 5 7 0 0 0 0 3 3 3 4 2 7 7 8 8 9 3 5 8 8 0 8 7 8 9 1 2 3 6 7 7 4 4 4
Carcass ID Number	6 7 6 7 6 6 6 6 6 6 6 6 6 6 6 7 6 6 6 7 6 7 6 6 6 6 0 7 2 5 6 4 4 2 5 9 1 9 8 8 2 2 1 2 0 7 2 1 1 1 1 1 1 1 1 2 1 2 1 2 1 1 2 1 2 2 2 2 3 2 2 3 3 4 5
Alimentary System	
Esophagus	+ +
Gallbladder	M + M + + + + + + M M + + + + + + + + + + +
Intestine large	M A + M A + + + + + + + + + + + + + + + + +
Intestine large, cecum	M A + M A + + + + + + + + + + + + + + + + +
Intestine large, colon	M A + M A + + + + + + + + + + + + + + + + +
Intestine large, rectum	M A + M A + + + + + + + + M + + + + + + + +
Intestine small	M A + + A + + + + + + + + + + + + + + + + +
Intestine small, duodenum	M A + + A + + + + + + + + + + + + + + + + +
Intestine small, ileum	M A + M A + + + + + + + + + + + + + + + + +
Intestine small, jejunum	M A + M A + + + + + + + + + + + + + + + + +
Liver	+ +
Hepatocellular carcinoma	
Hepatocellular adenoma	X
Mesentery	+ + + + + + +
Pancreas	+ A + + A + + + + + + + + + + + + + + + + +
Salivary glands	M + + M M + M + + + + + + + + + + + + + + +
Stomach	+ +
Stomach, forestomach	+ +
Stomach, glandular	+ + + + A + + + + + + + + + + + + + + + + + +
Cardiovascular System	
Heart	+ +
Endocrine System	
Adrenal gland	+ +
Adrenal gland, cortex	+ +
Adrenal gland, medulla	+ +
Islets, pancreatic	+ A + + A + + + + + + + + + + + + + + + + +
Parathyroid gland	+ + + M M + M + M M M + M M M M M M + + + M
Pituitary gland	M + + M M + + M + + + + + + + + + + + + + +
Pars distalis, adenoma	
Thyroid gland	+ + + M M + M + + + + + + + + + + + + + + +
General Body System	
None	
Genital System	
Ovary	+ +
Granulosa cell tumor benign	X
Uterus	+ +
Endometrium, sarcoma stromal	
Vagina	+ + + + +

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	6 2 4	6 2 5	6 4 3	6 4 4	6 4 5	6 5 3	6 5 4	6 5 5	6 6 3	6 6 4	6 6 5	6 7 3	6 7 4	6 7 5	6 8 3	6 8 4	6 8 5	6 9 3	6 9 4	6 9 5	7 0 3	7 0 4	7 0 5	7 2 4	7 2 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus																										22
Gallbladder																										18
Intestine large																										18
Intestine large, cecum																										18
Intestine large, colon																										18
Intestine large, rectum																										17
Intestine small									+																	20
Intestine small, duodenum																										19
Intestine small, ileum																										18
Intestine small, jejunum									+																	19
Liver		+	+															+								25
Hepatocellular carcinoma		X																								1
Hepatocellular adenoma																										1
Mesentery																										5
Pancreas						+														+						22
Salivary glands																										18
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Heart																										22
Endocrine System																										
Adrenal gland																										22
Adrenal gland, cortex																										22
Adrenal gland, medulla																										22
Islets, pancreatic																				+						21
Parathyroid gland																										9
Pituitary gland								+																+		20
Pars distalis, adenoma								X																X		2
Thyroid gland																										19
General Body System																										
None																										
Genital System																										
Ovary		+		+	+	+			+		+				+	+			+	+		+			+	+	36
Granulosa cell tumor benign																										1
Uterus	+		+	+		+	+					+	+	+	+	+	+	+	+	+	+	+	+	+			42
Endometrium, sarcoma stromal			X																								1
Vagina																											4

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80: 25,000 ppm (continued)

Number of Days on Study	1 1 2 4 4 4 4 5 5 5 5 6 6 6 6 6 6 6 7 7 7 7 7 7 7 1 9 4 3 5 9 9 2 8 8 8 1 2 3 3 3 5 7 0 0 0 0 3 3 3 4 2 7 7 8 8 9 3 5 8 8 0 8 7 8 9 1 2 3 6 7 7 4 4 4
Carcass ID Number	6 7 6 7 6 6 6 6 6 6 6 6 6 6 6 7 6 6 6 7 6 7 6 6 6 6 0 7 2 5 6 4 4 2 5 9 1 9 8 8 2 2 1 2 0 7 2 1 1 1 1 1 1 1 1 2 1 2 1 2 1 1 2 1 2 2 2 2 3 2 2 3 3 4 5
Hematopoietic System	
Bone marrow	+ +
Lymph node	+ + + + A + + + + + + + + + + + + + + + + + +
Lymph node, mandibular	M M M M M + M + + + + + + + + + + + + + + + +
Lymph node, mesenteric	+ + + M A + + + + + + + + + + + + + + + + + +
Spleen	M + + + A + + + + + + + + + + + + + + + + + +
Hemangiosarcoma	X
Thymus	+ + + M + + M + + + + + + + + + + + + + + + +
Integumentary System	
Mammary gland	M M M + M + + + + + + + + + + + + + + + + + + +
Skin	+ +
Musculoskeletal System	
Bone	+ +
Nervous System	
Brain	+ +
Respiratory System	
Lung	+ +
Alveolar/bronchiolar adenoma	X
Alveolar/bronchiolar carcinoma	X
Nose	+ +
Trachea	+ + + M + + + + + + + + + + + + + + + + + + +
Special Senses System	
Eye	+
Urinary System	
Kidney	+ +
Urinary bladder	+ M + M + + + + + M + + + + + + + + + + M +
Systemic Lesions	
Multiple organs	+ +
Lymphoma malignant lymphocytic	X X
Lymphoma malignant mixed	X X

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80:
25,000 ppm (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	6 2 4	6 2 5	6 4 3	6 4 4	6 4 5	6 5 3	6 5 4	6 5 5	6 6 3	6 6 4	6 6 5	6 7 3	6 7 4	6 7 5	6 8 3	6 8 4	6 8 5	6 9 3	6 9 4	6 9 5	7 0 3	7 0 4	7 0 5	7 2 4	7 2 5	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow											+				+											22
Lymph node															+		+	+		+						26
Lymph node, mandibular																										16
Lymph node, mesenteric															+		+	+								23
Spleen		+					+		+		+				+											25
Hemangiosarcoma																										1
Thymus																										20
Integumentary System																										
Mammary gland																										19
Skin		+		+		+	+	+			+	+		+	+			+	+				+	+	+	38
Musculoskeletal System																										
Bone																										22
Nervous System																										
Brain																										22
Respiratory System																										
Lung																										22
Alveolar/bronchiolar adenoma																										1
Alveolar/bronchiolar carcinoma																										1
Nose																										22
Trachea																										21
Special Senses System																										
Eye																										1
Urinary System																										
Kidney											+															23
Urinary bladder																										18
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic											X															3
Lymphoma malignant mixed		X	X						X									X								6

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm

Number of Days on Study	3 6 1	3 7 8	4 0 9	4 3 8	4 4 2	4 4 7	4 6 9	4 7 4	4 7 7	5 1 4	5 4 8	5 7 7	5 8 1	5 8 4	6 2 4	6 2 9	6 4 7	6 8 7	6 9 3	6 9 4	7 0 2	7 0 2	7 1 7	7 1 7	7 3 0
Carcass ID Number	5 3 1	5 3 2	5 9 1	5 1 1	5 5 1	5 8 1	5 1 2	5 4 1	5 4 2	5 9 2	5 0 1	5 0 2	4 9 1	5 3 3	5 1 3	5 9 3	5 8 3	6 0 2	6 6 1	5 3 4	5 0 3	5 1 4	5 5 2	5 6 2	4 9 2
Alimentary System																									
Esophagus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	M	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Intestine large, cecum	+	A	+	M	+	+	+	M	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Intestine large, colon	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Intestine large, rectum	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Leiomyoma																									
Intestine small	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Intestine small, duodenum	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Intestine small, ileum	+	A	+	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	M	+	+	A	+	+
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Fibrosarcoma, metastatic, mesentery						X																			
Liver	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma													X												
Mesentery			+	+	+	+		+	+	+		+			+				+	+		+			
Fibrosarcoma						X																			
Pancreas	+	M	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery						X																			
Salivary glands	+	M	M	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Glandular, fibrosarcoma, metastatic, mesentery						X																			
Stomach, glandular	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Tooth																+									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma NOS																									
Islets, pancreatic	+	M	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	M	+	+	+	+	+	M	M	+	M	M	M	M	+	M	+	M	+	M	+	+	+	+
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Pars distalis, adenoma													X			X	X						X	X	
Thyroid gland	+	M	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicle, adenoma																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	734	734	734	734	
Carcass ID Number	49 3	49 4	49 5	50 4	50 5	51 5	53 5	54 3	54 4	54 5	55 3	55 4	55 5	56 3	56 4	56 5	58 3	58 4	58 5	59 4	59 5	60 2	60 3	60 4	60 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	44
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	46
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Leiomyoma															X											1
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Fibrosarcoma, metastatic, mesentery																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma				X																						2
Mesentery							+																			13
Fibrosarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibrosarcoma, metastatic, mesentery																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Glandular, fibrosarcoma, metastatic, mesentery																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tooth																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma NOS													X												X	2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	M	+	M	+	M	+	M	+	M	M	M	+	+	+	+	+	M	M	+	M	M	M	+	+	27
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma				X				X		X					X		X	X	X	X			X		X	15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	46
Follicle, adenoma												X														1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	3 6 1	3 7 8	4 0 9	4 3 8	4 4 2	4 4 7	4 6 9	4 7 4	4 7 7	5 1 4	5 4 8	5 7 7	5 8 1	5 8 4	6 2 4	6 2 9	6 4 7	6 8 7	6 9 3	6 9 4	7 0 2	7 0 2	7 1 7	7 1 7	7 3 0
Carcass ID Number	5 3 1	5 3 2	5 9 1	5 1 1	5 5 1	5 8 1	5 1 2	5 4 1	5 4 2	5 9 2	5 0 1	5 0 2	5 9 1	5 3 3	5 1 3	5 9 3	5 8 2	6 0 1	5 6 1	5 3 4	5 0 3	5 1 4	5 5 2	5 6 2	4 9 2
General Body System																									
None																									
Genital System																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Luteoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																									
Vagina	+							+	+	+			+												
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	X	+	+	+	+	+
Hemangiosarcoma, metastatic, spleen																				X					
Lymph node	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	M	M	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Spleen	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																				X					
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sebaceous gland, carcinoma																									
Subcutaneous tissue, fibrosarcoma																									
Subcutaneous tissue, hemangioma								X																	
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																			+	+					
Hemangiosarcoma, metastatic, spleen																				X					

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	734	734	734	734	734	
Carcass ID Number	49 3	49 4	49 5	50 4	50 5	51 5	53 5	53 4	54 4	54 5	54 5	55 3	55 4	55 5	56 3	66 4	66 5	68 3	68 4	68 5	68 5	69 4	69 5	70 2	70 3	70 4	70 5	Total Tissues/ Tumors
General Body System																												
None																												
Genital System																												
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Luteoma		X																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyoma			X																									1
Vagina																												5
Hematopoietic System																												
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, spleen																												1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																												1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																												
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenocarcinoma																												1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sebaceous gland, carcinoma																			X									1
Subcutaneous tissue, fibrosarcoma												X	X															2
Subcutaneous tissue, hemangioma																												1
Musculoskeletal System																												
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																												2
Hemangiosarcoma, metastatic, spleen																												1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	3 6 1	3 7 1	4 0 1	4 3 1	4 4 1	4 4 1	4 6 1	4 7 1	4 7 1	5 1 1	5 4 1	5 7 1	5 8 1	5 8 1	6 2 1	6 2 1	6 4 1	6 8 1	6 9 1	6 9 1	7 0 1	7 0 1	7 1 1	7 1 1	7 3 1
Carcass ID Number	5 3 1	5 3 2	5 9 1	5 1 1	5 5 1	5 8 1	5 1 2	5 4 1	5 4 2	5 9 2	5 0 1	5 0 2	4 9 1	5 3 3	5 1 3	5 9 3	5 8 2	6 0 1	5 6 1	5 3 4	5 0 3	5 1 4	5 5 2	5 6 2	4 9 2
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord																+									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	M	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye			+																						
Harderian gland			+																						
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic			X													X				X					
Lymphoma malignant mixed																			X		X			X	

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Polysorbate 80: 50,000 ppm (continued)

Number of Days on Study	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	49 3	49 4	49 5	50 4	50 5	51 5	53 5	54 3	54 4	54 5	55 3	55 4	55 5	56 3	66 4	66 5	68 3	68 4	68 5	69 4	69 5	60 2	60 3	60 4	60 5	Total Tissues/ Tumors
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma				X																X						2
Alveolar/bronchiolar carcinoma								X																		1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Special Senses System																										
Eye																										1
Harderian gland																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic																										3
Lymphoma malignant mixed			X						X						X	X							X			8

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Polysorbate 80

	0 ppm	25,000 ppm	50,000 ppm
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rates[a]	3/50 (6%)	2/25 (8%)[e]	2/49 (4%)
Adjusted rates[b]	10.0%		6.4%
Terminal rates[c]	3/30 (10%)		1/26 (4%)
First incidence (days)	729 (T)		581
Life table tests[d]			P=0.569N
Logistic regression tests[d]			P=0.555N
Fisher exact test[d]			P=0.510N
Lung: Alveolar/bronchiolar Adenoma			
Overall rates	3/50 (6%)	1/22 (5%)[e]	2/50 (4%)
Adjusted rates	10.0%		7.7%
Terminal rates	3/30 (10%)		2/26 (8%)
First incidence (days)	729 (T)		729 (T)
Life table tests			P=0.566N
Logistic regression tests			P=0.566N
Fisher exact test			P=0.500N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rates	3/50 (6%)	2/22 (9%)[e]	3/50 (6%)
Adjusted rates	10.0%		11.5%
Terminal rates	3/30 (10%)		3/26 (12%)
First incidence (days)	729 (T)		729 (T)
Life table tests			P=0.597
Logistic regression tests			P=0.597
Fisher exact test			P=0.661N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	11/46 (24%)	2/20 (10%)[e]	15/48 (31%)
Adjusted rates	39.1%		47.5%
Terminal rates	10/27 (37%)		10/26 (38%)
First incidence (days)	708		581
Life table tests			P=0.192
Logistic regression tests			P=0.151
Fisher exact test			P=0.287
Skin (Subcutaneous Tissue): Fibrosarcoma			
Overall rates	4/50 (8%)	0/50 (0%)	2/50 (4%)
Adjusted rates	10.5%	0.0%	7.7%
Terminal rates	0/30 (0%)	0/28 (0%)	2/26 (8%)
First incidence (days)	637	–[f]	729 (T)
Life table tests	P=0.273N	P=0.085N	P=0.407N
Logistic regression tests	P=0.249N	P=0.060N	P=0.374N
Cochran-Armitage test[d]	P=0.222N		
Fisher exact test		P=0.059N	P=0.339N
Uterus: Stromal Polyp			
Overall rates	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rates	10.0%	0.0%	0.0%
Terminal rates	3/30 (10%)	0/28 (0%)	0/26 (0%)
First incidence (days)	729 (T)	–	–
Life table tests	P=0.046N	P=0.132N	P=0.146N
Logistic regression tests	P=0.046N	P=0.132N	P=0.146N
Cochran-Armitage test	P=0.037N		
Fisher exact test		P=0.121N	P=0.121N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Uterus: Stromal Polyp or Stromal Sarcoma			
Overall rates	4/50 (8%)	1/50 (2%)	0/50 (0%)
Adjusted rates	12.6%	3.6%	0.0%
Terminal rates	3/30 (10%)	1/28 (4%)	0/26 (0%)
First incidence (days)	707	729 (T)	-
Life table tests	P=0.036N	P=0.205N	P=0.086N
Logistic regression tests	P=0.035N	P=0.211N	P=0.081N
Cochran-Armitage test	P=0.026N		
Fisher exact test		P=0.181N	P=0.059N
All Organs: Malignant Lymphoma (Lymphocytic, Mixed, or Undifferentiated Cell Type)			
Overall rates	18/50 (36%)	9/50 (18%)	11/50 (22%)
Adjusted rates	49.6%	26.6%	32.9%
Terminal rates	12/30 (40%)	5/28 (18%)	5/26 (19%)
First incidence (days)	653	585	409
Life table tests	P=0.162N	P=0.073N	P=0.213N
Logistic regression tests	P=0.125N	P=0.065N	P=0.177N
Cochran-Armitage test	P=0.068N		
Fisher exact test		P=0.035N	P=0.093N
All Organs: Benign Tumors			
Overall rates	17/50 (34%)	5/50 (10%)	19/50 (38%)
Adjusted rates	52.9%	15.8%	58.4%
Terminal rates	15/30 (50%)	3/28 (11%)	13/26 (50%)
First incidence (days)	696	588	474
Life table tests	P=0.218	P=0.006N	P=0.225
Logistic regression tests	P=0.207	P=0.007N	P=0.204
Cochran-Armitage test	P=0.368		
Fisher exact test		P=0.004N	P=0.418
All Organs: Malignant Tumors			
Overall rates	22/50 (44%)	11/50 (22%)	17/50 (34%)
Adjusted rates	55.9%	30.4%	50.0%
Terminal rates	13/30 (43%)	5/28 (18%)	10/26 (38%)
First incidence (days)	609	499	409
Life table tests	P=0.352N	P=0.053N	P=0.415N
Logistic regression tests	P=0.261N	P=0.029N	P=0.347N
Cochran-Armitage test	P=0.170N		
Fisher exact test		P=0.016N	P=0.206N
All Organs: Benign or Malignant Tumors			
Overall rates	30/50 (60%)	16/50 (32%)	29/50 (58%)
Adjusted rates	76.6%	42.9%	77.8%
Terminal rates	21/30 (70%)	8/28 (29%)	18/26 (69%)
First incidence (days)	609	499	409
Life table tests	P=0.349	P=0.025N	P=0.346
Logistic regression tests	P=0.399	P=0.012N	P=0.353
Cochran-Armitage test	P=0.460N		
Fisher exact test		P=0.004N	P=0.500N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Polysorbate 80 (continued)

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus statistical comparisons with the controls are not appropriate.
[f] Not applicable; no tumors in animal group

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polysorbate 80[a]

	0 ppm	25,000 ppm	50,000 ppm
Disposition Summary			
Animals initially in study	60	60	60
15-month interim evaluation	9	10	10
Early deaths			
Natural death	13	15	15
Moribund	8	7	9
Survivors			
Terminal sacrifice	30	28	26
Animals examined microscopically	50[b]	50	50
Alimentary System			
Gallbladder	(46)	(18)	(47)
Infiltration cellular, lymphocyte	20 (43%)	2 (11%)	17 (36%)
Intestine large, cecum	(48)	(18)	(44)
Inflammation, chronic active	1 (2%)		
Intestine small, duodenum	(48)	(19)	(47)
Serosa, inflammation, chronic active	1 (2%)		
Intestine small, ileum	(47)	(18)	(45)
Serosa, inflammation, chronic active	1 (2%)		
Intestine small, jejunum	(48)	(19)	(47)
Serosa, inflammation, chronic active	1 (2%)		
Liver	(50)	(25)	(49)
Cytomegaly			1 (2%)
Fatty change	2 (4%)		
Focal cellular change	1 (2%)		1 (2%)
Hematopoietic cell proliferation	20 (40%)	6 (24%)	21 (43%)
Infiltration cellular, lymphocyte	23 (46%)	4 (16%)	23 (47%)
Inflammation, acute	3 (6%)	1 (4%)	5 (10%)
Necrosis	2 (4%)	3 (12%)	2 (4%)
Vacuolation cytoplasmic	2 (4%)		
Hepatocyte, hyperplasia	1 (2%)		
Serosa, inflammation, acute			1 (2%)
Serosa, inflammation, chronic	1 (2%)		
Mesentery	(10)	(5)	(13)
Cyst		1 (20%)	
Inflammation, acute	4 (40%)	3 (60%)	8 (62%)
Inflammation, chronic active	2 (20%)		1 (8%)
Fat, necrosis	2 (20%)		
Pancreas	(47)	(22)	(48)
Abscess	1 (2%)		
Infiltration cellular, lymphocyte	21 (45%)	1 (5%)	15 (31%)
Inflammation, acute			2 (4%)
Inflammation, chronic			1 (2%)
Inflammation, chronic active	1 (2%)	1 (5%)	1 (2%)
Acinus, atrophy	2 (4%)	1 (5%)	
Duct, cyst			2 (4%)
Duct, ectasia	2 (4%)		
Salivary glands	(46)	(18)	(46)
Infiltration cellular, lymphocyte	40 (87%)	12 (67%)	31 (67%)
Acinus, atrophy			1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Alimentary System (continued)			
Stomach, forestomach	(49)	(50)	(49)
Cyst epithelial inclusion		1 (2%)	
Erosion		1 (2%)	1 (2%)
Hyperplasia, squamous	4 (8%)	8 (16%)	26 (53%)
Infiltration cellular, lymphocyte	1 (2%)		6 (12%)
Inflammation, acute	2 (4%)	4 (8%)	3 (6%)
Inflammation, chronic active	2 (4%)		13 (27%)
Inflammation, subacute			1 (2%)
Mineralization	2 (4%)		
Ulcer	1 (2%)		7 (14%)
Stomach, glandular	(49)	(49)	(48)
Infiltration cellular, lymphocyte	15 (31%)	9 (18%)	13 (27%)
Inflammation, acute			1 (2%)
In flammation, chronic active	1 (2%)		3 (6%)
Mineralization	3 (6%)	2 (4%)	
Mucosa, ectasia	1 (2%)		
Mucosa, inflammation, acute			1 (2%)
Cardiovascular System			
Heart	(49)	(22)	(50)
Embolus bacterial			1 (2%)
Infiltration cellular, lymphocyte	9 (18%)	3 (14%)	8 (16%)
Inflammation, acute	3 (6%)		3 (6%)
Inflammation, chronic			1 (2%)
Inflammation, chronic active	1 (2%)		
Epicardium, inflammation, acute			2 (4%)
Ventricle, thrombus	1 (2%)		
Endocrine System			
Adrenal gland	(50)	(22)	(50)
Capsule, inflammation, acute			1 (2%)
Capsule, inflammation, chronic active	1 (2%)		
Adrenal gland, cortex	(50)	(22)	(50)
Congestion	1 (2%)		2 (4%)
Cyst	1 (2%)		
Degeneration, fatty	3 (6%)		4 (8%)
Hyperplasia	2 (4%)		
Inflammation, acute	2 (4%)		1 (2%)
Pigmentation	35 (70%)	5 (23%)	32 (64%)
Spindle cell, hyperplasia	50 (100%)	22 (100%)	49 (98%)
Adrenal gland, medulla	(50)	(22)	(50)
Hyperplasia	2 (4%)		2 (4%)
Islets, pancreatic	(47)	(21)	(48)
Infiltration cellular, lymphocyte	1 (2%)		
Inflammation, chronic			1 (2%)
Parathyroid gland	(26)	(9)	(27)
Infiltration cellular, lymphocyte	1 (4%)		

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Endocrine System (continued)			
Pituitary gland	(46)	(20)	(48)
Congestion	1 (2%)		
Pars distalis, angiectasis	1 (2%)	2 (10%)	
Pars distalis, cyst	4 (9%)		1 (2%)
Pars distalis, hyperplasia	8 (17%)	3 (15%)	8 (17%)
Thyroid gland	(49)	(19)	(46)
Infiltration cellular, lymphocyte	8 (16%)		5 (11%)
Inflammation, chronic			1 (2%)
Follicle, ectasia	8 (16%)	2 (11%)	5 (11%)
Follicle, hyperplasia	3 (6%)	1 (5%)	2 (4%)
Follicular cell, ectasia	2 (4%)		
General Body System			
None			
Genital System			
Ovary	(48)	(36)	(50)
Abscess	9 (19%)	13 (36%)	16 (32%)
Angiectasis	1 (2%)		
Hemorrhage	2 (4%)		1 (2%)
Mineralization	1 (2%)		1 (2%)
Follicle, cyst	3 (6%)	1 (3%)	3 (6%)
Periovarian tissue, cyst	5 (10%)	13 (36%)	8 (16%)
Uterus	(50)	(42)	(50)
Abscess	2 (4%)	3 (7%)	1 (2%)
Angiectasis	1 (2%)		1 (2%)
Hydrometra	1 (2%)	1 (2%)	1 (2%)
Endometrium, abscess			1 (2%)
Endometrium, angiectasis		1 (2%)	
Endometrium, hyperplasia, cystic	44 (88%)	37 (88%)	45 (90%)
Endometrium, inflammation, acute	9 (18%)	10 (24%)	17 (34%)
Endometrium, metaplasia, squamous	1 (2%)	1 (2%)	2 (4%)
Hematopoietic System			
Bone marrow	(50)	(22)	(50)
Angiectasis	1 (2%)		
Infiltration cellular, lymphocyte	1 (2%)		
Myelofibrosis	21 (42%)	1 (5%)	13 (26%)
Myeloid cell, hyperplasia	34 (68%)	14 (64%)	38 (76%)
Lymph node	(49)	(26)	(49)
Iliac, hyperplasia, lymphoid	2 (4%)	1 (4%)	
Mediastinal, hyperplasia, lymphoid	3 (6%)	5 (19%)	6 (12%)
Mediastinal, inflammation, acute		1 (4%)	2 (4%)
Pancreatic, hyperplasia, lymphoid		1 (4%)	
Renal, hyperplasia, lymphoid	2 (4%)	6 (23%)	6 (12%)
Renal, inflammation, acute	1 (2%)	2 (8%)	1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Hematopoietic System (continued)			
Lymph node, mandibular	(46)	(16)	(46)
Congestion			1 (2%)
Hyperplasia, lymphoid	39 (85%)	9 (56%)	39 (85%)
Inflammation, acute			1 (2%)
Pigmentation	24 (52%)	1 (6%)	22 (48%)
Lymph node, mesenteric	(48)	(23)	(48)
Angiectasis	1 (2%)		
Congestion	4 (8%)	2 (9%)	1 (2%)
Hyperplasia, lymphoid	24 (50%)	9 (39%)	32 (67%)
Inflammation, acute	4 (8%)		3 (6%)
Pigmentation	1 (2%)		1 (2%)
Spleen	(49)	(25)	(49)
Hematopoietic cell proliferation	46 (94%)	18 (72%)	44 (90%)
Hyperplasia, lymphoid	19 (39%)	5 (20%)	11 (22%)
Inflammation, acute	4 (8%)	1 (4%)	3 (6%)
Pigmentation	28 (57%)	4 (16%)	26 (53%)
Capsule, inflammation, chronic	1 (2%)	1 (4%)	
Thymus	(48)	(20)	(48)
Cyst	6 (13%)		7 (15%)
Edema	1 (2%)		
Hyperplasia, lymphoid	18 (38%)	6 (30%)	12 (25%)
Inflammation, acute	2 (4%)		1 (2%)
Integumentary System			
Mammary gland	(49)	(19)	(48)
Acinus, ectasia	9 (18%)	1 (5%)	13 (27%)
Duct, ectasia	1 (2%)	5 (26%)	4 (8%)
Skin	(50)	(38)	(50)
Inflammation, chronic	18 (36%)	6 (16%)	15 (30%)
Inflammation, chronic active	1 (2%)		1 (2%)
Inflammation, multifocal	1 (2%)		
Head, ulcer		1 (3%)	
Musculoskeletal System			
Bone	(50)	(22)	(50)
Cranium, inflammation, chronic			2 (4%)
Nervous System			
Brain	(50)	(22)	(50)
Compression			1 (2%)
Gliosis			1 (2%)
Hemorrhage			1 (2%)
Hydrocephalus		1 (5%)	1 (2%)
Infiltration cellular, lymphocyte			1 (2%)
Mineralization	26 (52%)	9 (41%)	27 (54%)
Meninges, hemorrhage			1 (2%)
Meninges, inflammation, chronic			1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polysorbate 80 (continued)

	0 ppm	25,000 ppm	50,000 ppm
Respiratory System			
Lung	(50)	(22)	(50)
Abscess	1 (2%)		
Congestion	2 (4%)	4 (18%)	4 (8%)
Hemorrhage	2 (4%)	1 (5%)	3 (6%)
Infiltration cellular, lymphocyte	48 (96%)	18 (82%)	42 (84%)
Alveolar epithelium, hyperplasia	2 (4%)		1 (2%)
Alveolus, infiltration cellular, histiocyte	4 (8%)	2 (9%)	2 (4%)
Arteriole, inflammation, chronic active	1 (2%)		
Bronchiole, inflammation, acute			1 (2%)
Interstitium, inflammation, acute	2 (4%)	2 (9%)	5 (10%)
Interstitium, inflammation, chronic			1 (2%)
Interstitium, inflammation, chronic active			1 (2%)
Mediastinum, atypical cells	1 (2%)		
Mediastinum, inflammation, acute	1 (2%)	1 (5%)	3 (6%)
Peribronchial, inflammation, acute		1 (5%)	1 (2%)
Pleura, inflammation, acute		1 (5%)	1 (2%)
Nose	(49)	(22)	(50)
Lumen, hemorrhage	20 (41%)	5 (23%)	13 (26%)
Mucosa, inflammation, acute	1 (2%)		
Nasolacrimal duct, hemorrhage	7 (14%)	1 (5%)	11 (22%)
Nasolacrimal duct, inflammation, acute			1 (2%)
Olfactory epithelium, degeneration	48 (98%)	19 (86%)	49 (98%)
Respiratory epithelium, degeneration	2 (4%)		
Special Senses System			
None			
Urinary System			
Kidney	(50)	(23)	(50)
Amyloid deposition		1 (4%)	
Infiltration cellular, lymphocyte	43 (86%)	14 (61%)	40 (80%)
Inflammation, acute	1 (2%)	2 (9%)	2 (4%)
Inflammation, chronic	1 (2%)		
Inflammation, chronic active	1 (2%)	1 (4%)	
Metaplasia, osseous	3 (6%)		1 (2%)
Mineralization			1 (2%)
Capsule, inflammation, chronic active	1 (2%)		
Renal tubule, casts protein	12 (24%)	1 (4%)	11 (22%)
Renal tubule, ectasia	20 (40%)	2 (9%)	15 (30%)
Renal tubule, mineralization	2 (4%)	1 (4%)	
Renal tubule, necrosis	16 (32%)		11 (22%)
Renal tubule, regeneration	15 (30%)		11 (22%)
Urinary bladder	(49)	(18)	(48)
Infiltration cellular, lymphocyte	32 (65%)	11 (61%)	35 (73%)
Inflammation, acute	1 (2%)		
Serosa, inflammation, chronic active	1 (2%)		

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Does not include one animal that died before the interim sacrifice

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA PROTOCOL

Testing was performed as reported by Mortelmans *et al.* (1986). Polysorbate 80 was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains (TA100, TA1535, TA1537, TA98) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C prior to the addition of soft agar supplemented with *l*-histidine and *d*-biotin and subsequent plating on minimal glucose agar plates. Incubation continued for an additional 48 hours.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of polysorbate 80. The high dose was limited to 10 mg per plate. All assays were repeated; all positive assays were repeated under the conditions that elicited the positive response.

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

RESULTS

Polysorbate 80 (100-10,000 μg/plate) was tested for gene mutation in four strains of *Salmonella typhimurium* (TA100, TA1535, TA1537, and TA98) in a preincubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9; no induction of mutations was observed (Table E1; Mortelmans *et al.*, 1986).

TABLE E1
Mutagenicity of Polysorbate 80 in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	137 ± 1.9	185 ± 4.7	132 ± 6.4	196 ± 2.3	124 ± 9.2	186 ± 9.2
	100	125 ± 9.0	172 ± 2.2	134 ± 13.3	183 ± 11.3	127 ± 3.6	188 ± 10.7
	333	137 ± 3.2	182 ± 3.5	122 ± 9.5	168 ± 6.9	121 ± 11.6	187 ± 3.2
	1,000	122 ± 8.5	197 ± 11.5	127 ± 3.8	189 ± 1.5	128 ± 13.6	187 ± 9.8
	3,333	116 ± 8.1	172 ± 3.2	110 ± 12.9	161 ± 3.5	121 ± 7.8	171 ± 4.9
	10,000	117 ± 4.8	184 ± 6.1	116 ± 8.0	163 ± 8.7	104 ± 6.9	160 ± 13.6
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		2,054 ± 41.6	788 ± 36.9	2,318 ± 96.3	1,140 ± 44.1	1,263 ± 44.1	907 ± 11.2
TA1535	0	25 ± 0.9	26 ± 3.3	10 ± 2.2	12 ± 0.7	10 ± 0.9	12 ± 1.9
	100	28 ± 3.7	24 ± 3.2	11 ± 1.5	12 ± 1.7	13 ± 2.9	10 ± 3.9
	333	20 ± 3.3	28 ± 4.1	10 ± 1.2	16 ± 2.7	12 ± 0.6	11 ± 0.9
	1,000	22 ± 1.8	21 ± 3.2	11 ± 1.2	13 ± 0.9	10 ± 1.8	11 ± 1.0
	3,333	20 ± 3.2	24 ± 1.7	11 ± 0.7	16 ± 1.2	9 ± 0.3	10 ± 2.2
	10,000	18 ± 3.8	21 ± 2.3	9 ± 1.3	8 ± 2.0	11 ± 0.9	8 ± 1.8
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,427 ± 31.1	661 ± 14.0	166 ± 20.9	63 ± 8.5	104 ± 9.3	48 ± 6.2
TA1537	0	7 ± 1.2	7 ± 1.0	9 ± 0.6	10 ± 1.0	7 ± 1.5	7 ± 0.0
	100	6 ± 0.6	8 ± 0.9	11 ± 3.0	5 ± 1.5	5 ± 1.5	8 ± 0.9
	333	7 ± 0.9	9 ± 0.9	7 ± 0.9	5 ± 0.0	8 ± 1.5	9 ± 1.0
	1,000	8 ± 2.0	9 ± 1.2	10 ± 2.0	5 ± 1.3	6 ± 1.5	8 ± 2.3
	3,333	7 ± 0.7	4 ± 0.9	8 ± 1.0	8 ± 1.8	10 ± 2.5	6 ± 1.5
	10,000	8 ± 1.0	9 ± 2.0	6 ± 0.9	9 ± 0.3	8 ± 1.2	5 ± 1.7
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		770 ± 65.4	258 ± 9.0	271 ± 13.8	72 ± 6.3	104 ± 10.0	48 ± 4.3

TABLE E1
Mutagenicity of Polysorbate 80 in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate[b] -S9 Trial 1	-S9 Trial 2	-S9 Trial 3	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2
TA98	0	17 ± 2.0	11 ± 2.3	19 ± 1.5	28 ± 3.8	27 ± 5.0
	100	20 ± 2.3	15 ± 3.5	17 ± 1.2	33 ± 4.2	26 ± 3.5
	333	24 ± 1.5	20 ± 2.4	21 ± 3.5	29 ± 1.9	36 ± 1.9
	1,000	23 ± 1.5	25 ± 1.0	21 ± 2.7	28 ± 3.6	32 ± 3.8
	3,333	20 ± 2.3	26 ± 2.8	15 ± 1.2	26 ± 2.6	25 ± 1.7
	10,000	18 ± 1.0	19 ± 2.8	19 ± 2.6	27 ± 4.0	32 ± 3.7
Trial summary		Negative	Weakly Positive	Negative	Negative	Negative
Positive control		2,047 ± 45.8	1,211 ± 54.8	1,250 ± 13.7	1,859 ± 72.6	1,129 ± 91.5

TA98 (continued)

Dose (μg/plate)	+10% rat S9 Trial 1	+10% rat S9 Trial 2
0	25 ± 1.2	23 ± 2.9
100	27 ± 4.1	23 ± 2.9
333	26 ± 1.2	29 ± 3.1
1,000	27 ± 1.2	27 ± 1.7
3,333	35 ± 0.9	28 ± 1.0
10,000	23 ± 3.5	22 ± 0.6
Trial summary	Negative	Negative
Positive control	833 ± 32.4	663 ± 48.5

[a] Study performed at EG&G Mason Research Institute. The detailed protocol and these data are presented in Mortelmans *et al.* (1986). Cells and polysorbate 80 or solvent (distilled water) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. High dose was limited by toxicity or solubility, but did not exceed 10 mg/plate; 0 μg/plate dose is the solvent control.

[b] Revertants are presented as mean ± standard error from three plates.

[c] 2-aminoanthracene was used on 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 TA1537.

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

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Day Feed Studies of Polysorbate 80[a]

	0 ppm	3,000 ppm	6,000 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
n	5	5	5	5	5	5
Necropsy body wt	231 ± 5	224 ± 6	224 ± 7	220 ± 4	226 ± 4	202 ± 2**
Brain						
Absolute	1.83 ± 0.04	1.80 ± 0.05	1.73 ± 0.03	1.74 ± 0.03	1.67 ± 0.07*	1.72 ± 0.02*
Relative	7.94 ± 0.24	8.09 ± 0.36	7.75 ± 0.12	7.94 ± 0.16	7.41 ± 0.34	8.54 ± 0.12
Heart						
Absolute	0.79 ± 0.02	0.80 ± 0.05	0.76 ± 0.02	0.75 ± 0.02	0.78 ± 0.03	0.76 ± 0.01
Relative	3.41 ± 0.10	3.59 ± 0.18	3.38 ± 0.08	3.41 ± 0.08	3.43 ± 0.07	3.74 ± 0.06*
R. Kidney						
Absolute	1.08 ± 0.05	1.07 ± 0.04	1.04 ± 0.04	0.99 ± 0.03	1.05 ± 0.03	1.02 ± 0.01
Relative	4.68 ± 0.17	4.78 ± 0.08	4.64 ± 0.08	4.49 ± 0.12	4.67 ± 0.12	5.06 ± 0.04
Liver						
Absolute	11.31 ± 0.49	11.20 ± 0.42	10.66 ± 0.38	9.71 ± 0.24*	9.79 ± 0.63*	9.98 ± 0.28*
Relative	49.0 ± 1.4	50.1 ± 1.7	47.7 ± 1.2	44.2 ± 0.6	43.4 ± 2.7	49.4 ± 1.1
Lungs						
Absolute	1.15 ± 0.03	1.17 ± 0.05	1.13 ± 0.03	1.09 ± 0.03	1.19 ± 0.09	1.04 ± 0.06
Relative	4.97 ± 0.12	5.24 ± 0.14	5.07 ± 0.11	4.96 ± 0.13	5.29 ± 0.42	5.13 ± 0.31
Thymus						
Absolute	0.41 ± 0.02	0.47 ± 0.03	0.49 ± 0.03	0.39 ± 0.02	0.53 ± 0.05**	0.53 ± 0.02**
Relative	1.78 ± 0.10	2.11 ± 0.14	2.22 ± 0.19	1.78 ± 0.10	2.35 ± 0.19*	2.61 ± 0.09**
Female						
n	5	5	5	5	5	5
Necropsy body wt	151 ± 2	155 ± 2	154 ± 2	156 ± 3	164 ± 4**	145 ± 3
Brain						
Absolute	1.68 ± 0.01	1.70 ± 0.01	1.63 ± 0.03	1.67 ± 0.02	1.65 ± 0.02	1.59 ± 0.06
Relative	11.1 ± 0.1	11.0 ± 0.2	10.6 ± 0.1	10.7 ± 0.2	10.0 ± 0.1	11.0 ± 0.5
Heart						
Absolute	0.50 ± 0.06	0.54 ± 0.01	0.57 ± 0.03	0.56 ± 0.01	0.57 ± 0.02	0.53 ± 0.02
Relative	3.31 ± 0.40	3.49 ± 0.07	3.73 ± 0.18	3.62 ± 0.08	3.47 ± 0.10	3.68 ± 0.13
R. Kidney						
Absolute	0.71 ± 0.02	0.78 ± 0.04	0.73 ± 0.01	0.75 ± 0.02	0.74 ± 0.02	0.73 ± 0.03
Relative	4.69 ± 0.06	5.06 ± 0.27	4.76 ± 0.06	4.80 ± 0.05	4.51 ± 0.14	5.04 ± 0.11
Liver						
Absolute	6.26 ± 0.11	6.22 ± 0.07	6.12 ± 0.16	6.43 ± 0.20	6.31 ± 0.66	6.38 ± 0.11
Relative	41.4 ± 0.9	40.2 ± 0.4	39.9 ± 0.8	41.3 ± 0.7	38.8 ± 4.3	44.2 ± 0.9
Lungs						
Absolute	0.89 ± 0.03	0.86 ± 0.03	0.88 ± 0.07	1.01 ± 0.05	0.92 ± 0.05	0.85 ± 0.04
Relative	5.86 ± 0.18	5.53 ± 0.14	5.71 ± 0.37	6.52 ± 0.30	5.63 ± 0.28	5.90 ± 0.22
Thymus						
Absolute	0.35 ± 0.03	0.35 ± 0.01	0.35 ± 0.01	0.33 ± 0.05	0.44 ± 0.03	0.40 ± 0.04
Relative	2.31 ± 0.18	2.24 ± 0.09	2.31 ± 0.05	2.14 ± 0.34	2.68 ± 0.14	2.76 ± 0.27

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[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 error).

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Feed Studies of Polysorbate 80[a]

	0 ppm	3,100 ppm	6,200 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	351 ± 8	367 ± 6	352 ± 3	355 ± 4	348 ± 6	350 ± 5
Brain						
Absolute	1.92 ± 0.02	1.93 ± 0.02	1.91 ± 0.02	1.94 ± 0.02	1.92 ± 0.04	1.88 ± 0.03
Relative	5.49 ± 0.10	5.27 ± 0.10	5.45 ± 0.06	5.47 ± 0.06	5.51 ± 0.08	5.36 ± 0.07
Heart						
Absolute	0.93 ± 0.03	0.98 ± 0.03	0.97 ± 0.02	0.91 ± 0.02	1.02 ± 0.04	0.94 ± 0.03
Relative	2.66 ± 0.08	2.67 ± 0.06	2.77 ± 0.06	2.57 ± 0.04	2.94 ± 0.11	2.69 ± 0.05
R. Kidney						
Absolute	1.26 ± 0.04	1.23 ± 0.03	1.29 ± 0.02	1.21 ± 0.02	1.30 ± 0.05	1.32 ± 0.02
Relative	3.58 ± 0.08	3.35 ± 0.08	3.66 ± 0.05	3.42 ± 0.05	3.72 ± 0.10	3.77 ± 0.05
Liver						
Absolute	13.71 ± 0.35	13.11 ± 0.33	14.00 ± 0.28	13.55 ± 0.29	15.30 ± 0.64	14.74 ± 0.48
Relative	39.1 ± 0.9	35.7 ± 0.6	39.8 ± 0.7	38.2 ± 0.5	44.0 ± 1.5*	42.1 ± 1.3*
Lungs						
Absolute	1.33 ± 0.02	1.43 ± 0.04*	1.53 ± 0.04**	1.43 ± 0.03**	1.43 ± 0.05*	1.49 ± 0.06*
Relative	3.82 ± 0.12	3.91 ± 0.11	4.36 ± 0.12**	4.03 ± 0.08*	4.12 ± 0.12	4.27 ± 0.18
R. Testis						
Absolute	1.43 ± 0.04	1.49 ± 0.03	1.42 ± 0.03	1.46 ± 0.02	1.40 ± 0.03	1.43 ± 0.05
Relative	4.06 ± 0.05	4.07 ± 0.05	4.04 ± 0.06	4.12 ± 0.09	4.04 ± 0.04	4.09 ± 0.13
Thymus						
Absolute	0.41 ± 0.02	0.38 ± 0.02	0.37 ± 0.02	0.34 ± 0.01**	0.43 ± 0.03	0.34 ± 0.03*
Relative	1.16 ± 0.05	1.02 ± 0.05	1.06 ± 0.06	0.95 ± 0.03*	1.23 ± 0.08	0.97 ± 0.08
Female						
n	10	10	10	10	10	10
Necropsy body wt	206 ± 3	210 ± 3	210 ± 3	201 ± 2	207 ± 2	205 ± 4
Brain						
Absolute	1.81 ± 0.02	1.81 ± 0.02	1.75 ± 0.02	1.74 ± 0.02	1.75 ± 0.02	1.82 ± 0.04
Relative	8.78 ± 0.13	8.64 ± 0.13	8.34 ± 0.09	8.66 ± 0.13	8.46 ± 0.07	8.93 ± 0.28
Heart						
Absolute	0.59 ± 0.02	0.62 ± 0.01	0.60 ± 0.02	0.59 ± 0.01	0.61 ± 0.02	0.63 ± 0.01
Relative	2.86 ± 0.05	2.94 ± 0.03	2.84 ± 0.06	2.91 ± 0.04	2.94 ± 0.09	3.08 ± 0.03**
R. Kidney						
Absolute	0.71 ± 0.03	0.75 ± 0.03	0.71 ± 0.01	0.71 ± 0.01	0.73 ± 0.01	0.76 ± 0.02
Relative	3.43 ± 0.14	3.56 ± 0.09	3.36 ± 0.05	3.55 ± 0.06	3.52 ± 0.05	3.73 ± 0.06
Liver						
Absolute	6.76 ± 0.22	7.22 ± 0.21	6.57 ± 0.18	6.33 ± 0.14	6.82 ± 0.23	7.41 ± 0.26
Relative	32.8 ± 0.7	34.4 ± 0.8	31.3 ± 0.7	31.5 ± 0.9	32.9 ± 1.0	36.1 ± 0.7
Lungs						
Absolute	0.95 ± 0.04	1.02 ± 0.02	1.05 ± 0.03	0.98 ± 0.02	1.00 ± 0.03	1.05 ± 0.03
Relative	4.63 ± 0.16	4.86 ± 0.05	5.00 ± 0.12	4.86 ± 0.09	4.80 ± 0.14	5.11 ± 0.14
Thymus						
Absolute	0.30 ± 0.02	0.30 ± 0.01	0.28 ± 0.02	0.26 ± 0.01	0.28 ± 0.01	0.30 ± 0.02
Relative	1.47 ± 0.07	1.42 ± 0.05	1.33 ± 0.06	1.31 ± 0.04	1.36 ± 0.06	1.48 ± 0.07

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Organ weights and body weights are given in grams; organ-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 15-Month Interim Evaluation in the 2-Year Feed Studies of Polysorbate 80[a]

	0 ppm	25,000 ppm	50,000 ppm
Male			
n	10	10	10
Necropsy body wt	444 ± 10	462 ± 10	460 ± 12
Brain			
Absolute	2.12 ± 0.03	2.14 ± 0.02	2.09 ± 0.03
Relative	4.79 ± 0.11	4.64 ± 0.09	4.56 ± 0.10
R. Kidney			
Absolute	1.70 ± 0.04	1.78 ± 0.04	1.66 ± 0.04
Relative	3.84 ± 0.09	3.86 ± 0.07	3.60 ± 0.06
Liver			
Absolute	17.02 ± 0.53	17.44 ± 0.40	16.85 ± 0.39
Relative	38.4 ± 0.9	37.8 ± 0.7	36.7 ± 0.6
Female			
n	10	10	10
Necropsy body wt	311 ± 8	324 ± 7	306 ± 9
Brain			
Absolute	1.92 ± 0.02	1.93 ± 0.03	1.91 ± 0.01
Relative	6.20 ± 0.12	5.97 ± 0.12	6.29 ± 0.21
R. Kidney			
Absolute	1.06 ± 0.02	1.05 ± 0.03	1.00 ± 0.03
Relative	3.41 ± 0.08	3.26 ± 0.07	3.30 ± 0.09
Liver			
Absolute	10.36 ± 0.24	10.59 ± 0.30	10.06 ± 0.22
Relative	33.4 ± 0.3	32.7 ± 0.6	33.0 ± 0.4

[a] Organ weights and body weights are given in grams; organ-wieght-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).

TABLE F4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Day Feed Studies of Polysorbate 80[a]

	0 ppm	3,000 ppm	6,000 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
n	5	5	5	5	5	5
Necropsy body wt	26.6 ± 1.3	26.4 ± 1.1	26.6 ± 1.3	26.4 ± 0.7	23.8 ± 1.7	28.0 ± 0.9
Brain						
Absolute	0.445 ± 0.012	0.441 ± 0.017	0.418 ± 0.024	0.452 ± 0.007	0.443 ± 0.010	0.425 ± 0.016
Relative	16.8 ± 0.5	16.8 ± 0.9	16.0 ± 1.4	17.1 ± 0.3	19.0 ± 1.4	15.2 ± 0.7
Heart						
Absolute	0.138 ± 0.005	0.134 ± 0.007	0.132 ± 0.006	0.129 ± 0.002	0.127 ± 0.008	0.138 ± 0.005
Relative	5.22 ± 0.27	5.08 ± 0.19	4.97 ± 0.07	4.89 ± 0.09	5.37 ± 0.20	4.93 ± 0.07
R. Kidney						
Absolute	0.250 ± 0.020	0.234 ± 0.014	0.233 ± 0.013	0.241 ± 0.012	0.235 ± 0.024	0.257 ± 0.016
Relative	9.34 ± 0.32	8.85 ± 0.35	8.76 ± 0.20	9.13 ± 0.37	9.80 ± 0.46	9.15 ± 0.33
Liver						
Absolute	1.38 ± 0.09	1.38 ± 0.03	1.11 ± 0.06	1.25 ± 0.06	1.21 ± 0.14	1.24 ± 0.10
Relative	51.8 ± 1.0	52.4 ± 2.4	41.9 ± 2.7	47.2 ± 1.4	50.1 ± 3.1	44.1 ± 2.5*
Lungs						
Absolute	0.180 ± 0.007	0.167 ± 0.006	0.222 ± 0.038	0.200 ± 0.011	0.231 ± 0.038	0.148 ± 0.008
Relative	6.79 ± 0.18	6.39 ± 0.45	8.65 ± 1.94	7.61 ± 0.52	10.52 ± 2.87	5.27 ± 0.14
Thymus						
Absolute	0.047 ± 0.011	0.050 ± 0.005	0.047 ± 0.007	0.051 ± 0.006	0.042 ± 0.011	0.046 ± 0.004
Relative	1.85 ± 0.53	1.92 ± 0.22	1.74 ± 0.19	1.93 ± 0.20	1.66 ± 0.42	1.65 ± 0.17
Female						
n	5	5	5	5	5	5
Necropsy body wt	19.8 ± 0.2	21.2 ± 0.6	20.2 ± 0.6	21.8 ± 0.8	21.0 ± 0.6	20.2 ± 0.4
Brain						
Absolute	0.443 ± 0.007	0.439 ± 0.011	0.442 ± 0.010	0.440 ± 0.012	0.459 ± 0.007	0.434 ± 0.026
Relative	22.4 ± 0.2	20.7 ± 0.6	21.9 ± 0.5	20.3 ± 1.0	21.9 ± 0.8	21.4 ± 1.0
Heart						
Absolute	0.100 ± 0.005	0.125 ± 0.007*	0.099 ± 0.003	0.108 ± 0.005	0.114 ± 0.008	0.103 ± 0.008
Relative	5.06 ± 0.29	5.91 ± 0.31	4.90 ± 0.06	4.95 ± 0.08	5.46 ± 0.45	5.08 ± 0.34
R. Kidney						
Absolute	0.159 ± 0.002	0.167 ± 0.006	0.154 ± 0.006	0.172 ± 0.010	0.180 ± 0.013	0.168 ± 0.009
Relative	8.03 ± 0.06	7.87 ± 0.14	7.62 ± 0.21	7.88 ± 0.26	8.64 ± 0.79	8.32 ± 0.41
Liver						
Absolute	1.05 ± 0.03	1.13 ± 0.04	0.92 ± 0.05	1.09 ± 0.05	1.11 ± 0.05	0.99 ± 0.08
Relative	53.2 ± 1.5	53.2 ± 0.8	45.5 ± 1.1	50.1 ± 0.8	53.1 ± 3.3	48.9 ± 3.8[b]
Lungs						
Absolute	0.176 ± 0.006	0.156 ± 0.005	0.148 ± 0.007	0.179 ± 0.007	0.187 ± 0.019	0.188 ± 0.023
Relative	8.88 ± 0.26	7.39 ± 0.37	7.34 ± 0.34	8.23 ± 0.31	8.99 ± 1.04	9.28 ± 1.05
Thymus						
Absolute	0.062 ± 0.006	0.066 ± 0.007	0.061 ± 0.003	0.057 ± 0.005	0.069 ± 0.006	0.065 ± 0.013
Relative	3.14 ± 0.32	3.09 ± 0.29	3.02 ± 0.11	2.63 ± 0.29	3.28 ± 0.26	3.17 ± 0.60[b]

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
[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 error).
[b] n=4

TABLE F5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Feed Studies of Polysorbate 80[a]

	0 ppm	3,100 ppm	6,200 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	33.6 ± 0.9	33.7 ± 0.8	34.8 ± 0.8	34.8 ± 1.1	33.1 ± 0.8	33.9 ± 0.7
Brain						
Absolute	0.454 ± 0.008	0.451 ± 0.005	0.444 ± 0.009	0.464 ± 0.009	0.451 ± 0.006	0.446 ± 0.006
Relative	13.6 ± 0.5	13.4 ± 0.3	12.8 ± 0.3	13.4 ± 0.3	13.7 ± 0.3	13.2 ± 0.3
Heart						
Absolute	0.164 ± 0.007	0.157 ± 0.006	0.160 ± 0.006	0.172 ± 0.011	0.163 ± 0.007	0.155 ± 0.006
Relative	4.89 ± 0.19	4.66 ± 0.11	4.60 ± 0.16	4.92 ± 0.22	4.91 ± 0.11	4.57 ± 0.14
R. Kidney						
Absolute	0.287 ± 0.012	0.312 ± 0.013	0.303 ± 0.011	0.293 ± 0.009	0.303 ± 0.015	0.305 ± 0.010
Relative	8.59 ± 0.41	9.24 ± 0.23	8.73 ± 0.33	8.45 ± 0.24	9.17 ± 0.40	9.02 ± 0.29
Liver						
Absolute	1.44 ± 0.07	1.57 ± 0.08	1.66 ± 0.07	1.41 ± 0.06	1.63 ± 0.08	1.51 ± 0.08
Relative	42.7 ± 1.7	46.6 ± 1.9	47.7 ± 1.5	40.5 ± 1.5	49.0 ± 1.4	44.7 ± 2.4
Lungs						
Absolute	0.188 ± 0.014	0.178 ± 0.009	0.187 ± 0.010	0.213 ± 0.015	0.171 ± 0.008	0.202 ± 0.011
Relative	5.59 ± 0.35	5.28 ± 0.24	5.37 ± 0.27	6.09 ± 0.35	5.17 ± 0.20	5.98 ± 0.35
R. Testis						
Absolute	0.120 ± 0.004	0.115 ± 0.003	0.111 ± 0.003	0.116 ± 0.003	0.112 ± 0.003	0.106 ± 0.003**
Relative	3.58 ± 0.13	3.41 ± 0.05	3.20 ± 0.11*	3.36 ± 0.12	3.39 ± 0.09	3.14 ± 0.12**
Thymus						
Absolute	0.047 ± 0.005	0.040 ± 0.004	0.054 ± 0.004	0.047 ± 0.005	0.056 ± 0.004	0.065 ± 0.011
Relative	1.42 ± 0.16	1.19 ± 0.12	1.55 ± 0.11	1.36 ± 0.13	1.69 ± 0.12	1.94 ± 0.35
Female						
n	10	10	10	10	10	10
Necropsy body wt	25.1 ± 0.6	26.4 ± 0.4	25.3 ± 0.6	24.6 ± 0.6	27.2 ± 0.6*	24.7 ± 0.3
Brain						
Absolute	0.463 ± 0.006	0.469 ± 0.006	0.469 ± 0.010	0.454 ± 0.009	0.483 ± 0.007	0.465 ± 0.008
Relative	18.5 ± 0.5	17.8 ± 0.2	18.6 ± 0.2	18.5 ± 0.5	17.8 ± 0.3	18.9 ± 0.4
Heart						
Absolute	0.116 ± 0.003	0.118 ± 0.004	0.121 ± 0.005	0.112 ± 0.004	0.124 ± 0.005	0.120 ± 0.005
Relative	4.64 ± 0.15	4.47 ± 0.15	4.78 ± 0.12	4.56 ± 0.15	4.57 ± 0.19	4.86 ± 0.18
R. Kidney						
Absolute	0.181 ± 0.006	0.197 ± 0.006	0.186 ± 0.005	0.195 ± 0.006	0.199 ± 0.004*	0.199 ± 0.003*
Relative	7.25 ± 0.27	7.46 ± 0.18	7.35 ± 0.10	7.94 ± 0.20	7.29 ± 0.14	8.06 ± 0.13**
Liver						
Absolute	1.01 ± 0.02	1.23 ± 0.02**	1.06 ± 0.03	1.20 ± 0.07	1.15 ± 0.04	1.14 ± 0.03
Relative	40.5 ± 1.0	46.6 ± 1.0**	41.9 ± 0.6	48.8 ± 2.5*	42.2 ± 0.9	46.1 ± 1.1*
Lungs						
Absolute	0.158 ± 0.006	0.181 ± 0.009	0.166 ± 0.005	0.166 ± 0.007	0.181 ± 0.007	0.172 ± 0.007
Relative	6.32 ± 0.25	6.84 ± 0.30	6.58 ± 0.23	6.76 ± 0.28	6.66 ± 0.25	6.98 ± 0.32
Thymus						
Absolute	0.056 ± 0.002	0.046 ± 0.003	0.049 ± 0.005	0.044 ± 0.006	0.045 ± 0.004	0.054 ± 0.004
Relative	2.24 ± 0.11	1.74 ± 0.12	1.91 ± 0.16	1.77 ± 0.24	1.66 ± 0.15*	2.18 ± 0.16

* Significantly different ($P \leq 0.05$) from the control group by Dunn's test
** $P \leq 0.01$
[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 error).

TABLE F6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 15-Month Interim Evaluation in the 2-Year Feed Studies of Polysorbate 80[a]

	0 ppm	25,000 ppm	50,000 ppm
Male			
n	7	6	10
Necropsy body wt	37.9 ± 2.0	36.7 ± 1.6	36.6 ± 1.2
Brain			
Absolute	0.476 ± 0.007	0.485 ± 0.004	0.477 ± 0.007
Relative	12.7 ± 0.4	13.4 ± 0.7	13.1 ± 0.4
R. Kidney			
Absolute	0.386 ± 0.021	0.398 ± 0.023	0.405 ± 0.019
Relative	10.2 ± 0.4	10.8 ± 0.3	11.1 ± 0.3
Liver			
Absolute	2.00 ± 0.12	1.99 ± 0.13	1.99 ± 0.08
Relative	52.8 ± 1.3	54.0 ± 1.8	54.3 ± 1.1
Female			
n	9	10	10
Necropsy body wt	37.3 ± 1.2	37.4 ± 1.6	34.1 ± 1.4
Brain			
Absolute	0.497 ± 0.012	0.495 ± 0.006	0.485 ± 0.008
Relative	13.4 ± 0.4	13.4 ± 0.5	14.4 ± 0.5
R. Kidney			
Absolute	0.239 ± 0.011	0.247 ± 0.007	0.236 ± 0.010
Relative	6.43 ± 0.29	6.65 ± 0.17	6.94 ± 0.16
Liver			
Absolute	1.60 ± 0.05	1.64 ± 0.05	1.57 ± 0.07
Relative	43.1 ± 1.2	44.2 ± 1.0	46.2 ± 1.2

[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 error).

APPENDIX G
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF POLYSORBATE 80

Polysorbate 80 was obtained from McKesson Chemical Company (Kansas City, MO) in two lots. Lot 250-1 was used during the 14-day, 13-week, and 2-year studies, and lot 7230-C was used throughout the remainder of the 2-year studies. Characterization and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (MRI; Kansas City, MO). MRI reports on analyses performed in support of the polysorbate 80 studies are on file at the National Institute of Environmental Health Sciences.

Both lots of the study chemical, a translucent, pale yellow, viscous fluid, was identified as polysorbate 80 by infrared, ultraviolet/visible, and nuclear magnetic resonance (NMR) spectroscopy. All spectra (Figures G1 and G2) were consistent with those expected for the structure and with the literature spectra of polysorbate 80 (*Sadtler Standard Spectra*).

The bulk chemical was further characterized by elemental analysis; Karl Fischer water analysis; United States Pharmacopeia/National Formulary (USP/NF) and Food Chemicals Codex (second edition) methods of titration for free fatty acids, saponification value, and hydroxyl value; American Oil Chemists Society (AOCS) Official Method Cd 8-53 of analysis for peroxide value; colorimetric assay; and thin-layer chromatography (TLC). The colorimetric assay was performed by partitioning the sample between ethyl acetate and 5 N aqueous sodium chloride. Complexation of the resultant compounds was accomplished with ammonium cobaltous thiocyanate. The relative amounts of sorbitan polyethylene glycols fatty acid esters and sorbitan polyethylene glycols were measured at an absorbance of 320 nm. TLC was performed with two systems: A) on silica gel 60 F-254 plates with methanol as the solvent and B) on Whatman KC_{18} plates, reverse phase, with a solvent system of diethyl ether:methanol: concentrated ammonium hydroxide (50:40:10). Visualization was accomplished with visible light, short-wave (254 nm) and long-wave (366 nm) ultraviolet light, iodine vapor, and a spray of 0.5% potassium permanganate in 1 N sodium hydroxide.

For lot 250-1, elemental analyses for carbon and hydrogen were in agreement with theoretical values. Karl Fischer analysis indicated 2.4 ± 0.2% water. The USP XIX method of analysis for free fatty acids indicated 0.57 ± 0.02% as oleic acid. The USP XIX method of analysis indicated a saponification value of 53.1 ± 0.6 mg KOH/g sample. The Food Chemicals Codex method of analysis indicated a hydroxyl value of 68.1 ± 0.6 mg KOH/g sample. Oleic acid, saponification, and hydroxyl values were within USP and Food Chemicals Codex specifications. The AOCS method of analysis indicated 35.8 ± 0.5 meq peroxide/1,000 g sample. Colorimetric assay indicated 85.0 ± 0.6% sorbitan polyethylene glycols fatty acid esters and 15.0 ± 0.6% sorbitan polyethylene glycols. TLC indicated one major spot and one trace impurity by one system. Four spots, with no major spot distinguished, as well as three trace impurities and one slight impurity, were indicated by a second system.

For lot 7230-C, elemental analyses for carbon and hydrogen were in agreement with theoretical values. Karl Fischer analysis indicated 2.5 ± 0.3% water. The USP XX/NF XV method of analysis for free fatty acids indicated 0.742 ± 0.008% as oleic acid. The USP XX/NF XV method of analysis indicated a saponification value of 48.8 ± 0.7 mg KOH/g sample. The USP XX/NF XV method of analysis indicated a hydroxyl value of 66.7 ± 3.7 mg KOH/g sample. Oleic acid, saponification, and hydroxyl values were within USP/NF specifications. The AOCS method of analysis indicated 29.9 ± 1.1 meq peroxide/1,000 g sample. Colorimetric assay indicated 82.7 ± 0.7% sorbitan polyethylene glycols fatty acid esters and 17.3 ± 0.7% sorbitan polyethylene glycols. TLC indicated one major spot, one minor impurity, and one trace impurity by one system and a major spot, one slight trace and four minor impurities by a second system. As a supplement to the characterization analyses, the complete battery

of National Formulary tests was performed on lot 7230-C. All tests indicated that this lot met the NF requirements for polysorbate 80.

Stability studies were performed by free fatty acid determination and colorimetric assay. The stability studies indicated that polysorbate 80, when stored protected from light, was stable as a bulk chemical for 2 weeks at temperatures up to 60° C. During the 2-year studies, the stability of the bulk chemical was monitored by the study laboratory using infrared spectrometry and by titration to determine free fatty acids and peroxide. No degradation of polysorbate 80 was seen throughout the studies.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing appropriate quantities of polysorbate 80 with feed in a Patterson-Kelly twin-shell blender (Table G1). Dose formulations were prepared weekly during the 2-year studies.

Homogeneity and stability analyses of the dosed feed preparations were conducted by the analytical chemistry laboratory. For the homogeneity analyses, 10 g aliquots of the formulations were extracted with 50 mL methanol. Aliquots (2 mL) were diluted with 50 mL methanol; 2 mL samples were then evaporated to dryness. The residues were dissolved in 8 mL methylene chloride and reacted with 2.5 mL of cobalt thiocyanate solution (1.5 g cobalt nitrate and 10 g ammonium thiocyanate dissolved in 50 mL water). The absorbance of the methylene chloride layer was determined at 320 nm. For the stability studies, feed samples were extracted with methanol, then mixed with 25 mL ethyl acetate and 25 mL of 5 N sodium chloride solution (292 g sodium chloride dissolved and diluted to 1 L with water). The ethyl acetate layer of each sample was then extracted with 25 mL of the 5 N sodium chloride solution and evaporated, and the residues were dissolved in 10 mL methylene chloride and mixed with 3 mL cobalt thiocyanate solution. The samples were then centrifuged and the absorbance measured by spectroscopy at 320 nm. The aqueous sodium chloride layer of each sample was extracted with methylene chloride and centrifuged, then extracted with 3 mL of the cobalt thiocyanate solution. The absorbance was measured by the same procedure used for the ethyl acetate layer. Homogeneity of these formulations was confirmed; stability of the formulation was established for at least 2 weeks when stored in the dark at temperatures up to 5° C.

Periodic analyses of the dose formulations of polysorbate 80 were conducted at the study laboratory and at the analytical chemistry laboratory using ultraviolet/visible spectroscopy at 320 nm. Dose formulations were analyzed once during the 14-day studies and twice during the 13-week studies. During the 14-day studies, all dose formulations for rats and mice were within the acceptable range of ± 10% of target concentrations (Table G2). During the 13-week studies, 11 of the 14 dose formulations for rats and mice were within the specified ± 10% of the target concentrations (Table G3). During the 2-year studies, the dose formulations were analyzed at least once every 8 weeks; 84 of the 87 dose formulations for rats and mice were within the specified ± 10% of the target concentrations. Results of the dose formulation analyses studies for the 2-year studies are presented in Table G4. Results of periodic referee analysis performed by the analytical chemistry laboratory indicated good agreement with the results obtained by the study laboratory (Table G5).

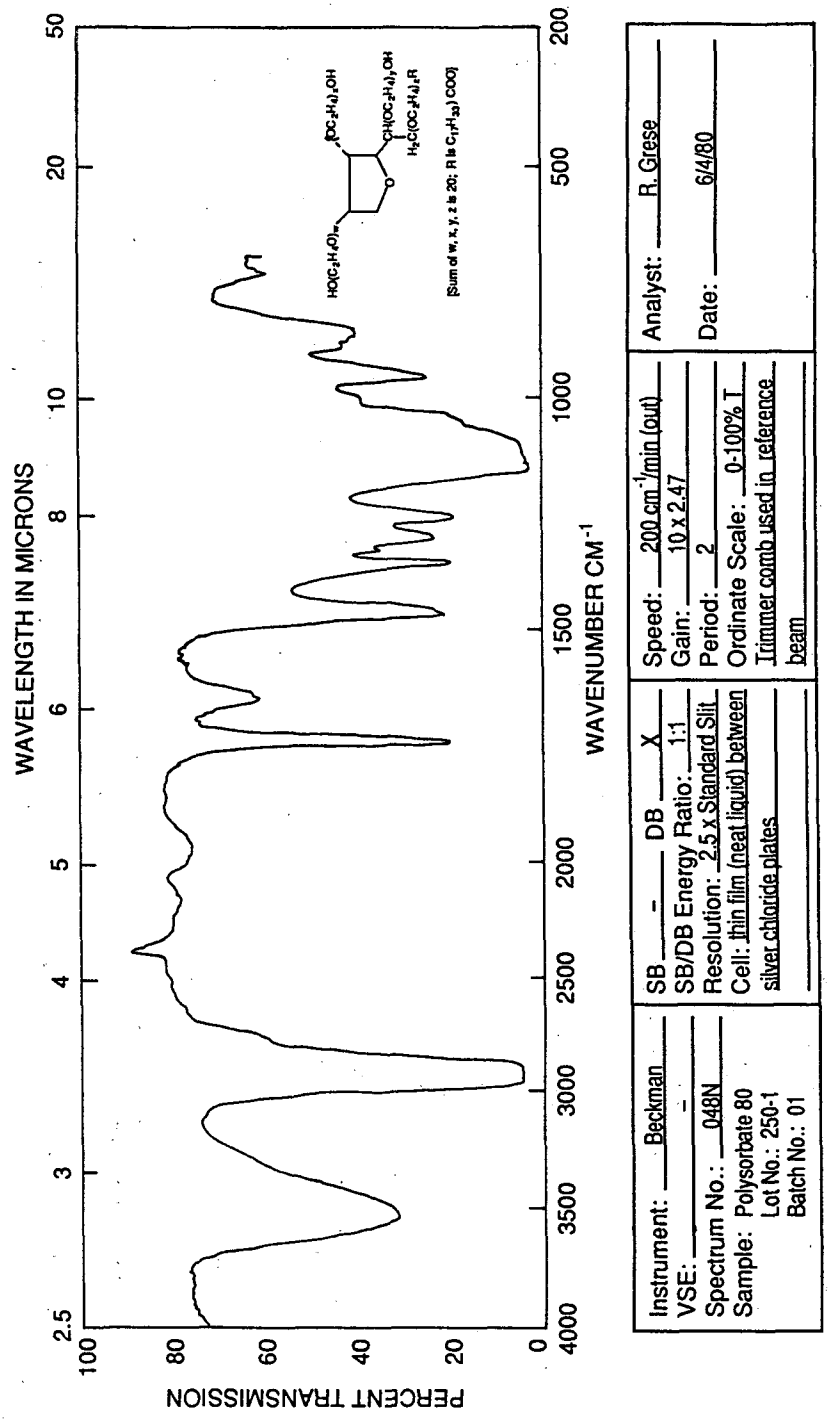

FIGURE G1
Infrared Absorption Spectrum of Polysorbate 80

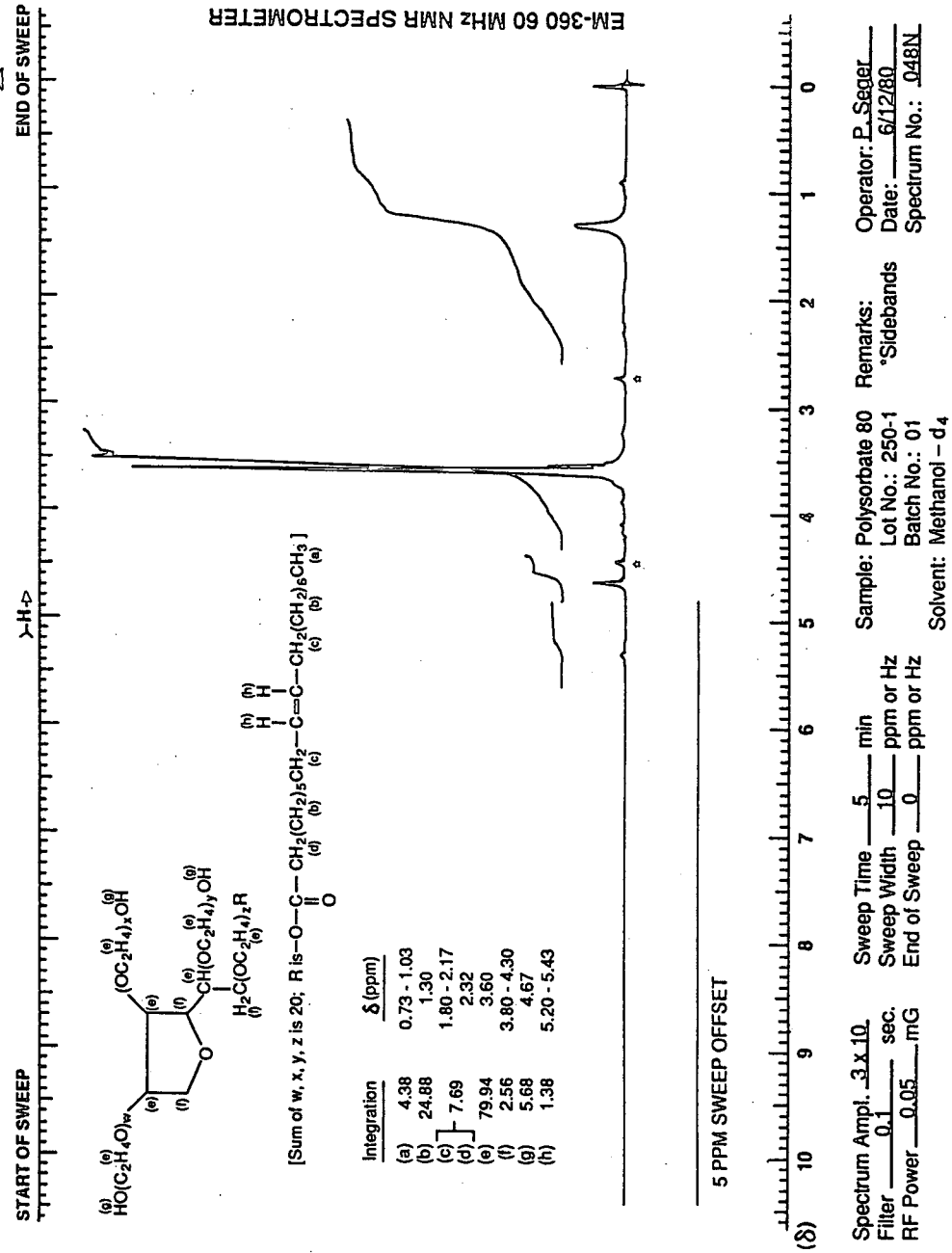

FIGURE G2
Nuclear Magnetic Resonance Spectrum of Polysorbate 80

TABLE G1
Preparation and Storage of Dose Formulations in the Feed Studies of Polysorbate 80

14-Day Studies	13-Week Studies	2-Year Studies
Preparation		
A premix with polysorbate 80 and feed was prepared by blending with a spatula; premix and remainder of feed was layered into a blender and mixed for 15 minutes with an intensifier bar. Dose formulations were prepared once.	Same as 14-day studies. Dose formulations were prepared weekly.	Same as 14-day studies. Dose formulations were prepared weekly.
Chemical Lot Number		
250-1	Same as 14-day studies	250-1 and 7230-C
Maximum Storage Time		
14 days from date of preparation	Same as 14-day studies	Same as 14-day studies
Storage Conditions		
Stored in plastic bags, in the dark, at 5° C	Same as 14-day studies	Same as 14-day studies
Study Laboratory		
Southern Research Institute, Birmingham, AL	Same as 14-day studies	Same as 14-day studies
Referee Laboratory		
Midwest Research Institute, Kansas City, MO	Same as 14-day studies	Same as 14-day studies

TABLE G2
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 14-Day Feed Studies of Polysorbate 80

Date Prepared	Date Analyzed	Target Concentration (wt/wt%)	Determined Concentration[a] (wt/wt%)	% Difference from Target
12 March 1981	18 March 1981	0.3	0.300	0
		0.6	0.636	+6
		1.25	1.12	−10
		2.5	2.36	−6
		5.0	4.62	−8

[a] Results of duplicate analyses

TABLE G3
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 13-Week Feed Studies of Polysorbate 80

Date Prepared	Date Analyzed	Target Concentration[a] (wt/wt%)	Determined Concentration[b] (wt/wt%)	% Difference from Target
10 June 1981	23 June 1981	0.31[c]	0.336	+8
		0.31[d]	0.204	−34
		0.31[e]	0.308	−1
		0.62	0.605	−2
		1.25	1.73	+38
		2.5	2.48	−1
		5.0[c]	5.16	+3
		5.0[d]	5.07	+1
		5.0[e]	4.89	−2
29 July 1981	30 July 1981	0.31	0.267	−14[f]
		0.62	0.572	−8
		1.25	1.22	−2
		2.5	2.70	+8
		5.0	5.28	+6

[a] Target concentrations: 0.31% = 3,100 ppm; 0.62% = 6,200 ppm; 1.25% = 12,500 ppm; 2.5% = 25,000 ppm; 5.0% = 50,000 ppm
[b] Results of duplicate analyses
[c] Sample selection from top left of twin-shell blender
[d] Sample selection from top right of twin-shell blender
[e] Sample selection from bottom of twin-shell blender
[f] Used for dosing due to lack of time for remixing

TABLE G4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Polysorbate 80

Date Prepared	Date Analyzed	Target Concentration[a] (wt/wt%)	Determined Concentration[b] (wt/wt%)	% Difference from Target
16 August 1982	18 August 1982	2.5	2.42	-3
		2.5	2.48	-1
		2.5	2.44	-2
		5.0	5.02	0
		5.0	4.62	-8
		5.0	4.62	-8
4 October 1982	6 October 1982	2.5	2.65	+6
		2.5	2.34	-6
		2.5	2.30	-8
		5.0	4.76	-5
		5.0	4.74	-5
		5.0	4.68	-6
29 November 1982	1 December 1982	2.5	2.50	0
		2.5	2.51	0
		2.5	2.43	-3
		5.0	4.08	-18[c]
		5.0	4.96	-1
		5.0	4.82	-4
3 December 1982[d]	3 December 1982	5.0	4.98	0
24 January 1983	24-25 January 1983	2.5	2.34	-6
		2.5	2.30	-8
		2.5	2.32	-7
		5.0	5.22	+4
		5.0	5.12	+2
		5.0	5.16	+3
21 March 1983	22 March 1983	2.5	2.50	0
		2.5	2.44	-2
		2.5	2.30	-8
		5.0	4.78	-4
		5.0	4.71	-6
		5.0	4.66	-7
16 May 1983	17 May 1983	2.5	2.46	-2
		2.5	2.96	+18[e]
		2.5	2.70	+8[e]
		5.0	4.75	-5
		5.0	4.87	-3
		5.0	4.69	-6
	19 May 1983[f]	2.5	2.35	-6
		2.5	2.47	-1

TABLE G4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Polysorbate 80 (continued)

Date Prepared	Date Analyzed	Target Concentration (wt/wt%)	Determined Concentration (wt/wt%)	% Difference from Target
11 July 1983	11-12 July 1983	2.5	2.51	0
		2.5	2.32	-7
		2.5	2.50	0
		5.0	4.64	-7
		5.0	4.89	-2
		5.0	4.56	-9
12 September 1983	13 September 1983	2.5	2.56	+2
		2.5	2.42	-3
		2.5	2.62	+5
		5.0	5.06	+1
		5.0	5.34	+7
		5.0	5.22	+4
31 October 1983	1 November 1983	2.5	2.61	+4
		2.5	2.44	-2
		2.5	2.49	0
		5.0	5.22	+4
		5.0	5.18	+4
		5.0	5.02	0
19 December 1983	19-20 December 1983	2.5	2.54	+2
		2.5	2.52	+1
		2.5	2.44	-2
		5.0	5.12	+2
		5.0	5.08	+2
		5.0	5.12	+2
6 February 1984	6-7 February 1984	2.5	2.52	+1
		2.5	2.63	+5
		2.5	2.54	+2
		5.0	4.95	-1
		5.0	4.96	-1
		5.0	5.00	0
9 April 1984	9-10 April 1984	2.5	2.63	+5
		2.5	2.58	+3
		2.5	2.52	+1
		5.0	5.00	0
		5.0	4.87	-3
		5.0	4.86	-3
21 May 1984	21-22 May 1984	2.5	2.46	-2
		2.5	2.46	-2
		2.5	2.76	+10[c]
		2.5	2.62	+5
		5.0	4.94	-1
		5.0	4.92	-2
		5.0	5.00	0
		5.0	5.00	0

TABLE G4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Polysorbate 80 (continued)

Date Prepared	Date Analyzed	Target Concentration (wt/wt%)	Determined Concentration (wt/wt%)	% Difference from Target
24 May 1984[d]	24-25 May 1984	2.5	2.46	-2
23 July 1984	23-24 July 1984	2.5	2.52	+1
		2.5	2.46	-2
		2.5	2.42	-3
		5.0	4.90	-2
		5.0	4.76	-5
		5.0	4.70	-6
		5.0	4.80	-4

[a] Target concentrations: 2.5% = 25,000 ppm; 5.0% = 50,000 ppm
[b] Results of duplicate analyses
[c] Sample remixed
[d] Analysis results of remix
[e] Poor duplication; samples returned to blender for additional 15 minutes mixing
[f] Analysis results of reblended samples

TABLE G5
Results of Referee Analysis of Dose Formulations in the 13-Week and 2-Year Feed Studies of Polysorbate 80

Date Prepared	Target Concentration (wt/wt%)	Determined Concentration (wt/wt%)	
		Study Laboratory[a]	Referee Laboratory[b]
29 July 1981	5.0	5.28	4.88 ± 0.13
9 August 1982	2.5	2.41	2.41 ± 0.07
24 January 1983	5.0	5.22	4.85 ± 0.06
11 July 1983	2.5	2.50	2.42 ± 0.16
31 October 1983	5.0	5.02	4.89 ± 0.11
9 April 1984	5.0	5.00	4.91 ± 0.09

[a] Results of duplicate analysis
[b] Results of triplicate analysis; mean ± standard deviation

APPENDIX H
FEED AND COMPOUND CONSUMPTION IN THE 2-YEAR FEED STUDIES

TABLE H1
Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of Polysorbate 80

Week	0 ppm		25,000 ppm			50,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
1	14.2	149	14.9	151	2,473	15.2	149	5,098
2	17.1	185	17.4	184	2,362	17.7	186	4,774
4	14.9	223	15.7	224	1,755	15.9	223	3,575
5	17.5	242	17.0	241	1,767	17.3	239	3,627
8	13.3	277	15.2	278	1,370	15.4	276	2,786
9	15.4	292	16.4	292	1,403	17.2	290	2,962
12	15.5	321	16.8	323	1,301	15.1	320	2,363
13	16.5	330	17.0	333	1,273	15.6	327	2,389
17	16.4	360	16.1	361	1,114	17.1	356	2,405
21	17.3	384	17.5	387	1,132	17.5	384	2,282
25	17.3	406	16.8	406	1,035	16.7	402	2,073
29	17.3	420	17.8	420	1,059	17.6	416	2,111
33	18.2	436	18.9	434	1,088	18.8	430	2,188
37	17.5	446	18.6	447	1,038	17.9	444	2,011
41	18.6	452	18.6	450	1,035	18.7	447	2,091
45	18.4	456	18.3	458	999	18.6	452	2,058
49	18.9	459	19.2	463	1,039	18.2	453	2,012
53	18.1	451	18.0	464	970	18.5	457	2,018
57	20.5	458	19.6	466	1,049	20.2	456	2,213
61	18.8	461	18.7	467	1,002	18.4	459	2,007
65	18.3	459	18.5	473	976	18.4	457	2,010
69	19.7	469	18.1	472	959	19.8	465	2,126
73	18.0	472	17.8	479	927	17.7	465	1,899
77	18.6	471	17.9	479	934	20.3	465	2,184
81	16.9	469	16.6	481	863	18.2	470	1,932
85	17.7	480	16.4	478	856	17.4	473	1,840
89	18.3	481	17.3	473	916	18.5	467	1,978
93	17.7	476	18.1	474	957	18.7	470	1,985
97	15.2	450	13.1	452	727	14.2	444	1,596
101	15.8	434	14.8	441	839	16.4	437	1,869
Weeks 1-13:								
Mean	15.5	252.3	16.3	253.1	1,713	16.2	251.2	3,447
SD[c]	1.4		0.9		475	1.1		1,035
CV[d]	9.2		5.6		27.7	6.6		30.0
Weeks 14-52:								
Mean	17.8	424.3	18.0	425.1	1,060	17.9	420.3	2,137
SD	0.8		1.0		43	0.7		132
CV	4.4		5.7		4.1	4.2		6.2
Weeks 53-101:								
Mean	18.0	463.8	17.3	469.2	921	18.2	460.5	1,974
SD	1.4		1.7		83	1.6		160
CV	7.9		10.0		9.0	9.0		8.1

[a] Grams of feed consumed per animal per day
[b] Milligrams of polysorbate 80 consumed per day per kilogram body weight
[c] Standard deviation of weekly means
[d] Coefficient of variation = (standard deviation/mean) x 100

TABLE H2
Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of Polysorbate 80

Week	0 ppm		25,000 ppm			50,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
1	12.4	122	12.5	124	2,535	12.4	122	5,067
2	13.4	138	11.4	138	2,062	11.9	137	4,333
4	9.5	150	10.6	150	1,764	10.3	150	3,419
5	14.9	160	14.2	158	2,255	15.4	158	4,866
8	10.5	174	11.3	172	1,641	10.5	172	3,040
9	15.2	179	13.3	177	1,872	14.1	177	3,988
12	11.1	188	10.6	189	1,395	10.0	187	2,682
13	12.9	191	12.8	191	1,676	13.2	189	3,503
17	11.3	202	11.8	202	1,465	11.9	200	2,977
21	12.4	212	12.2	214	1,420	12.2	213	2,869
25	11.2	222	11.4	224	1,267	11.8	222	2,655
29	12.5	227	11.6	231	1,259	11.8	227	2,612
33	12.7	237	12.3	238	1,292	13.3	233	2,870
37	12.6	246	12.1	251	1,211	12.2	247	2,468
41	13.4	254	13.4	254	1,317	13.3	251	2,650
45	12.9	262	12.8	264	1,207	13.1	256	2,560
49	13.7	276	13.1	278	1,176	13.4	266	2,528
53	13.0	284	12.9	285	1,134	13.4	275	2,434
57	13.5	297	13.2	296	1,111	13.3	284	2,352
61	13.1	305	13.6	302	1,123	13.2	292	2,264
65	13.6	316	13.6	313	1,089	13.9	305	2,272
69	14.2	326	15.2	323	1,177	14.6	314	2,321
73	13.6	332	13.4	333	1,004	13.4	324	2,074
77	13.2	341	13.6	337	1,008	13.2	331	1,995
81	13.4	347	14.2	339	1,044	13.2	335	1,973
85	13.3	354	13.7	347	984	12.9	343	1,883
89	13.7	353	12.9	349	926	13.6	346	1,962
93	13.8	351	14.8	349	1,056	14.5	351	2,071
97	12.3	347	12.7	347	916	12.1	345	1,748
101	12.2	348	12.9	348	929	13.2	345	1,909
Weeks 1-13:								
Mean	12.5	162.9	12.1	162.3	1,900	12.2	161.5	3,862
SD[c]	2.0		1.3		368	1.9		853
CV[d]	16.4		11.1		19.4	15.9		22.1
Weeks 14-52:								
Mean	12.5	237.6	12.3	239.5	1,291	12.6	234.8	2,688
SD	0.8		0.7		97	0.7		176
CV	6.7		5.4		7.5	5.7		6.6
Weeks 53-101:								
Mean	13.3	330.8	13.6	328.2	1,039	13.4	322.3	2,097
SD	0.6		0.7		86	0.7		211
CV	4.3		5.5		8.3	4.9		10.1

[a] Grams of feed consumed per animal per day
[b] Milligrams of polysorbate 80 consumed per day per kilogram body weight
[c] Standard deviation of weekly means
[d] Coefficient of variation = (standard deviation/mean) x 100

TABLE H3
Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of Polysorbate 80

Week	0 ppm		25,000 ppm			50,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
1	7.7	22.2	7.5	22.7	8,207	8.0	22.4	17,860
2	6.5	25.1	6.0	25.6	5,824	6.6	25.5	12,889
4	5.3	27.0	4.7	27.5	4,281	6.1	26.5	11,481
5	7.7	27.5	7.6	28.0	6,769	7.5	27.5	13,641
9	7.4	29.8	8.3	30.5	6,839	8.1	30.3	13,316
13	7.8	31.2	8.5	31.8	6,691	8.6	31.6	13,553
16	9.9	32.7	10.2	33.5	7,649	10.1	32.9	15,365
20	9.9	34.4	9.7	34.6	7,001	8.6	34.4	12,513
25	8.2	35.0	8.8	35.7	6,175	9.5	34.9	13,637
29	9.8	34.3	9.5	35.5	6,688	9.8	35.1	13,984
33	10.3	35.8	9.4	36.8	6,414	10.0	35.8	13,952
37	9.2	35.8	8.2	35.4	5,807	9.3	35.8	13,039
41	12.0	36.6	10.2	37.4	6,803	11.6	36.3	15,936
45	14.5	37.7	11.3	36.8	7,690	13.0	36.7	17,724
49	13.3	38.9	10.6	38.4	6,919	12.4	38.4	16,166
53	11.2	38.3	9.3	38.3	6,093	10.3	37.7	13,637
58	4.5	37.3	4.3	37.4	2,903	4.1	37.5	5,509
62	5.2	38.5	4.5	36.2	3,113	5.0	37.5	6,614
66	5.4	38.3	4.7	38.4	3,031	5.2	38.3	6,851
70	5.1	37.6	5.0	36.8	3,385	5.0	37.2	6,718
74	5.6	38.3	4.3	38.5	2,817	4.8	38.2	6,339
78	5.3	39.0	4.0	38.3	2,615	4.4	37.7	5,859
82	5.1	38.9	4.3	37.5	2,842	4.9	37.0	6,564
86	5.8	37.4	3.9	36.1	2,731	4.5	36.4	6,119
90	4.1	37.6	3.8	37.7	2,527	4.0	36.9	5,364
94	5.3	37.5	4.3	36.4	2,956	4.3	36.9	5,761
98	4.6	37.6	3.8	36.5	2,623	4.4	37.6	5,915
102	4.7	36.2	3.9	36.2	2,703	4.5	35.0	6,399
Weeks 1-13:								
Mean	7.1	27.1	7.1	27.7	6,435	7.5	27.3	13,790
SD[c]	1.0		1.5		1,304	1.0		2,145
CV[d]	13.8		20.8		20.3	12.8		15.6
Weeks 14-52:								
Mean	10.8	35.7	9.8	36.0	6,794	10.5	35.6	14,702
SD	2.1		0.9		623	1.5		1,696
CV	19.0		9.6		9.2	14.3		11.5
Weeks 53-102:								
Mean	5.5	37.9	4.6	37.3	3,103	5.0	37.2	6,742
SD	1.8		1.5		928	1.6		2,124
CV	31.9		31.4		29.9	32.3		31.5

[a] Grams of feed consumed per animal per day
[b] Milligrams of polysorbate 80 consumed per day per kilogram body weight
[c] Standard deviation of weekly means
[d] Coefficient of variation = (standard deviation/mean) x 100

TABLE H4
Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of Polysorbate 80

Week	0 ppm		25,000 ppm			50,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
1	7.6	16.8	7.6	16.4	11,624	7.9	16.5	24,050
2	5.8	18.9	7.0	18.7	9,354	6.5	18.9	17,159
4	4.4	20.5	5.6	20.3	6,838	5.1	20.3	12,529
5	7.0	20.2	7.2	20.6	8,787	7.1	20.3	17,417
9	7.3	21.9	9.2	23.0	9,980	8.8	22.8	19,403
13	7.6	23.0	8.4	22.8	9,208	9.3	22.8	20,308
16	10.1	24.9	10.7	24.2	11,094	10.7	24.7	21,642
20	9.0	26.4	10.2	26.1	9,816	9.7	25.3	19,224
25	7.5	27.7	9.0	27.5	8,173	10.2	26.6	19,106
29	8.0	28.6	9.5	28.0	8,498	8.4	27.7	15,104
33	8.0	29.6	10.3	29.3	8,763	10.0	28.1	17,710
37	7.2	30.2	7.9	29.2	6,772	7.8	28.2	13,749
41	8.2	31.1	9.5	30.9	7,696	10.0	29.4	17,019
45	9.9	30.9	12.8	30.7	10,429	11.0	28.8	19,157
49	8.5	32.6	9.7	32.1	7,543	9.7	30.7	15,873
53	7.9	33.3	9.0	33.0	6,835	7.6	30.8	12,322
58	3.4	33.2	3.8	32.9	2,864	3.5	31.3	5,600
62	5.5	34.8	5.4	35.0	3,880	5.8	32.9	8,797
66	5.5	36.4	5.4	35.8	3,746	5.8	33.1	8,791
70	5.7	36.9	5.9	36.1	4,076	5.4	33.5	8,003
74	4.8	37.6	5.1	36.0	3,542	5.0	34.2	7,338
78	5.3	40.0	4.5	37.0	3,045	4.8	34.4	6,945
82	4.7	39.9	4.8	37.7	3,211	4.9	34.4	7,176
86	5.0	40.4	5.0	37.0	3,407	6.0	34.7	8,655
90	3.3	39.9	3.4	36.6	2,337	3.7	34.4	5,375
94	5.8	38.9	5.1	37.0	3,439	5.3	35.1	7,524
98	4.7	39.5	5.5	37.2	3,689	4.9	34.7	7,125
102	5.2	40.0	4.6	38.1	2,993	4.6	35.6	6,404
Weeks 1-13								
Mean	6.6	20.2	7.5	20.3	9,299	7.4	20.3	18,477
SD[c]	1.3		1.2		1,562	1.6		3,834
CV[d]	19.2		16.6		16.8	20.9		20.8
Weeks 14-52								
Mean	8.5	29.1	10.0	28.7	8,754	9.7	27.7	17,620
SD	1.0		1.3		1,429	1.0		2,451
CV	11.8		13.5		16.3	10.7		13.9
Weeks 53-102								
Mean	5.1	37.8	5.2	36.1	3,620	5.2	33.8	7,697
SD	1.1		1.3		1,073	1.0		1,770
CV	22.2		25.8		29.6	20.1		23.0

[a] Grams of feed consumed per animal per day
[b] Milligrams of polysorbate 80 consumed per day per kilogram body weight
[c] Standard deviation of weekly means
[d] Coefficient of variation = (standard deviation/mean) x 100

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

TABLE I1
Ingredients of NIH-07 Rat and Mouse Ration[a]

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

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

TABLE I2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[a]

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

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

TABLE I3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrients	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	22.86 ± 1.17	21.2–25.9	25
Crude fat (% by weight)	5.09 ± 0.51	4.2–6.2	25
Crude fiber (% by weight)	3.50 ± 0.37	2.8–4.5	25
Ash (% by weight)	6.70 ± 0.24	6.3–7.3	25
Amino Acids (% of total diet)			
Arginine	1.308 ± 0.606	1.210–1.390	8
Cystine	0.306 ± 0.084	0.181–0.400	8
Glycine	1.150 ± 0.047	1.060–1.210	8
Histidine	0.576 ± 0.024	0.531–0.607	8
Isoleucin	0.917 ± 0.029	0.881–0.944	8
Leucine	1.946 ± 0.055	1.850–2.040	8
Lysine	1.270 ± 0.058	1.200–1.370	8
Methionine	0.448 ± 0.128	0.306–0.699	8
Phenylalanine	0.987 ± 0.140	0.665–1.110	8
Threonine	0.877 ± 0.042	0.824–0.940	8
Tryptophane	0.236 ± 0.176	0.107–0.671	8
Tyrosine	0.676 ± 0.105	0.564–0.794	8
Valine	1.103 ± 0.040	1.050–1.170	8
Essential Fatty Acids (% of total diet)			
Linoleic	2.393 ± 0.258	1.830–2.570	7
Linolenic	0.280 ± 0.040	0.210–0.320	7
Vitamins			
Vitamin A (IU/kg)	11,588 ± 4,351	4,200–22,000	25
Vitamin D (IU/kg)	4,450 ± 1,382	3,000–6,300	4
α–Tocopherol (ppm)	37.95 ± 9.406	22.5–48.9	8
Thiamine (ppm)	18.36 ± 3.89	12.0–31.0	25
Riboflavin (ppm)	7.92 ± 0.87	6.10–9.00	8
Niacin (ppm)	103.38 ± 26.59	65.0–150.0	8
Pantothenic acid (ppm)	29.54 ± 3.60	23.0–34.0	8
Pyridoxine (ppm)	9.55 ± 3.48	5.60–14.0	8
Folic acid (ppm)	2.25 ± 0.73	1.80–3.70	8
Biotin (ppm)	0.254 ± 0.042	0.19–0.32	8
Vitamin B_{12} (ppb)	38.45 ± 22.01	10.6–65.0	8
Choline (ppm)	3,089 ± 328.69	2,400–3,430	8
Minerals			
Calcium (%)	1.25 ± 0.11	1.04–1.43	25
Phosphorus (%)	0.95 ± 0.05	0.86–1.10	25
Potassium (%)	0.883 ± 0.078	0.772–0.971	6
Chloride (%)	0.526 ± 0.092	0.380–0.635	8
Sodium (%)	0.313 ± 0.390	0.258–0.371	8
Magnesium (%)	0.168 ± 0.010	0.151–0.181	8
Sulfur (%)	0.280 ± 0.064	0.208–0.420	8
Iron (ppm)	360.54 ± 100	255.0–523.0	8
Manganese (ppm)	91.97 ± 6.01	81.70–99.40	8
Zinc (ppm)	54.72 ± 5.67	46.10–64.50	8
Copper (ppm)	11.06 ± 2.50	8.090–15.39	8
Iodine (ppm)	3.37 ± 0.92	1.52–4.13	6
Chromium (ppm)	1.79 ± 0.36	1.04–2.09	8
Cobalt (ppm)	0.681 ± 0.14	0.490–0.780	4

TABLE I4
Contaminant Levels in NIH-07 Rat and Mouse Ration[a]

Contaminants	Mean ± Standard Deviation[a]	Range	Number of Samples
Arsenic (ppm)	0.53 ± 0.14	0.18–0.74	25
Cadmium (ppm)[b]	0.12 ± 0.04	<0.10–0.20	25
Lead (ppm)[c]	0.63 ± 0.52	0.27–2.93	25
Mercury (ppm)	<0.05		25
Selenium (ppm)	0.31 ± 0.06	0.21–0.45	25
Aflatoxins (ppb)	<5.0		25
Nitrate nitrogen (ppm)	9.59 ± 4.30	2.50–19.0	25
Nitrite nitrogen (ppm)[d]	1.25 ± 1.46	<0.10–6.10	25
BHA (ppm)[e,f]	4.12 ± 5.06	<2.00–20.00	25
BHT (ppm)[e,g]	2.96 ± 2.64	<1.00–13.00	25
Aerobic plate count (CFU/g)[h]	151,668 ± 144,321	6,200–420,000	25
Coliform (MPN/g)[i]	512 ± 791	<3.00–2,400	25
E. coli[j]	9.68 ± 29	<3.00–150.0	25
E. coli[k]	3.83 ± 2.68	<3.00–15.0	24
Total nitrosoamines (ppb)[l]	5.78 ± 5.03	0.80–30.30	25
N-Nitrosodimethylamine (ppb)[l]	4.89 ± 6.02	0.50–30.00	25
N-Nitrosopyrrolidine (ppb)[l]	0.89 ± 0.75	0.30–2.70	25
Pesticides (ppm)			
α_rBHC[m]	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.1		25
Estimated PCBs	<0.2		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.1		25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion[n]	0.15 ± 0.18	0.05–0.81	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

TABLE I4
Contaminant Levels in NIH-07 Rat and Mouse Ration (continued)

[a] For values less than the limit of detection, the detection limit is given for the mean.
[b] Four batches (milled on 22 February 1984, 14 March 1984, 9 May 1984, and 13 June 1984) contained 0.20 ppm; all others contained <0.10 ppm.
[c] Includes one large value of 2.93 ppm obtained in the batch milled on 27 July 1982.
[d] Includes one large value of 6.10 ppm obtained in the batch milled on 18 August 1983.
[e] Sources of contamination: soy oil and fish meal
[f] Values from 26 August 1982 to 30 November 1982 range from 10 to 20 ppm; all other values are less than 3 ppm.
[g] Includes one large value of 13 ppm obtained in the batch milled on 30 November 1982.
[h] CFU = colony forming unit
[i] MPN = most probable number
[j] Includes one large value of 150 MPN/g obtained in the batch milled on 26 August 1982
[k] Excludes the large value obtained in the batch milled on 26 August 1982
[l] All values were corrected for percent recovery
[m] BHC = hexachlorocyclohexane or benzene hexachloride
[n] Fifteen lots contained more than 0.05 ppm.

APPENDIX J
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

Rats

During the 13-week studies, samples for viral screening were collected from five diet control animals of each sex. At the termination of the 13-week studies, the animals were bled. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Inc. (Bethesda, MD) for determination of the antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Hemagglutination Inhibition	
PVM (pneumonia virus of mice)	Study termination
Sendai	Study termination
KRV (Kilham rat virus)	Study termination
H-1 (Toolan's H-1 virus)	Study termination
ELISA	
RCV/SDA (rat corona virus/ sialodacryoadenitis virus)	Study termination

During the 2-year studies, 15 F344/N rats of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group were killed at 6, 12, and 18 months on study. Samples for viral screening at 24 months were collected from five diet control animals of each sex. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Inc. (Bethesda, MD) for determination of the antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Hemagglutination Inhibition	
PVM	6, 12, 18, and 24 months
Sendai	6, 12, 18, and 24 months
KRV	6, 12, 18, and 24 months
H-1	6, 12, 18, and 24 months
Complement Fixation	
RCV	6 months
ELISA	
RCV/SDA	6, 12, 18, and 24 months
Mycoplasma pulmonis	12, 18, and 24 months

Mice

During the 13-week studies, samples for viral screening were collected from five diet control animals of each sex. At the termination of the 13-week studies, the animals were bled. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Inc. (Bethesda, MD) for determination of the antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Hemagglutination Inhibition	
PVM	Study termination
Reovirus 3	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
Polyoma virus	Study termination
MVM (minute virus of mice)	Study termination
Ectromelia virus (mouse pox)	Study termination
Complement Fixation	
Sendai	Study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
ELISA	
MHV (mouse hepatitis virus)	Study termination

During the 2-year studies, 15 B6C3F$_1$ mice of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group were killed at 6 months; five males and four females were killed at 12 months; and two males and five females were killed at 18 months on study. Samples for viral screening at 24 months were collected from five diet control animals of each sex. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Inc. (Bethesda, MD) for determination of the antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Hemagglutination Inhibition	
PVM	6, 12, 18, and 24 months
Reovirus 3	6, 12, 18, and 24 months
GDVII	6, 12, and 18 months
Polyoma virus	6, 12, 18, and 24 months
Sendai	6, 12, 18, and 24 months
MVM	6, 12, 18, and 24 months
Ectromelia virus	6, 12, 18, and 24 months
Complement Fixation	
Mouse adenoma virus	6, 12, 18, and 24 months
LCM	6, 12, 18, and 24 months
Method of Analysis (continued)	**Time of Analysis**
ELISA	
GDVII	24 months
Mycoplasma pulmonis	12, 18, and 24 months
MHV	6, 12, 18, and 24 months

RESULTS

The serology results for sentinel animals are presented in Table J1.

TABLE J1
Murine Virus Antibody Determinations for Rats and Mice in the 13-Week and 2-Year Feed Studies of Polysorbate 80

	Interval	Incidence of Antibody in Sentinel Animals	Positive Serologic Reaction for
13-Week Studies			
Rats	13 weeks	0/10	None positive
Mice	13 weeks	0/10	None positive
2-Year Studies			
Rats	6 months	9/10	PVM
	12 months	9/10	PVM
		7/10	*M. pulmonis*[a]
	18 months	10/10	PVM
	24 months	7/10	PVM
		2/10	*M. pulmonis*[a]
Mice	6 months	3/10	PVM
	12 months	3/9	PVM
	18 months	2/7	PVM
	24 months	0/10	None positive

[a] Further evaluation of this assay indicated that it was not specific for *Mycoplasma pulmonis*, and these results were considered to be false positive.

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TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Dermal)
206	1,2-Dibromo-3-chloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butyl Benzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-ethylhexyl)phthalate
219	2,6-Dichloro-p-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
228	Vinylidene Chloride
229	Guar Gum
230	Agar
231	Stannous Chloride
232	Pentachloroethane
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	*D*-Mannitol
237	1,1,1,2-Tetrachloroethane
238	Ziram
239	Bis(2-chloro-1-methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
243	Trichloroethylene (Rats and Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
246	Chrysotile Asbestos (Hamsters)
247	L-Ascorbic Acid
248	4,4'-Methylenedianiline Dihydrochloride
249	Amosite Asbestos (Hamsters)
250	Benzyl Acetate
251	2,4- & 2,6-Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
254	Dichloromethane (Methylene Chloride)
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
266	Monuron
267	1,2-Propylene Oxide
269	1,3-Dichloropropane (Telone II®)
271	HC Blue No. 1
272	Propylene
273	Trichloroethylene (Four Rat Strains)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
277	Tremolite
278	2,6-Xylidine
279	Amosite Asbestos
280	Crocidolite Asbestos
281	HC Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethyl) phosphonium Sulfate & Tetrakis(hydroxymethyl) phosphonium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	*o*-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane (Methylene Chloride)
307	Ephedrine Sulfate
308	Chlorinated Paraffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
313	Mirex
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
320	Rotenone
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
328	Methyl Carbamate
329	1,2-Epoxybutane
330	4-Hexylresorcinol
331	Malonaldehyde, Sodium Salt
332	2-Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
335	C.I. Acid Orange 3
336	Penicillin VK
337	Nitrofurazone

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338	Erythromycin Stearate
339	2-Amino-4-nitrophenol
340	Iodinated Glycerol
341	Nitrofurantoin
342	Dichlorvos
343	Benzyl Alcohol
344	Tetracycline Hydrochloride
345	Roxarsone
346	Chloroethane
347	D-Limonene
348	*a*-Methyldopa Sesquihydrate
349	Pentachlorophenol
350	Tribromomethane
351	*p*-Chloroaniline Hydrochloride
352	N-Methylolacrylamide
353	2,4-Dichlorophenol
354	Dimethoxane
355	Diphenhydramine Hydrochloride
356	Furosemide
357	Hydrochlorothiazide
358	Ochratoxin A
359	8-Methoxypsoralen
360	N,N-Dimethylaniline
361	Hexachloroethane
362	4-Vinyl-1-Cyclohexene Diepoxide
363	Bromoethane (Ethyl Bromide)
364	Rhodamine 6G (C.I. Basic Red 1)
365	Pentaerythritol Tetranitrate
366	Hydroquinone
367	Phenylbutazone
368	Nalidixic Acid
369	Alpha-Methylbenzyl Alcohol
370	Benzofuran
371	Toluene
372	3,3'-Dimethoxybenzidine Dihydrochloride
373	Succinic Anhydride
374	Glycidol
375	Vinyl Toluene
376	Allyl Glycidyl Ether
377	*o*-Chlorobenzalmalononitrile
378	Benzaldehyde
379	2-Chloroacetophenone
380	Epinephrine Hydrochloride
381	*d*-Carvone
382	Furfural
386	Tetranitromethane
387	Amphetamine Sulfate
389	Sodium Azide
390	3,3'-Dimethylbenzidine Dihydrochloride
391	Tris(2-chloroethyl) Phosphate
393	Sodium Fluoride
395	Probenecid
399	Titanocene Dichloride

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DEPARTMENT OF
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Public Health Service
National Toxicology Program
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P.O. Box 12233, MD A0-01
Research Triangle Park, NC 27709

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