NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 359

TOXICOLOGY AND CARCINOGENESIS STUDIES OF 8-METHOXYPSORALEN

(CAS NO. 298-81-7)

IN F344/N RATS

(GAVAGE 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 chemical health and safety requirements and must meet or exceed all applicable Federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All NTP toxicology and carcinogenesis studies are subjected to a comprehensive audit before being presented for public review. This Technical Report has been reviewed and approved by the NTP Board of Scientific Counselors' Peer Review Panel in public session; the interpretations described herein represent the official scientific position of the NTP.

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.

Anyone who is aware of related ongoing or published studies not mentioned in this report, or of any errors in this report, is encouraged to make this information known to the NTP. Comments and questions about these Technical Reports on Toxicology and Carcinogenesis Studies should be directed to Dr. J.E. Huff, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-5722).

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF 8-METHOXYPSORALEN

(CAS NO. 298-81-7)

IN F344/N RATS

(GAVAGE STUDIES)

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P.O. Box 12233
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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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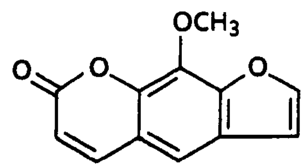

8-METHOXYPSORALEN

CAS No. 298-81-7

$C_{12}H_8O_4$ Molecular weight 216.2

Synonyms: 9-methoxy-7*H*-furo[3,2-*g*]benzopyran-7-one; 6-hydroxy-7-methoxy-5-benzofuranacrylic acid δ-lactone; 8-MP; 8-MOP; 8-methoxy-(furano-3′,2′:6,7-coumarin); 8-methoxy-4′,5′:6,7-furocoumarin; 9-methoxypsoralen; 8-methoxypsoralene; methoxsalen; oxypsoralen

Trade Names: Ammoidin; Meladinin (VAN); Meladinine; Meladoxen; Meloxine; Methoxa-Dome; Mopsoralen; Oxsoralen; Soloxsalen; Trioxun; Xanthotoxin; Xanthotoxine

ABSTRACT

Oral administration of 8-methoxypsoralen followed by exposure to longwave ultraviolet light (primarily ultraviolet A, 320-400 nm) is used in the treatment of vitiligo and psoriasis. 8-Methoxypsoralen also occurs naturally in a variety of vegetables. Toxicology and carcinogenesis studies of 8-methoxypsoralen without ultraviolet A were conducted by administering USP-grade 8-methoxypsoralen (99% pure) in corn oil by gavage to groups of F344/N rats once or for 16 days, 13 weeks, or 2 years. In vitro genetic toxicology tests were performed with bacteria and mammalian cells.

Single-Administration, Sixteen-Day, and Thirteen-Week Studies: In the single-administration studies, the chemical was administered at doses of 0 and 63-1,000 mg/kg. Four of five male rats and 5/5 female rats that received 1,000 mg/kg 8-methoxypsoralen died within 2 days.

In the 16-day studies, the chemical was administered at doses of 0 and 50-800 mg/kg. All rats receiving 800 mg/kg died within 5 days, and one male and one female at 400 mg/kg and one female at 200 mg/kg also died before the end of the studies. The final mean body weights of animals at 200 or 400 mg/kg were 14% or 30% lower than those of vehicle controls. No compound-related effects were observed at necropsy.

In the 13-week studies, the chemical was administered at doses of 0 and 25-400 mg/kg. Six of 10 male rats and 8/10 female rats that received 400 mg/kg died before the end of the studies. The final mean body weight of male rats that received 100, 200, or 400 mg/kg was 12%, 22%, or 45% lower than that of vehicle controls. The final mean body weight of female rats that received 200 or 400 mg/kg was 15% or 35% lower than that of vehicle controls. The liver weight to body weight ratios for all dosed groups of rats except the lowest (25 mg/kg) were greater than those for vehicle controls. Compound-related effects included fatty change in the liver in males and females and atrophy of the testis, seminal vesicles, and prostate.

Based on these results, 2-year studies were conducted by administering 0, 37.5, or 75 mg/kg 8-methoxypsoralen in corn oil by gavage, 5 days per week for 103 weeks, to groups of 50 F344/N rats of each sex.

Body Weight and Survival in the Two-Year Studies: The mean body weights of dosed male rats were generally 3%-14% lower than those of vehicle controls, and the mean body weights of high dose female rats were 5%-17% lower. The survival of both the low and the high dose groups of male rats was lower than that of the vehicle controls (male: vehicle control, 30/50; low dose, 16/50; high dose, 16/50; female: 39/50; 33/50; 36/50), likely because of kidney toxicity and neoplasia.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Mineralization of the renal papilla was observed in high dose male rats (vehicle control, 0/50; low dose, 0/50; high dose, 31/49). The severity of nephropathy was increased in dosed male rats. Focal hyperplasia of renal tubular cells was observed in dosed male rats (0/50; 8/50; 8/49). The incidences of tubular cell adenomas (1/50; 11/50; 8/49), adenocarcinomas (0/50; 1/50; 3/49), and adenomas or adenocarcinomas (combined) (1/50; 12/50; 11/49) were increased in dosed male rats. Hyperplasia of the parathyroid glands (2/49; 22/47; 18/48) and fibrous osteodystrophy (2/50; 10/50; 12/49) in male rats were secondary to chronic nephropathy.

The incidences of carcinomas or squamous cell carcinomas (combined) of the Zymbal gland were increased in dosed male rats (1/50; 7/50; 4/49). The mean historical incidence for carcinomas or squamous cell carcinomas (combined) in corn oil vehicle control male F344/N rats is 0.8% (16/1,949); the highest incidence in any one group is 4% (2/49).

Fibromas of the subcutaneous tissue in male rats occurred with a positive trend (1/50; 5/50; 7/49). An additional high dose male had a sarcoma. The mean historical incidence of fibromas or fibrosarcomas (combined) of subcutaneous tissue in corn oil vehicle control male F344/N rats is 9% (171/1,949).

Alveolar/bronchiolar adenomas occurred with a positive trend in male rats (4/50; 9/50; 9/49). The mean historical incidence of alveolar/bronchiolar neoplasms in corn oil vehicle control male F344/N rats is 3% (68/1,944); the highest observed incidence is 10% (5/50).

Chronic inflammation, ulcers, and epithelial hyperplasia of the forestomach were observed at increased incidences in dosed male rats (chronic inflammation: 1/50; 6/50; 5/49; ulcers: 5/50; 13/50; 11/49; epithelial hyperplasia: 4/50; 19/50; 20/49). Squamous cell papillomas were observed in two low dose male rats.

Squamous cell papillomas were observed in the palate or tongue of one low dose and three high dose female rats; none was observed in vehicle controls. These papillomas were not considered to be related to chemical administration.

Diffuse hypertrophy of the thyroid gland was observed at increased incidences in dosed male rats (2/50; 31/50; 39/49).

Genetic Toxicology: 8-Methoxypsoralen was mutagenic in *Salmonella typhimurium* strain TA104 in the presence and absence of activation and in strains TA98, TA100, and TA102 when tests were conducted with exogenous metabolic activation; 8-methoxypsoralen was not mutagenic with or without activation in strain TA1535. Treatment with 8-methoxypsoralen induced both sister chromatid exchanges (SCEs) and chromosomal aberrations in Chinese hamster ovary (CHO) cells in the absence of exogenous metabolic activation; in the presence of activation, induction of SCEs occurred, but no significant increase in chromosomal aberrations was observed.

Audit: The data, documents, and pathology materials from the 2-year studies of 8-methoxypsoralen have been audited at the NTP Archives. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity** of 8-methoxypsoralen (without ultraviolet radiation) for male F344/N rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney and carcinomas of the Zymbal gland. Subcutaneous tissue fibromas and alveolar/bronchiolar adenomas of the lung in male F344/N rats may have been related to chemical administration. Dose-related nonneoplastic lesions in male F344/N rats included increased severity of nephropathy and mineralization of the kidney and forestomach lesions. There was *no evidence of carcinogenic activity* of 8-methoxypsoralen for female F344/N rats given the chemical at 37.5 or 75 mg/kg per day for 2 years.

SUMMARY OF THE TWO-YEAR GAVAGE AND GENETIC TOXICOLOGY STUDIES OF 8-METHOXYPSORALEN

Male F344/N Rats	Female F344/N Rats
Doses	
0, 37.5, or 75 mg/kg 8-methoxypsoralen in corn oil, 5 d/wk	0, 37.5, or 75 mg/kg 8-methoxypsoralen in corn oil, 5 d/wk
Body weights in the 2-year study	
Dosed lower than vehicle controls	High dose lower than vehicle controls
Survival rates in the 2-year study	
30/50; 16/50; 16/50 (decreased survival of dosed groups probably due to kidney toxicity)	39/50; 33/50; 36/50
Nonneoplastic effects	
Mineralization of the renal papilla (0/50; 0/50; 31/49); increased severity of nephropathy; forestomach lesions	None
Neoplastic effects	
Tubular cell adenomas (1/50; 11/50; 8/49) and adenocarcinomas (0/50; 1/50; 3/49) of the kidney; carcinomas of the Zymbal gland (1/50; 7/50; 4/49); fibromas of the subcutaneous tissue (1/50; 5/50; 7/49); alveolar/bronchiolar adenomas (4/50; 9/50; 9/49)	None
Level of evidence of carcinogenic activity	
Clear evidence	No evidence

Genetic toxicology

Salmonella (gene mutation)	CHO cells in vitro: SCE	CHO cells in vitro: Aberration
Positive with and without S9	Positive with and without S9	Positive without S9; negative with S9

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 6.

A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 9.

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

- **Clear Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.

- **Some Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.

- **Equivocal Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.

- **No Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.

- **Inadequate Study of Carcinogenic Activity** is demonstrated by studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

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

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

CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of 8-Methoxypsoralen is based on the 13-week studies that began in May 1980 and ended in August 1980 and on the 2-year studies that began in May 1981 and ended in May 1983 at SRI International (Menlo Park, California).

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PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on 8-methoxypsoralen on April 18, 1988, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenicity and other observed toxic responses.

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SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF 8-METHOXYPSORALEN

On April 18, 1988, the draft Technical Report on the toxicology and carcinogenesis studies of 8-methoxypsoralen received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina.

Dr. J.K. Dunnick, NIEHS, began the discussion by reviewing the experimental design, results, and proposed conclusions (clear evidence of carcinogenic activity for male rats, no evidence of carcinogenic activity for female rats).

Dr. Gallo, a principal reviewer, agreed with the conclusions. He asked why the studies were not conducted with concurrent administration of ultraviolet (UV) light, since the Food and Drug Administration (FDA) had approved use of this drug in humans only with exposure to UV light. He recommended that the conclusions state that the studies were performed without UV light. Dr. Dunnick reported that the chemical was nominated by the FDA with the request that it be studied without UV light to determine if there were tumorigenic effects from 8-methoxypsoralen alone. She noted that there are appreciable concentrations of 8-methoxypsoralen in some vegetables, e.g., up to 1,000 ppm in parsnips. Dr. Gallo asked that more details on dermal effects be added to the discussion [page 53].

Dr. Sivak, the second principal reviewer, agreed with the conclusions. He proposed that a description of the NTP short-term mouse studies with 8-methoxypsoralen and UV light be added to the discussion [see Appendix E].

Dr. Chinchilli, the third principal reviewer, agreed with the conclusions.

Dr. Lijinsky asked whether there was any indication of $\alpha_{2\mu}$-globulin involvement in the tumorigenic effects. Dr. Dunnick said that there was no evidence of kidney toxicity or hyaline droplet formation in the 13-week studies. Dr. J. Ashby pointed out that this was another chemical containing a furan moiety which was clearly genotoxic.

Dr. Gallo moved that the Technical Report on 8-methoxypsoralen be accepted with the revisions discussed and the conclusions as written for male rats, clear evidence of carcinogenic activity, and for female rats, no evidence of carcinogenic activity. Dr. Hooper seconded the motion, which was approved unanimously with nine votes.

I. INTRODUCTION

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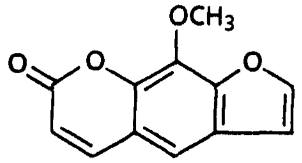

8-METHOXYPSORALEN

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Synonyms: 9-methoxy-7*H*-furo[3,2-*g*]benzopyran-7-one; 6-hydroxy-7-methoxy-5-benzofuranacrylic acid δ-lactone; 8-MP; 8-MOP; 8-methoxy-(furano-3′,2′:6,7-coumarin); 8-methoxy-4′,5′:6,7-furocoumarin; 9-methoxypsoralen; 8-methoxypsoralene; methoxsalen; oxypsoralen

Trade Names: Ammoidin; Meladinin (VAN); Meladinine; Meladoxen; Meloxine; Methoxa-Dome; Mopsoralen; Oxsoralen; Soloxsalen; Trioxun; Xanthotoxin; Xanthotoxine

This report describes studies of the toxicity and carcinogenicity of 8-methoxypsoralen administered by gavage without concomitant ultraviolet radiation exposure. No 2-year studies on the toxicity or carcinogenicity of orally administered 8-methoxypsoralen in rodents have been reported in the literature.

Production and Use

Oral administration of 8-methoxypsoralen followed by exposure to longwave ultraviolet light (primarily ultraviolet A, 320-400 nm) is used to facilitate repigmentation in persons with vitiligo and to treat skin disorders such as psoriasis and mycosis fungoidis (Swinyard and Pathak, 1985). 8-Methoxypsoralen was licensed for use in the United States for vitiligo in 1981 (Fed. Regist., 1981) and for psoriasis in 1982 (personal communication from V. Glocklin, Food and Drug Administration, FDA, to J. Dunnick, NIEHS, 1982). The recommended dose for the treatment of vitiligo is two 10-mg capsules taken 2-4 hours before ultraviolet radiation. For psoriasis, the recommended therapy is one 10-mg capsule for a patient weighing less than 30 kg to four 10-mg capsules for a patient weighing more than 66 kg, taken 2 hours before ultraviolet exposure. The daily dose of 8-methoxypsoralen could be up to 1 mg/kg or a dose of approximately 37 mg/m^2 (Swinyard and Pathak, 1985; PDR, 1988). This treatment is usually given two or three times per week, with the ultraviolet A radiation varying from 0.5 to 3.0 J/cm^2, depending on skin type. More recently, 8-methoxypsoralen followed by ultraviolet A therapy has been used in the treatment of T-cell lymphomas (Edelson et al., 1987). It is estimated that 35,000 prescriptions were written for 8-methoxypsoralen in 1986 (personal communication from C. Baum, FDA, to J. Dunnick, NIEHS, 1987).

Exposure to psoralen derivatives also occurs through ingestion of foods such as parsnips and parsley (Pathak et al., 1962; Ivie et al., 1981). The concentration of 8-methoxypsoralen in parsnip root is reported to be between 26 and 29 ppm (Ivie et al., 1981) or up to 1,100 ppm (Ceska et al., 1986).

The use of plant extracts that now are known to contain psoralen derivatives, including 8-methoxypsoralen, for the treatment of skin diseases dates back to 1200-2000 B.C. in Egypt and India, where boiled extracts from fruits (*Ammi majus* Linnaeus or *Psoralea corylifolia* L.) were applied to the skin or ingested, followed by exposure to the sun (Fitzpatrick and Pathak, 1984). 5-Methoxypsoralen (or Bergapten) may be used in sunscreen formulations produced in Europe but is not among those products approved for this use in the United States (Pathak, 1981).

Toxicity in Humans

Oral administration of 8-methoxypsoralen followed by ultraviolet A radiation has been shown to clear general psoriasis (a disorder of epidermal cell proliferation of unknown etiology), and the mechanism for this effect is thought to be by inhibition of epidermal DNA synthesis (Parrish et al., 1974). Studies showed a dose-dependent increase in the development of cutaneous squamous cell carcinomas after combined 8-methoxypsoralen/ultraviolet A treatment (Stern et al., 1984). Other side effects from treatment with 8-methoxypsoralen and ultraviolet A radiation include erythema and impairment of the immune system (Morison, 1984; Kripke, 1984; Gange and Parrish, 1984). Toxicity from 8-methoxypsoralen alone has not been reported.

8-Methoxypsoralen with ultraviolet A radiation forms crosslinks with DNA (Song, 1984). This combined therapy (PUVA) is one of the few therapies that cause proliferation (melanogenesis) and growth arrest (inhibition of cell growth) in the same tissue (basal layer of epidermis) at the same time (Pathak et al., 1984; Wolff and Honigsmann, 1984).

The International Agency for Research on Cancer reports that the available data are inadequate to make an evaluation on the carcinogenicity of 8-methoxypsoralen alone (or other psoralen or isopsoralen derivatives alone) in humans or in animals (IARC, 1980, 1982, 1986, 1987). There is sufficient evidence that 8-methoxypsoralen given in combination with longwave ultraviolet light is carcinogenic to the skin of mice and humans (IARC, 1982, 1987; Wilbourn et al., 1986).

Toxicity in Animals

The majority of studies on the toxicity of 8-methoxypsoralen have dealt with toxicity after exposure to a combination of 8-methoxypsoralen and ultraviolet A. Topically applied 8-methoxypsoralen followed by ultraviolet A radiation produces skin tumors in mice (Grube et al., 1977; Young et al., 1983). 8-Methoxypsoralen given orally at doses up to 50 mg/kg, 6 days per week, to hairless C3H/HeN-hr mice for 8 months in conjunction with ultraviolet A radiation twice per week did not produce an increase in skin tumors (skin, liver, kidney, and stomach were examined histologically) (Langner et al., 1977). Earlier studies also showed that orally administered psoralen at 0.6-40 mg/kg body weight or 200-400 ppm in feed for 4-12 months in combination with ultraviolet A radiation (250-400 nm) did not produce skin tumors in mice (O'Neal and Griffin, 1957; Griffin et al., 1958; Pathak et al., 1959; Langner et al., 1977).

The oral LD_{50} of micronized 8-methoxypsoralen in male Holtzman rats was reported to be 791 mg/kg, and the oral LD_{50} in Swiss Webster mice was reported to be 449 mg/kg for males and 423 mg/kg for females (Apostolou et al., 1979). After intraperitoneal injection, the LD_{50} of 8-methoxypsoralen in Swiss albino rats was 470 mg/kg (Hakim et al., 1961). 8-Methoxypsoralen was shown to inhibit the in vitro proliferative response of human lymphocytes to phytohemagglutinin and concanavalin A (Cox et al., 1987).

Metabolism and Distribution

Maximum blood levels (3,000 ng/ml) occurred 2 hours after oral administration of Oxsoralen® to patients with vitiligo at a level of 0.6 mg/kg body weight (Chakrabarti et al., 1986). The highest plasma level achieved in psoriatic patients after a topical dose of 0.32 mg/kg was 69 ng/ml, 4 hours after application of the drug (Neild and Scott, 1982). The maximum plasma level after an oral dose of 40 mg was greater than 1,000 ng/ml (Busch et al., 1978). 8-Methoxypsoralen labeled with carbon-14 at the 8 position was administered at 40 mg per person. Sixty percent of the radioactivity was excreted in the urine within 8 hours; by 96 hours, 74% was excreted in the urine and 14% in the feces. The main metabolic transformation of 8-methoxypsoralen takes place at the 2′,3′ position of the furan ring (Schmid et al., 1980).

Maximum concentrations of [^{3}H]8-methoxypsoralen were seen in serum 10 minutes after rats were administered [^{3}H]8-methoxypsoralen orally at 1 mg/kg; the peak serum level was 686 ng/ml. Maximum concentrations were seen in the skin and liver from 30 minutes to 3 hours after chemical administration (Wulf and

Andreasen, 1981; Engel and Wulf, 1982). After Sprague Dawley rats were given an intravenous dose of [^{14}C]8-methoxypsoralen (10 mg/kg), 71% and 26% of the dose was recovered in the urine and feces, respectively, within 72 hours (Mays et al., 1986). Metabolites in the urine identified by enzymatic hydrolysis were 8-hydroxypsoralen, 5-hydroxy-8-methoxypsoralen, 5,8-dihydroxypsoralen, 5,8-dioxopsoralen, and 6-(7-hydroxy-8-methoxycoumaryl)-acetic acid, and 8-methoxypsoralen.

Reproductive Toxicity

No studies on the reproductive toxicity or the teratogenic potential of 8-methoxypsoralen have been located in the literature.

Genetic Toxicology

Results from in vitro DNA-binding studies demonstrated that approximately 2.5% of the available 8-methoxypsoralen (added at a ratio of one 8-methoxypsoralen molecule for every 30 DNA base pairs) intercalated with DNA in the absence of ultraviolet light (Isaacs et al., 1984). In contrast, photoactivation by near (i.e., longwave) ultraviolet light caused 68% of the added 8-methoxypsoralen (initially present in a ratio of one 8-methoxypsoralen molecule for every 22 DNA base pairs) to covalently bind to DNA. This demonstrated a greater than 25-fold increase in the binding of photoactivated 8-methoxypsoralen compared with the binding of 8-methoxypsoralen without ultraviolet radiation (0.82 molecules "dark bound" vs. 31 molecules covalently bound per 1,000 DNA base pairs). The covalent binding that occurs with photoactivation is an irreversible reaction that produces DNA monoadducts and crosslinks, whereas intercalation is a reversible association.

The mutagenicity and toxicity of 8-methoxypsoralen plus ultraviolet A radiation has been thoroughly documented in studies with phage (Esipova et al., 1978; Belogurov and Zavilgelsky, 1981), bacteria (Igali et al., 1970; Townsend et al., 1971; Ashwood-Smith and Grant, 1974; Bridges et al., 1979; Peshekhonov and Tarasov, 1981), fungi (Swanbeck and Thyresson, 1974; Averbeck et al., 1975; Simpson and Caten, 1979a,b; Muronets et al., 1980), and algae (Schimmer, 1979). Schimmer and Fischer (1980) indicated that the addition of S9 to a culture would diminish the toxic and mutagenic properties of 8-methoxypsoralen plus ultraviolet radiation; however, Kirkland et al. (1983) demonstrated that very similar levels of reactivity were exhibited by 8-methoxypsoralen plus ultraviolet radiation in *Escherichia coli* WP2*uvrA*$^-$ with and without induced rat liver S9. Induction of gene mutations, as measured by increased resistance to thioguanine and azaguanine, was reported for Chinese hamster V79 cells after treatment with 8-methoxypsoralen plus ultraviolet radiation without S9 (Burger and Simons, 1979; Arlett et al., 1980; Frank and Williams, 1982). In vitro induction of chromosomal aberrations in human lymphocytes (Sasaki and Tonomura, 1973; Swanbeck et al., 1975; Waksvik et al., 1977) and human fibroblasts (Natarajan et al., 1981) has been reported, along with induction of sister chromatid exchanges (SCEs) (Carter et al., 1976; Shafer et al., 1977; Mourelatos et al., 1977a; Abel and Schimmer, 1981; West et al., 1982; Bredberg and Lambert, 1983) and DNA strand breaks (Bredberg et al., 1982). Several additional studies with mammalian cells confirmed the clastogenicity of 8-methoxypsoralen plus ultraviolet A radiation in vitro (Latt and Loveday, 1978; MacRae et al., 1980; Natarajan et al., 1981; Ashwood-Smith et al., 1982; Hook et al., 1983).

The in vitro mutagenic activity of 8-methoxypsoralen without ultraviolet radiation ("dark" treatment) has been investigated by a number of researchers, with varying results. Induction of gene mutations by 8-methoxypsoralen alone has been reported in bacteria exposed during the growth phase when DNA replication was occurring (Bridges and Mottershead, 1977; Ashwood-Smith et al., 1982; Ellenberger, 1982; Kirkland et al., 1983). Bridges and Mottershead (1977) reported that 8-methoxypsoralen (30 μg/ml liquid preincubation concentration) without ultraviolet radiation was a frameshift mutagen without S9 activation in *E. coli* K12/ND160 and *Salmonella typhimurium* TA98. Kirkland et al. (1983) reproduced the "dark" mutagenesis by 8-methoxypsoralen (5 μg/plate or 2.5 mg/ml) in *E. coli* WP2*uvrA*$^-$(pKM101) but only in the presence of induced rat liver S9; they could not duplicate the positive response in *S. typhimurium*

TA98, but this might be the result of the lower doses used. The activity observed in the presence of S9, also demonstrated by Kirkland et al. with 8-methoxypsoralen plus ultraviolet A radiation, indicates that metabolism does not necessarily destroy the mutagenic potential of 8-methoxypsoralen. Gene mutation, as measured by increased azaguanine and thioguanine resistance, has been reported in Chinese hamster V79 cells treated with 8-methoxypsoralen alone (Arlett et al., 1980). Small increases in SCE frequency were reported for human lymphocytes treated with therapeutically relevant doses of 10^{-6} M 8-methoxypsoralen (Wulf, 1978), and larger increases in SCEs were observed in human lymphocytes treated with 50-100 times the clinical concentration (0.4 µg/ml in the peripheral blood) of 8-methoxypsoralen (Faed and Peterson, 1980). Treatment of the Syrian hamster cell line, BHK-21, with 22-220 µg/liter 8-methoxypsoralen induced a marginal increase in SCEs which was related to the dose (MacRae et al., 1980). These same doses, in conjunction with ultraviolet radiation, produced a much greater increase in SCEs (at the high dose, 15 SCEs per cell without ultraviolet radiation and 106 SCEs per cell with ultraviolet radiation).

Several studies have reported negative mutagenicity results after 8-methoxypsoralen treatment in the dark. Probst et al. (1981) observed no increase in revertant colonies in several strains of *S. typhimurium* after treatment with a 10,000-fold concentration gradient of 8-methoxypsoralen with and without exogenous metabolic activation. West et al. (1982) reported no induction of SCEs in human epidermal cells treated with up to 2.5 µg/ml 8-methoxypsoralen (similar to the serum concentration of the drug in patients treated with 8-methoxypsoralen plus ultraviolet A radiation), and Latt and Loveday (1978) observed no induction of SCEs in Chinese hamster ovary cells treated with 6×10^{-6} M (therapeutic dose) 8-methoxypsoralen in the dark. Burger and Simons (1979) and Babudri et al. (1981) compared the effects of dark treatment with 8-methoxypsoralen versus 8-methoxypsoralen plus ultraviolet A radiation in Chinese hamster cells; both studies reported induction of gene mutation by 8-methoxypsoralen plus ultraviolet A radiation but not by 8-methoxypsoralen alone. In the Babudri study, 5 µg/ml 8-methoxypsoralen in the dark produced no increase in gene mutation, but when the treatment was accompanied by 2 minutes of ultraviolet radiation, a positive response was obtained. Washing of the cells to remove nonbound 8-methoxypsoralen followed by additional irradiation for up to 6 minutes greatly increased both the cell killing and the mutation frequency. The authors speculated that the second radiation treatment converted monoadducts to crosslinks. Although both events lead to mutation, crosslinking results in greater lethality to cells. In summary, the published reports of genetic effects induced in vitro by 8-methoxypsoralen alone generally indicate a lower level of activity than that seen when 8-methoxypsoralen exposure is in the presence of ultraviolet radiation.

Studies of the in vivo genetic effects of 8-methoxypsoralen and ultraviolet A radiation have, in general, been negative. Analysis of human lymphocytes obtained from psoriatic patients treated with 8-methoxypsoralen plus ultraviolet A radiation showed no increase in SCEs or chromosomal aberrations compared with controls (Wolff-Schreiner et al., 1977; Mourelatos et al., 1977a,b; Lambert et al., 1978; Brogger et al., 1978a,b; Faed et al., 1980). However, Albertini (1979) reported an elevated frequency of thioguanine-resistant variants in lymphocytes from a group of psoriatic and vitiligo patients compared with controls, and Friedmann and Rogers (1980) observed inhibition of phytohemagglutinin-stimulated DNA synthesis in lymphocytes of psoriatic patients treated with 8-methoxypsoralen plus ultraviolet A radiation. Shuler and Latt (1979) reported a dose-related increase in SCEs in Chinese hamster cheek pouch mucosal cells after an intraperitoneal injection of 0.5, 1.0, 2.5, or 5.0 mg/kg 8-methoxypsoralen, followed after 45 minutes by 5 minutes of ultraviolet irradiation of the cheek pouches, but they detected no increase in the bone marrow cell SCE frequency. No increase in SCEs was seen in animals administered 8-methoxypsoralen alone (highest dose used was 2.5 mg/kg).

Study Rationale

8-Methoxypsoralen was nominated by the National Cancer Institute and the Food and Drug Administration in 1978 for toxicity and carcinogenicity studies in rats because no data were available on the carcinogenicity of this compound in rodents after oral administration. Orally administered 8-methoxypsoralen in combination with ultraviolet radiation was being considered for use in the treatment of psoriasis at the time of nomination. This report describes the studies of the toxicity and carcinogenicity of 8-methoxypsoralen alone. The oral route of administration was chosen because the drug is given orally to humans. The NTP also performed studies in HRA/Skh mice in which 8-methoxypsoralen was administered orally in combination with ultraviolet radiation for 13-weeks (Dunnick et al., 1987; Appendix E) and for 1 year (currently ongoing).

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF 8-METHOXYPSORALEN

8-Methoxypsoralen was obtained in two lots (lot nos. 21335 and 21784) from Elder Pharmaceuticals (Table 1). Purity and identity determinations on both lots were conducted at Midwest Research Institute (MRI) (Kansas City, Missouri). MRI reports on the analyses performed in support of the 8-methoxypsoralen studies are on file at NIEHS.

Both lots of the study chemical were identified as 8-methoxypsoralen by spectroscopy; the infrared, ultraviolet/visible, and nuclear magnetic resonance spectra (Figures 1 to 4) were consistent with those expected for the structure and with literature spectra (Sadtler Standard Spectra; Abu-Mustafa and Fayez, 1967; Lee and Soine, 1969) of 8-methoxypsoralen.

Purity for both lots was determined by elemental analysis, Karl Fischer water analysis, titration for free acid with 0.1 N sodium hydroxide, thin-layer chromatography, and gas chromatography. Thin-layer chromatography was performed on silica gel plates with two solvent systems, 100% anhydrous diethyl ether (system 1) and hexanes:ethyl acetate:methanol (65:26:9) (system 2). Visualization was performed under visible light, under ultraviolet light at 254 and 366 nm, and with a spray reagent of alkaline potassium permanganate. Gas chromatographic analysis was performed with flame ionization detection and a nitrogen carrier on a 1% SP1000 column (system 1) and on a 3% SP2100 column (system 2). The results of the elemental analyses for carbon and hydrogen were in agreement with the theoretical values for both lots. The water content of both lots was less than 0.1%. Titration of free acid indicated concentrations of less than 0.1% for lot no. 21335 and 0.20% for lot no. 21784. Gas chromatography of lot no. 21335 indicated one impurity with an area of 0.42% that of the major peak (system 1) or one impurity with a relative area of 0.28% (system 2); lot no. 21784 also had one impurity by each system, with relative areas of 0.20% (system 1) and 0.16% (system 2). Thin-layer chromatography detected only a single spot in both lots with system 1 and a major spot and a trace impurity in both lots with system 2. Comparison of the molar absorptivity at 300 nm of the study chemical with a USP standard indicated a purity of 96.9% for lot no. 21335 and 99.5% for lot no. 21784. USP specifications are 98%-102%.

Reanalysis of lot no. 21335 was conducted in September 1984 with the standard USP battery of tests. The study material met USP requirements for the infrared spectrum, had a melting point of 144.9°-146.4° C, and contained 0.07% water; a 0.02% residue after ignition was observed. The material was within specifications for heavy metal content and showed a single spot by thin-layer chromatography with benzene: ethyl acetate (9:1) as a solvent system. Comparison of the molar absorbance at 300 nm with a USP standard indicated a purity of 100.4%. Results of all tests were within USP specifications.

Stability studies performed by gas chromatography with the same system as described before for system 2 indicated that 8-methoxypsoralen was stable as the bulk chemical within the limits of experimental error, when stored for 2 weeks protected from light at temperatures up

TABLE 1. IDENTITY AND SOURCE OF 8-METHOXYPSORALEN USED IN THE GAVAGE STUDIES

Single-Administration Studies	Sixteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
Lot Numbers			
21335	21335	21335	21335; 21784
Date of Initial Use			
8/23/79	3/18/80	5/28/80	21335--5/28/81; 21784--11/23/81
Supplier			
Elder Pharmaceuticals (Bryan, OH)	Elder Pharmaceuticals (Bryan, OH)	Elder Pharmaceuticals (Bryan, OH)	21335--Elder Pharmaceuticals (Bryan, OH); 21784--Elder Pharmaceuticals (Covina, CA)

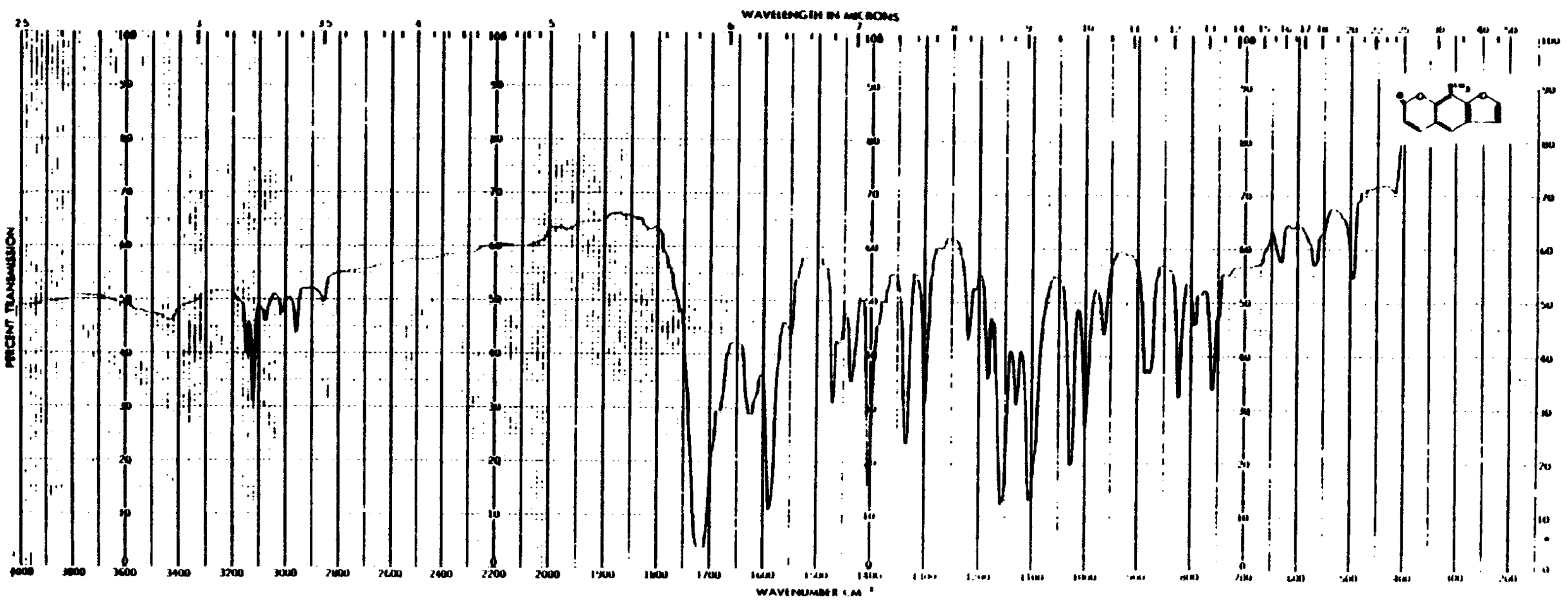
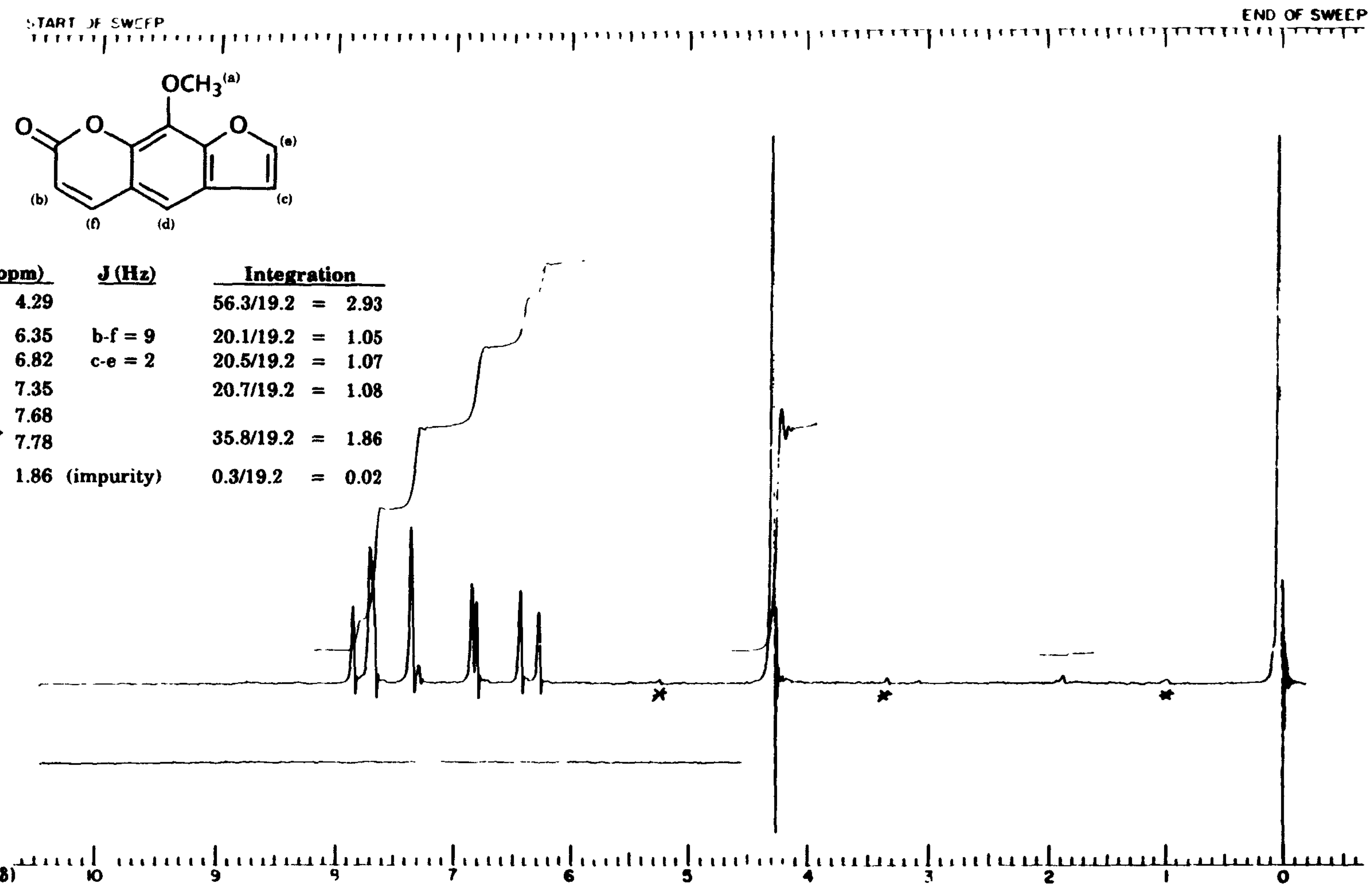


FIGURE 1. INFRARED ABSORPTION SPECTRUM OF 8-METHOXYPSORALEN (LOT NO. 21335)



δ (ppm)		J (Hz)	Integration
(a)	4.29		56.3/19.2 = 2.93
(b)	6.35	b-f = 9	20.1/19.2 = 1.05
(c)	6.82	c-e = 2	20.5/19.2 = 1.07
(d)	7.35		20.7/19.2 = 1.08
(e)	7.68		
(f)	7.78		35.8/19.2 = 1.86
(g)	1.86	(impurity)	0.3/19.2 = 0.02

FIGURE 2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF 8-METHOXYPSORALEN (LOT NO. 21335)

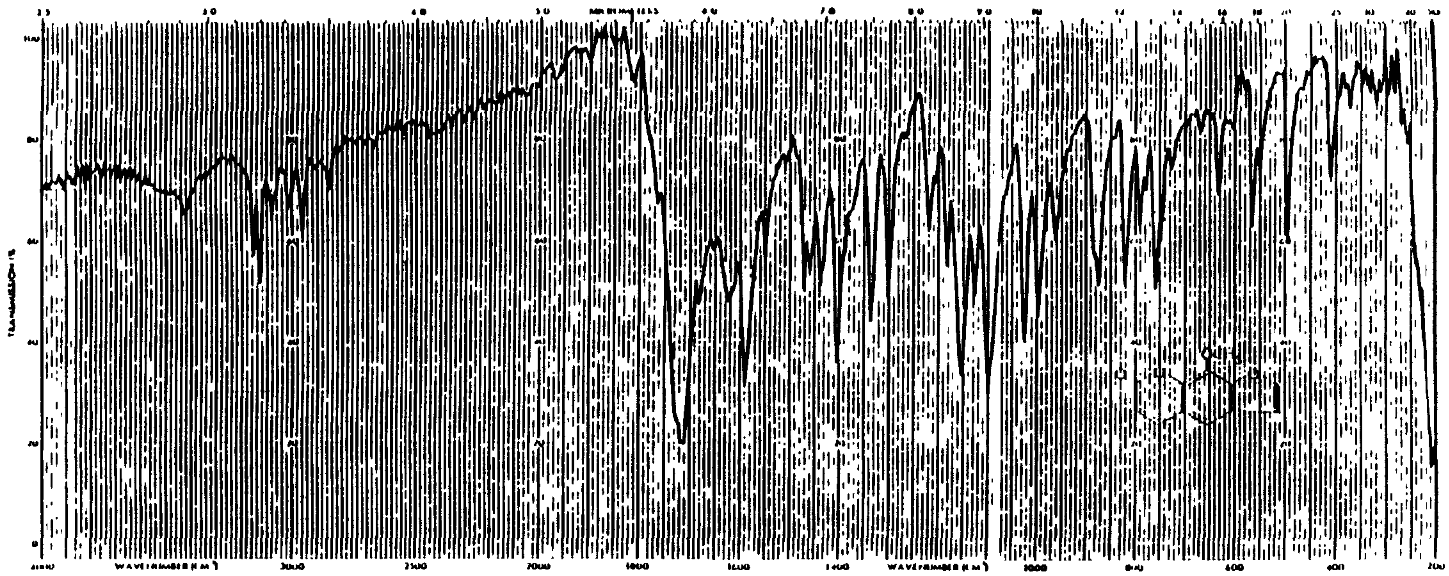


FIGURE 3. INFRARED ABSORPTION SPECTRUM OF 8-METHOXYPSORALEN (LOT NO. 21784)

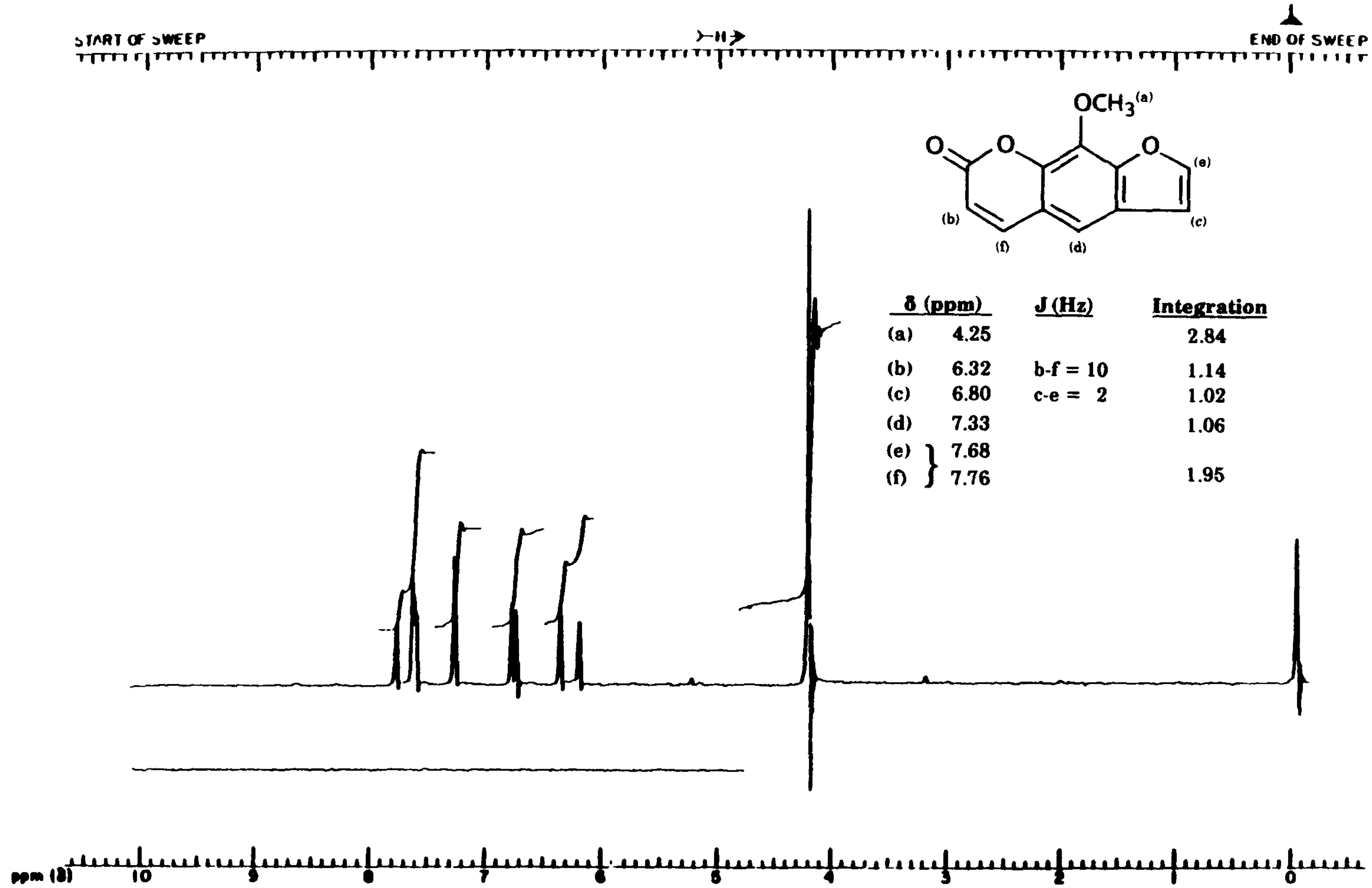


FIGURE 4. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF 8-METHOXYPSORALEN (LOT NO. 21784)

to 60° C. 8-Methoxypsoralen was kept protected from light in sealed plastic containers at room temperature. Periodic characterization of 8-methoxypsoralen by ultraviolet spectroscopy at 249 nm and with the same gas chromatographic system as described for system 1 detected no deterioration over the course of the studies.

PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES

For the single-administration studies, 8-methoxypsoralen was suspended in corn oil by homogenizing the mixture in a blender (Table 2). 8-Methoxypsoralen was difficult to mix with corn oil; in the rest of the studies, it was dissolved in acetone and then mixed with corn oil; the acetone was then removed under vacuum at 40° C, leaving a milky suspension. The stability of 8-methoxypsoralen in corn oil (10 mg/ml) was determined by gas chromatography with flame ionization detection and a 5% NPGSB plus 1% phosphoric acid column with nitrogen as the carrier, after extraction with acetonitrile, centrifugation, and the addition of triphenylethylene as an internal standard. The chemical, dispersed in corn oil as a finely divided amorphous solid, exhibited a 2% decrease in concentration after 7 days' storage in the dark at room temperature. The dose mixtures were stored for up to 10 days in the dark at 5° C for the 16-day and most of the 13-week studies. For the remainder of the 13-week studies, they were stored at room temperature. For the 2-year studies, dose mixtures were kept at room temperature usually for no longer than 13 days. The analytical chemistry laboratory performed an additional study by high-performance liquid chromatography to determine the stability of 8-methoxypsoralen in corn oil; a Waters µBondapak C_{18} column with detection at 254 nm and with a mobile phase of water:acetonitrile (55:45) was used. 8-Methoxypsoralen in corn oil at a concentration of 7.5 mg/ml was stable in the dark at room temperature for 14 days.

TABLE 2. PREPARATION AND STORAGE OF DOSE MIXTURES IN THE GAVAGE STUDIES OF 8-METHOXYPSORALEN

Single-Administration Studies	Sixteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
Preparation			
Suspensions of 8-methoxypsoralen were prepared by mixing the appropriate weight of chemical with the appropriate volume of corn oil for 3 min in a Waring blender	The chemical was dissolved in acetone and mixed with corn oil; acetone was removed by rotary evaporation until constant weight was obtained. Samples were prepared under white fluorescent light, but flasks were covered with tin foil during evaporation. The suspensions were kept in foil-wrapped round-bottom flasks and were stirred constantly with a magnetic stirrer during dosing	Similar to 16-d studies except prepared under yellow fluorescent light	Chemical dissolved in acetone and mixed with corn oil; acetone removed by rotary evaporation at 40° C; all procedures conducted under yellow fluorescent light
Maximum Storage Time			
Overnight	10 d	10 d	13 d (on two occasions stored for 19-21 d)
Storage Conditions			
In refrigerator in the dark	5° C in the dark	5° C in the dark until 6/16/80; room temperature thereafter	At room temperature in glass-stoppered, foil-wrapped flasks

Periodic analysis of formulated 8-methoxypsoralen in corn oil was conducted at the study laboratory and at the analytical chemistry laboratory. For the 13-week studies, dose mixtures were analyzed three times by gas chromatography with flame ionization detection after the dose mixture was extracted with acetonitrile. The first analysis was performed with an external standard and a 10% DC200 column with nitrogen as the carrier; the other analyses were performed with triphenylethylene as an internal standard with the same column as described above. Samples mixed on July 17, 1980, were analyzed with both internal and external standards; results ranged from 88% to 98.5% of the target concentrations (Table 3). Concentrations were consistently low, although generally within the ±10% limits, because of crystallization of 8-methoxypsoralen on the wall of the flask during evaporation of the acetone in preparation of the dose mixtures. Samples were kept at room temperature protected from light to minimize the problem of crystallization.

During the 2-year studies, the dose mixtures were analyzed at approximately 8-week intervals by gas chromatography or ultraviolet absorption at 249 nm after extraction with acetonitrile. For the 8-methoxypsoralen studies, the mixtures were formulated within ±10% of the target concentrations 39/42 times (approximately 93%) throughout the studies (Table 4). Results of periodic referee analyses performed by the analytical chemistry laboratory indicated agreement with the results from the study laboratory (Table 5).

TABLE 3. RESULTS OF ANALYSIS OF DOSE MIXTURES IN THE THIRTEEN-WEEK GAVAGE STUDIES OF 8-METHOXYPSORALEN

Date Mixed	Concentration of 8-Methoxypsoralen in Corn Oil (mg/ml) Target	Determined (a)	Determined as a Percent of Target
05/30/80	5.0	(b,c) 4.7	94
	10.0	(b,c) 9.0	90
	20.0	(b,c) 18.0	90
	40.0	(b,c) 36.0	90
	80.0	(b,c) 78.8	98.5
07/17/80	10.0	8.8	(d) 88
	20.0	18.8	94
	40.0	38.8	97
	80.0	74.2	93
	10.0	(c) 8.8	(d) 88
	20.0	(c) 19.4	97
	40.0	(c) 36.9	92
	80.0	(c) 74.8	93.5
08/21/80	10.0	(b) 9.7	96
	20.0	(b) 19.4	97
	40.0	(b) 38.5	96
	80.0	(b) 73.8	92

(a) Results of duplicate analysis unless otherwise specified
(b) Results of single analysis
(c) Performed with external standard
(d) Out of specifications

TABLE 4. RESULTS OF ANALYSIS OF DOSE MIXTURES IN THE TWO-YEAR GAVAGE STUDIES OF 8-METHOXYPSORALEN

Date Mixed	Concentration of 8-Methoxypsoralen in Corn Oil for Target Concentration (mg/ml) (a) 7.5	15
05/19/81	(b) 7.78	(b) 16.2
	(b) 7.98	(b) 16.0
05/26/81	7.99	16.2
	7.82	(c) 16.9
07/14/81	7.69	16.4
	7.98	16.0
09/22/81	7.45	14.8
	7.34	14.8
11/17/81	(d) 7.49	(d) 14.6
01/12/82	(d) 7.42	(d) 14.4
03/16/82	(d) 7.62	(d) 14.8
05/18/82	(d) 7.17	(d) 14.8
07/14/82	(d) 7.42	(d) 14.8
09/07/82	(d) 6.92	(d) 14.3
11/02/82	(d) 8.03	(d) 16.4
01/05/83	7.67	15.7
01/25/83	(e) 8.09	(e) 16.4
02/15/83	(f) 8.15	(f) 16.0
03/11/83	7.50	15.3
04/19/83	(c) 8.28	(c) 17.3
05/12/83	7.82	16.0
Mean (mg/ml)	7.70	15.6
Standard deviation	0.347	0.87
Coefficient of variation (percent)	4.5	5.6
Range (mg/ml)	6.92-8.28	14.3-17.3
Number of samples	21	21

(a) Results of duplicate analysis
(b) Results of reanalysis 1 week after original analysis; mixture not used in the studies.
(c) Outside of specifications; used in the studies.
(d) Results of triplicate analysis
(e) Analysis performed on archival sample 1 month after mixing
(f) Results of single analysis

TABLE 5. RESULTS OF REFEREE ANALYSIS OF DOSE MIXTURES IN THE TWO-YEAR GAVAGE STUDIES OF 8-METHOXYPSORALEN

Date Mixed	Target Concentration (mg/ml)	Determined Concentration (mg/ml) Study Laboratory (a)	Referee Laboratory (b)
05/19/81	7.5	7.78	7.70
07/14/81	15.0	16.0	14.94
01/25/83	7.5	(c) 8.09	7.74
05/12/83	15.0	16.0	15.2

(a) Results of duplicate analysis
(b) Results of triplicate analysis
(c) Analysis performed on an archival sample 1 month after the mix date

II. MATERIALS AND METHODS

SINGLE-ADMINISTRATION STUDIES

Male and female F344/N rats were obtained from Charles River Breeding Laboratories and held for 14 days before the studies began. Groups of five males and five females were administered a single dose of 0, 63, 125, 250, 500, or 1,000 mg/kg 8-methoxypsoralen in corn oil by gavage. Rats were observed once per day and were weighed on day 1. Necropsies were not performed. Details of animal maintenance are presented in Table 6.

SIXTEEN-DAY STUDIES

Male and female F344/N rats were obtained from Charles River Breeding Laboratories and held for 12 days before the studies began. Animals were 7 weeks old when placed on study. Groups of five males and five females were administered 0, 50, 100, 200, 400, or 800 mg/kg 8-methoxypsoralen in corn oil by gavage on 12 days over a 16-day period. Rats were observed once per day and were weighed on days 0, 7, and 15. A necropsy was performed on all animals. Details of animal maintenance are presented in Table 6.

THIRTEEN-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxic effects of repeated administration of 8-methoxypsoralen and to determine the doses to be used in the 2-year studies.

Four-week-old male and female F344/N rats were obtained from Charles River Breeding Laboratories, observed for 13 days, distributed to weight classes, and assigned to groups according to a table of random numbers. Groups of 10 males and 10 females were administered 0, 25, 50, 100, 200, or 400 mg/kg 8-methoxypsoralen in corn oil by gavage, 5 days per week for 13 weeks. Rats were observed once per day and were weighed on days 0, 7, and 15. Moribund animals were killed. Individual animal weights were recorded once per week. At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Tissues and groups examined are listed in Table 6.

TWO-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats were administered 0, 37.5, or 75 mg/kg 8-methoxypsoralen in corn oil by gavage, 5 days per week for 103 weeks.

Source and Specifications of Animals

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

Animal Maintenance

Animals were housed five per cage. Feed and water were available ad libitum. Cages were rotated once per week. Further details of animal maintenance are given in Table 6. Each dose group was housed on a separate rack. Each rack had five tiers (five cages per tier). Study groups were housed on the top four tiers, and sentinel animals were housed on the bottom tier. Once per week, all cages on the fourth tier were moved to the top tier and cages on the top three rows were moved down one tier. Special yellow fluorescent lighting in animal rooms was on for 12 hours (6:30 a.m.-6:30 p.m.) and off for 12 hours. General Electric F40/GO (40 W yellow) bulbs were used to reduce exposure to ultraviolet radiation known to be emitted by conventional fluorescent bulbs. The emission spectrum of F40/GO bulbs is about 500-700 nm, whereas that

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF 8-METHOXYPSORALEN

Single-Administration Studies	Sixteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
EXPERIMENTAL DESIGN			
Size of Study Groups			
5 males and 5 females	5 males and 5 females	10 males and 10 females	50 males and 50 females
Doses			
0, 63, 125, 250, 500, or 1,000 mg/kg 8-methoxypsoralen in corn oil by gavage; dose vol--5 ml/kg; multiple doses of 250 mg/kg were given to the 500 and 1,000 mg/kg groups; vehicle controls received 4 doses of 5 ml/kg corn oil	0, 50, 100, 200, 400, or 800 mg/kg 8-methoxypsoralen in corn oil by gavage; dose vol-- 5 ml/kg	0, 25, 50, 100, 200, or 400 mg/kg 8-methoxypsoralen in corn oil by gavage; dose vol-- 5 ml/kg except for wk 6 when all groups except 400 mg/kg received 2.5 ml/kg	0, 37.5, or 75 mg/kg 8-methoxypsoralen in corn oil by gavage; dose vol--5 ml/kg
Date of First Dose			
8/23/79	3/18/80	5/28/80	5/28/81
Date of Last Dose			
N/A	4/2/80	8/27/80	75 mg/kg groups--5/18/83; 37.5 mg/kg groups--5/23/83
Duration of Dosing			
Single dose	5 d/wk for 12 doses over 16 d	5 d/wk for 13 wk	5 d/wk for 103 wk
Type and Frequency of Observation			
Observed 1 × d; weighed before dosing	Observed 1 × d; weighed initially and 1 × wk thereafter	Observed 1 × d; weighed initially and 1 × wk thereafter	Observed 1 × d; weighed initially, 1 × wk for 13 wk, and then 1 × mo
Necropsy and Histologic Examinations			
No necropsy performed	Necropsy performed on all animals; histologic exams not performed	Necropsy performed on all animals; histologic exams performed on the following tissues of the vehicle control, 200 mg/kg, and 400 mg/kg groups: adrenal glands, brain, colon, esophagus, eyes, gross lesions and tissue masses with regional lymph nodes, heart, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular or mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes or ovaries/uterus, salivary glands, small intestine, spinal cord (if neurologic signs present), spleen, sternebrae or vertebrae or femur including marrow, stomach, thymus, thyroid gland, trachea, and urinary bladder; liver examined for lower dose groups; liver weighed at necropsy	Necropsy and histologic exams performed on all animals; the following tissues were examined: adrenal glands, brain, cecum, clitoral or preputial glands, colon, costochondral junction, duodenum, esophagus, eyes, gross lesions and tissue masses with regional lymph nodes, heart and aorta, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroids, pituitary gland, rectum, salivary glands, sciatic nerve, scrotal sac/tunica vaginalis/seminal vesicles/prostate/epididymis/testes or ovaries/uterus, spinal cord, spleen, sternebrae or vertebrae or femur including marrow, stomach, thigh muscle, thymus, thyroid gland, tongue, trachea, urinary bladder, and Zymbal gland

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF 8-METHOXYPSORALEN (Continued)

Single-Administration Studies	Sixteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE			
Strain and Species			
F344/N rats	F344/N rats	F344/N rats	F344/N rats
Animal Source			
Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Kingston, NY)
Study Laboratory			
SRI International	SRI International	SRI International	SRI International
Method of Animal Identification			
Ear punch	Ear punch	Ear punch	Ear punch
Time Held Before Study			
14 d	12 d	13 d	14 d
Age When Placed on Study			
6 wk	7 wk	6 wk	6-7 wk
Age When Killed			
8 wk	9 wk	19 wk	110-113 wk
Necropsy or Kill Dates			
9/7/79	4/3/80	8/28/80-8/29/80	Vehicle control--6/6/83-6/9/83; 37.5 mg/kg--6/1/83-6/3/83; 75 mg/kg--5/26/83-5/31/83
Method of Animal Distribution			
Animals distributed to weight classes and then assigned to cages by one table of random numbers and to groups by another table of random numbers	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
Feed			
Purina Rodent Laboratory Chow #5001®	NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum	Same as 16-d studies	Same as 16-d studies
Bedding			
Hardwood chips (P.W.I. Inc., Lowville, NY)	Ab-Sorb-Dri (Lab Products, Inc., Maywood, NY)	Ab-Sorb-Dri (Lab Products, Inc., Rochelle Park, NJ)	Ab-Sorb-Dri hardwood chips (Lab Products, Inc., Maywood, NY)
Water			
Automatic watering system deionized water, sterilized by UV; available ad libitum	Same as single-administration studies	Automatic watering system (Systems Engineering, Napa, CA); available ad libitum	Automatic watering system (SRI International and Systems Engineering, Napa, CA); deionized, filtered, UV-sterilized water available ad libitum
Cages			
Polyethylene (Lab Products, Inc., Rochelle Park, NJ)	Same as single-administration studies	Polycarbonate (Lab Products, Inc., Rochelle Park, NJ)	Same as 13-wk studies
Cage Filters			
Nonwoven polyester fiber (Lab Products, Inc., Rochelle Park, NJ)	Same as single-administration studies	Same as single-administration studies	Polyester filter sheets (Snow Filtration, Cincinnati, OH)

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF 8-METHOXYPSORALEN (Continued)

Single-Administration Studies	Sixteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)			
Cage Rotation			
None	None	1 × wk	1 × wk
Animals per Cage			
5	5	5	5
Other Chemicals on Study in the Same Room			
None	None	None	None
Animal Room Environment			
Temp--22° ± 4° C; hum--50%-65%; fluorescent light 12 h/d; 12-15 room air changes/h	Temp--24° ± 2° C; hum--40%-60%; yellow fluorescent light 12 h/d; 12-15 room air changes/h	Temp--74°-78° F; hum--46%-76%; yellow fluorescent light 12 h/d; 13-15 room air changes/h	Temp--68°-84° F; hum--20%-93%; yellow fluorescent light 12 h/d; 13.5-15 room air changes/h

of conventional white bulbs (F40 CW) ranges from 310 to 700 nm. No ultraviolet radiation was detected at the level of the top animal cages in the yellow-lighted room.

Clinical Examinations and Pathology

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

During necropsy, all organs and tissues were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Tissues examined are listed in Table 6.

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

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

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

Statistical Methods

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

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

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

Analysis of Tumor Incidence: Three statistical methods are used to analyze tumor incidence data: life table tests, incidental tumor analysis, and Fisher exact/Cochran-Armitage trend analyses. Tests of significance include pairwise comparisons of high dose and low dose groups with vehicle controls and tests for overall dose-response trends. For studies in which administration of the study compound has little effect on survival, the results of the three alternative analyses will generally be similar. When differing results are obtained by the three methods, the final interpretation of the data will depend on the extent to which the tumor under consideration is regarded as being the cause of death. Continuity-corrected tests are used in the analysis of tumor incidence, and reported P values are one-sided. For statistical purposes, all animals that died or were killed after the first day of the terminal kill were considered to have died on the first day of the terminal kill. The procedures described below also were used to evaluate selected nonneoplastic lesions.

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

Incidental Tumor Analyses--The second method of analysis assumed that all tumors of a given type observed in animals that died before the end of the study were "incidental"; i.e., they were merely observed at necropsy in animals dying of an unrelated cause. According to this approach, the proportions of tumor-bearing animals in dosed and vehicle control groups were compared in each of five time intervals: weeks 0-52, weeks 53-78, weeks 79-92, week 93 to the week before the terminal-kill period, and the terminal-kill period. The denominators of these proportions were the number of animals actually examined for tumors during the time interval. The individual time interval comparisons were then combined by the previously described method to obtain a single overall result. (See Haseman, 1984, for the computational details of both methods.) A recently developed method for the analysis of incidental tumors based on logistic regression (Dinse and Lagakos, 1983) was also employed as a supplemental test in some instances. This method has the advantage of not requiring time intervals in the statistical evaluation.

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

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

GENETIC TOXICOLOGY

Salmonella Protocol: Testing was performed as reported by Ames et al. (1975) with modifications listed below and described in greater detail by Haworth et al. (1983) and Mortelmans et al. (1986). All tests were performed under yellow light to eliminate interaction of 8-methoxypsoralen with ultraviolet radiation. Chemicals were sent to the laboratories as coded aliquots from Radian Corporation (Austin, Texas). The study chemical was incubated with the *Salmonella typhimurium* tester strains (TA98, TA100, TA102, TA104, and TA1535) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver) for 20 minutes at 37° C before the addition of soft agar supplemented with L-histidine and D-biotin and subsequent plating on minimal glucose agar plates. Incubation was continued for an additional 48 hours.

Chemicals were tested in a hierarchy, with initial screening performed in TA98 and TA100. Because the positive responses obtained in these two strains were not particularly strong, additional tests were conducted with TA1535. The negative results in this strain prompted further testing with TA102 and TA104.

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

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

Chinese Hamster Ovary Cytogenetics Assays: Testing was performed as reported by Galloway et al. (1985, 1987) and is described briefly below. Chemicals were sent to the laboratories as coded aliquots from Radian Corporation (Austin, Texas). Chemicals were tested in cultured

Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations both in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine (BrdU)-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of the study chemical; the high dose was limited by toxicity or solubility but did not exceed 5 mg/ml.

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

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

For the SCE test, if significant chemical-induced cell cycle delay was seen, incubation time was lengthened to ensure a sufficient number of scorable cells. The harvest time for the chromosomal aberration test was based on the cell cycle information obtained in the SCE test; if cell cycle delay was anticipated, the incubation period was extended approximately 5 hours.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind, and those from a single test were read by the same person. For the SCE test, 50 second-division metaphase cells were usually scored for frequency of SCEs per cell from each dose; 100 or 200 first-division metaphase cells were scored at each dose for the chromosomal aberration test. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

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

III. RESULTS

RATS

SINGLE-ADMINISTRATION STUDIES

SIXTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

GENETIC TOXICOLOGY

SINGLE-ADMINISTRATION STUDIES

Four of five males and all five females that received 1,000 mg/kg 8-methoxypsoralen died by day 2. No compound-related clinical signs were observed.

SIXTEEN-DAY STUDIES

All rats that received 800 mg/kg, one male and one female at 400 mg/kg, and one female at 200 mg/kg died before the end of the studies (Table 7). Rats that received 400 mg/kg lost weight. The final mean body weights of rats at 200 or 400 mg/kg were 14% or 30% lower than those of vehicle controls. Decreased activity was noted after rats were dosed with 200 mg/kg or more 8-methoxypsoralen. No compound-related effects were observed at necropsy.

TABLE 7. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE SIXTEEN-DAY GAVAGE STUDIES OF 8-METHOXYPSORALEN

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	5/5	139 ± 6	214 ± 7	+75 ± 5	
50	5/5	138 ± 13	212 ± 16	+74 ± 2	99
100	5/5	145 ± 7	208 ± 7	+63 ± 2	97
200	5/5	138 ± 8	185 ± 14	+47 ± 13	86
400	(d) 4/5	144 ± 10	149 ± 6	−4 ± 4	70
800	(e) 0/5	125 ± 6	(f)	(f)	(f)
FEMALE					
0	5/5	113 ± 4	151 ± 3	+38 ± 3	
50	5/5	114 ± 6	145 ± 5	+31 ± 3	96
100	5/5	114 ± 6	149 ± 6	+35 ± 1	99
200	(g) 4/5	114 ± 6	130 ± 5	+11 ± 1	86
400	(h) 4/5	110 ± 5	106 ± 7	−7 ± 4	70
800	(i) 0/5	115 ± 6	(f)	(f)	(f)

(a) Number surviving/number initially in the group.
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Day of death: 11
(e) Day of death: 3,3,3,3,5
(f) No data are reported due to the 100% mortality in this group.
(g) Day of death: 15
(h) Day of death: 4
(i) Day of death: all 3

THIRTEEN-WEEK STUDIES

Six of 10 males and 8/10 females that received 400 mg/kg died before the end of the studies (Table 8). The final mean body weight of male rats that received 100, 200, or 400 mg/kg was 12%, 22%, or 45% lower than that of vehicle controls. The final mean body weight of female rats that received 200 or 400 mg/kg was 15% or 35% lower than that of vehicle controls. The liver weight to body weight ratios for all dosed groups of rats except the lowest (25 mg/kg) were significantly greater than those of vehicle controls (Table 9). Rats that received 400 mg/kg had rough hair coats and a hunched appearance; they appeared depressed after they were dosed.

TABLE 8. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF 8-METHOXYPSORALEN

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	10/10	176 ± 5	352 ± 8	+176 ± 6	
25	10/10	175 ± 6	369 ± 7	+194 ± 7	105
50	10/10	174 ± 6	361 ± 5	+187 ± 6	103
100	10/10	172 ± 5	311 ± 5	+139 ± 5	88
200	10/10	176 ± 5	275 ± 10	+99 ± 9	78
400	(d) 4/10	171 ± 5	194 ± 14	+14 ± 15	55
FEMALE					
0	10/10	132 ± 3	210 ± 6	+78 ± 5	
25	10/10	135 ± 2	210 ± 3	+75 ± 3	100
50	10/10	131 ± 2	205 ± 3	+74 ± 1	98
100	10/10	134 ± 2	213 ± 3	+79 ± 4	101
200	10/10	135 ± 2	179 ± 7	+44 ± 6	85
400	(e) 2/10	132 ± 2	136 ± 8	+3 ± 12	65

(a) Number surviving/number initially in the group.
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Week of death: 2,2,2,2,8,9
(e) Week of death: 1,1,2,2,2,2,2,2

TABLE 9. LIVER WEIGHTS FOR RATS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF 8-METHOXYPSORALEN (a)

Dose (mg/kg)	Number Weighed	Necropsy Body Weight (grams)	Liver Weight (mg)	Liver Weight/ Necropsy Body Weight (mg/g)
MALE				
0	10	344.8 ± 8.33	11,739 ± 529	34.0 ± 1.04
25	10	364.2 ± 5.73	(b) 13,970 ± 354	38.4 ± 0.90
50	10	350.9 ± 4.10	(c) 15,008 ± 526	(c) 42.8 ± 1.35
100	10	(c) 311.2 ± 4.53	(c) 14,877 ± 603	(c) 47.7 ± 1.48
200	10	(c) 271.8 ± 8.83	(c) 16,437 ± 420	(c) 60.8 ± 1.55
400	4	(c) 188.3 ± 18.26	(c) 15,198 ± 848	(c) 82.3 ± 6.27
FEMALE				
0	10	206.2 ± 5.00	6,722 ± 322	32.5 ± 1.11
25	10	203.0 ± 3.07	7,243 ± 171	35.7 ± 0.72
50	10	202.4 ± 2.35	7,715 ± 242	(c) 38.1 ± 1.00
100	10	209.7 ± 3.10	(c) 9,741 ± 215	(c) 46.5 ± 0.81
200	10	(c) 175.8 ± 6.14	(c) 11,315 ± 293	(c) 64.7 ± 1.51
400	2	(c) 129.5 ± 12.50	(c) 12,885 ±1,735	(c) 99.1 ± 3.83

(a) Mean ± standard error; P values vs. the vehicle controls by Dunnett's test (Dunnett, 1955).
(b) P<0.05
(c) P<0.01

Compound-related histopathologic effects were observed in the liver, adrenal glands, testis, seminal vesicles, and prostate. Minimal-to-mild fatty changes in the liver were observed in 9/10 males and 10/10 females that received 400 mg/kg and in 6/10 males and 8/10 females that received 200 mg/kg but not in any vehicle controls. Fatty changes in the adrenal glands were observed in 7/10 females that received 400 mg/kg. Atrophy of the testis, seminal vesicles, and prostate was observed in 9/10 male rats that received 400 mg/kg and 2/10 male rats that received 200 mg/kg. Because kidney neoplasms were seen in male rats in the 2-year studies, the kidney slides from the 13-week studies were reexamined, and this reexamination confirmed that there was no evidence of toxicity or hyaline droplets in the kidney of male rats after 13 weeks of dosing with 8-methoxypsoralen.

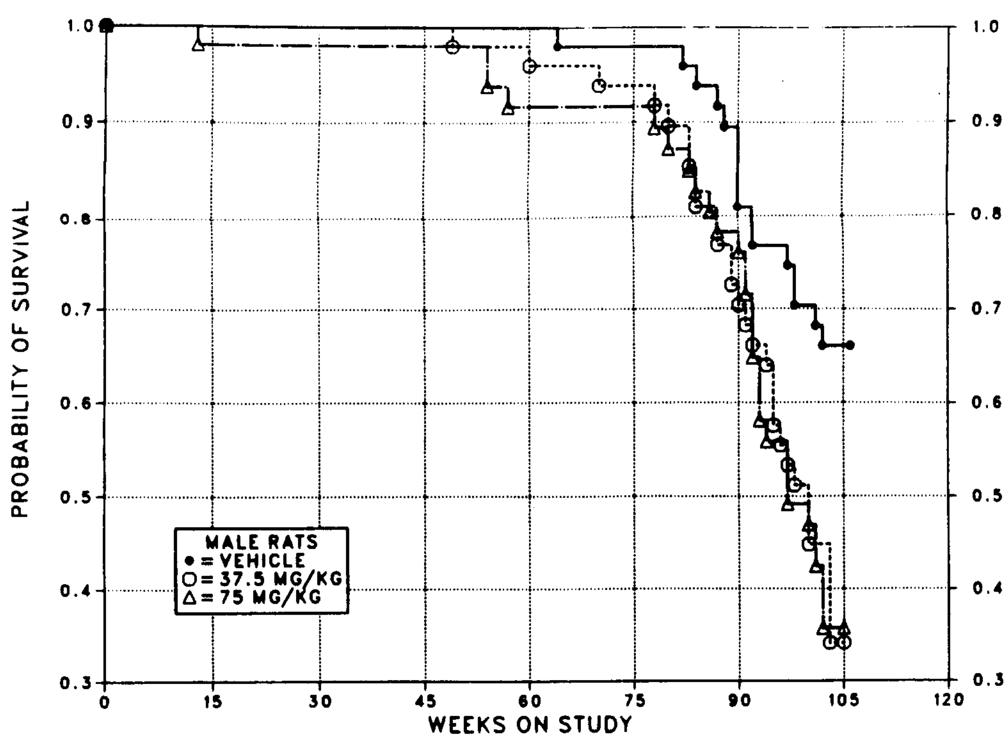
TWO-YEAR STUDIES

Body Weights and Clinical Signs

The mean body weights of dosed male rats were generally 3%-14% lower than those of vehicle controls from week 5 to the end of the study (Table 10 and Figure 5). The mean body weights of high dose female rats were 5%-17% lower than those of vehicle controls from week 5 to the end of the study. Mean body weights of low dose and vehicle control female rats were generally similar. No compound-related clinical signs were observed.

TABLE 10. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR GAVAGE STUDIES OF 8-METHOXYPSORALEN

Weeks on Study	Vehicle Control Av. Wt. (grams)	Vehicle Control No. of Survivors	37.5 mg/kg Av. Wt. (grams)	37.5 mg/kg Wt. (percent of veh. controls)	37.5 mg/kg No. of Survivors	75 mg/kg Av. Wt. (grams)	75 mg/kg Wt. (percent of veh. controls)	75 mg/kg No. of Survivors
MALE								
0	122	50	120	98	50	122	100	50
1	149	50	147	99	50	148	99	50
2	187	50	185	99	50	182	97	50
3	224	50	220	98	50	213	95	50
4	248	50	241	97	50	231	93	50
5	270	50	257	95	50	245	91	50
6	286	50	270	94	50	259	91	50
7	300	50	282	94	50	268	89	50
8	313	50	291	93	50	277	88	50
9	326	50	298	91	50	285	87	50
10	334	50	303	91	49	291	87	50
11	346	50	307	89	49	300	87	50
12	355	50	313	88	49	307	86	50
13	360	50	317	88	49	312	87	49
17	386	50	338	88	49	338	88	49
21	402	49	376	94	49	369	92	49
25	422	49	399	95	49	393	93	47
30	447	49	425	95	49	413	92	47
34	462	49	443	96	49	428	93	47
37	471	49	453	96	49	432	92	47
41	480	49	462	96	49	437	91	47
47	493	49	475	96	49	451	91	47
50	503	49	488	97	48	459	91	46
56	513	49	496	97	48	463	90	44
60	517	49	498	96	47	468	91	43
65	522	47	504	97	46	470	90	43
69	527	47	508	96	46	471	89	42
73	529	47	507	96	45	470	89	41
77	528	47	499	95	45	464	88	41
81	524	47	500	95	42	469	90	39
84	527	44	506	96	38	465	88	38
88	517	43	498	96	36	467	90	35
94	516	36	472	91	31	452	88	26
97	505	36	470	93	25	447	89	23
103	492	30	431	88	20	437	89	16
FEMALE								
0	110	50	110	100	50	109	99	50
1	125	50	125	100	50	122	98	50
2	143	50	143	100	50	139	97	50
3	154	50	154	100	50	147	95	50
4	165	50	162	98	50	156	95	50
5	173	50	169	98	50	163	94	50
6	180	50	176	98	50	170	94	50
7	185	50	181	98	50	172	93	50
8	189	50	185	98	50	180	95	50
9	193	50	189	98	50	182	94	50
10	194	50	193	99	50	184	95	50
11	197	50	194	98	50	186	94	50
12	199	50	198	99	50	188	94	50
13	204	50	199	98	50	188	92	50
17	214	50	201	94	50	194	91	50
21	226	50	212	94	50	206	91	50
25	228	50	217	95	50	213	93	50
30	232	50	225	97	50	219	94	50
34	238	50	231	97	50	224	94	50
37	239	50	234	98	50	224	94	50
41	242	50	236	98	50	224	93	50
47	252	50	243	96	50	232	92	50
50	258	50	250	97	50	234	91	50
56	268	50	259	97	50	239	89	50
60	276	50	266	96	50	241	87	50
65	283	50	270	95	49	246	87	49
69	291	50	280	96	48	249	86	49
73	298	49	286	96	48	254	85	49
77	301	47	291	97	45	257	85	48
81	305	47	299	98	44	261	86	48
84	310	47	305	98	43	265	85	48
88	316	47	310	98	40	268	85	46
94	320	46	315	98	38	270	84	41
97	323	41	322	100	36	269	83	40
103	326	39	324	99	33	282	87	36



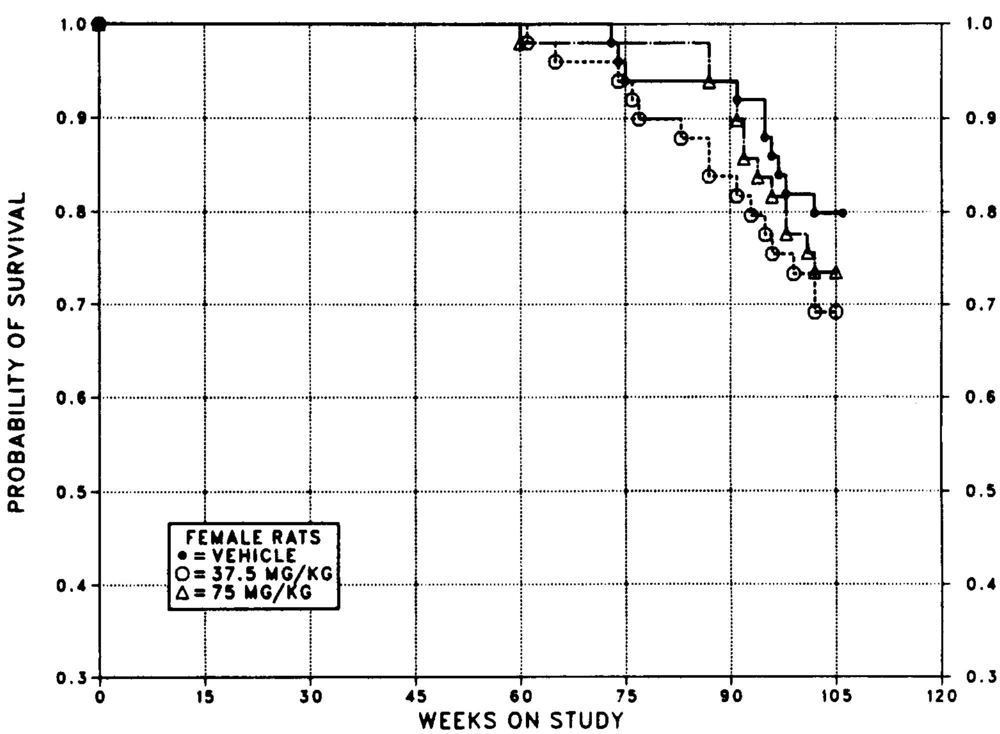


FIGURE 5. GROWTH CURVES FOR RATS ADMINISTERED 8-METHOXYPSORALEN IN CORN OIL BY GAVAGE FOR TWO YEARS

Survival

Estimates of the probabilities of survival for male and female rats administered 8-methoxypsoralen at the doses used in these studies and for vehicle controls are shown in Table 11 and in the Kaplan and Meier curves in Figure 6. The survival of both the low (after week 96) and the high (after week 97) dose groups of male rats was significantly lower than that of the vehicle controls. No significant differences in survival were observed between any groups of female rats.

Pathology and Statistical Analyses of Results

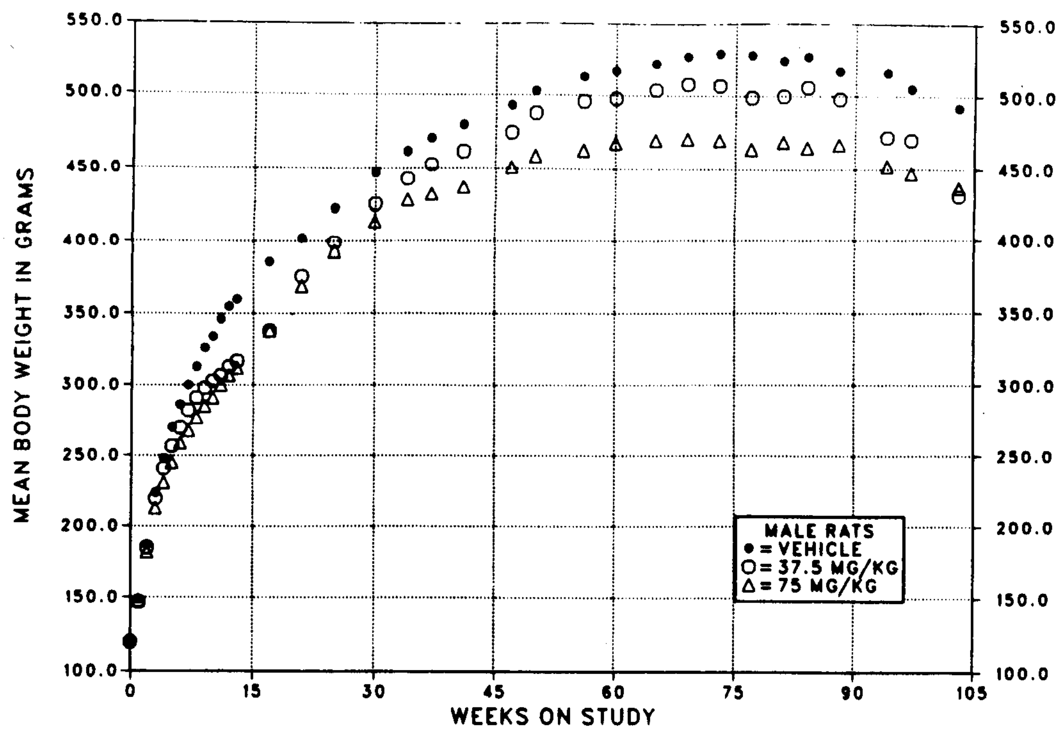
This section describes the statistically significant or biologically noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions of the kidney, parathyroids, bone, Zymbal gland, subcutaneous tissue, lung, oral cavity, forestomach, thyroid gland, preputial gland, eye, anterior pituitary gland, and testis.

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

TABLE 11. SURVIVAL OF RATS IN THE TWO-YEAR GAVAGE STUDIES OF 8-METHOXYPSORALEN

	Vehicle Control	37.5 mg/kg	75 mg/kg
MALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	16	31	29
Accidentally killed	4	3	5
Killed at termination	30	14	16
Died during termination period	0	2	0
Survival P values (c)	0.005	0.005	0.007
FEMALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	10	15	13
Accidentally killed	1	2	1
Killed at termination	38	33	36
Died during termination period	1	0	0
Survival P values (c)	0.560	0.286	0.607

(a) Terminal-kill period: weeks 104-106
(b) Includes animals killed in a moribund condition
(c) The result of the life table trend test is in the vehicle control column, and the results of the life table pairwise comparisons with the vehicle controls are in the dosed columns.



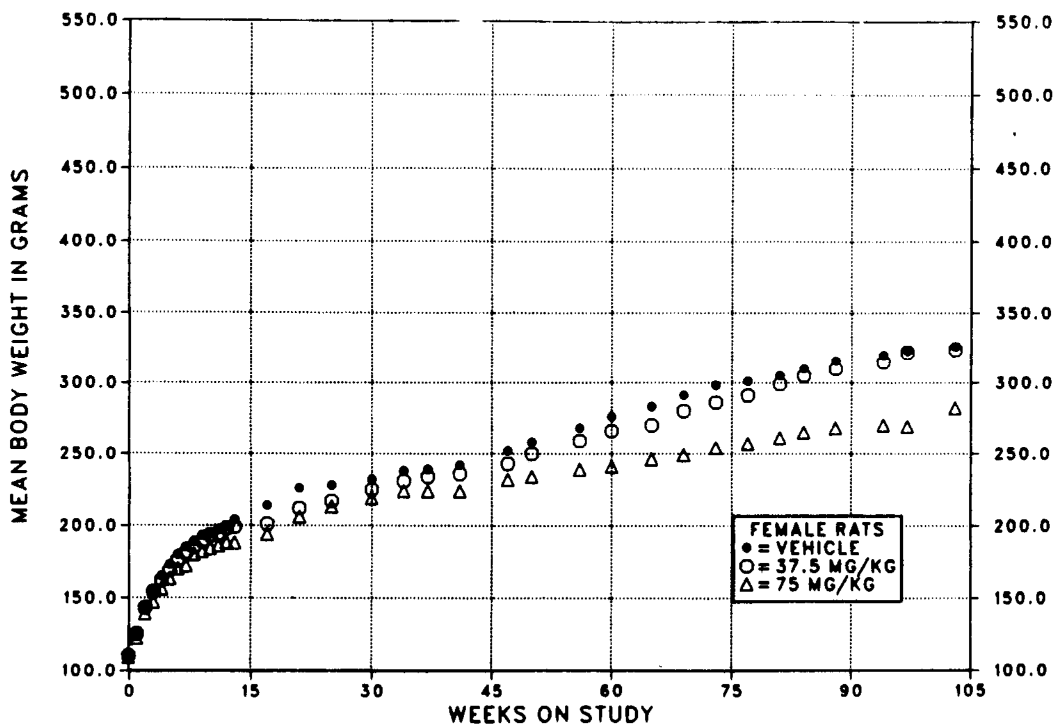


FIGURE 6. KAPLAN-MEIER SURVIVAL CURVES FOR RATS ADMINISTERED 8-METHOXYPSORALEN IN CORN OIL BY GAVAGE FOR TWO YEARS

Kidney: A spectrum of degenerative and proliferative changes in the kidneys of male rats was associated with the administration of 8-methoxypsoralen (Table 12). Nephropathy occurred in nearly all male rats, but the average severity and extent of this spontaneous disease were greater in dosed rats. Nephropathy consisted of degeneration and regeneration of the tubular epithelium with dilatation and atrophy of tubules, formation of hyaline and granular casts, thickening of basement membranes, interstitial fibrosis, and glomerulosclerosis. Linear accumulations of mineral within the inner medulla and papilla occurred only in high dose male rats.

Hyperplasia, adenomas, and adenocarcinomas of tubular epithelial cells are part of a morphologic continuum. Focal hyperplasia of the renal tubular epithelium occurred in dosed male rats but not in vehicle controls (Table 12). The lesion consisted of focally enlarged individual tubules filled with epithelial cells. The epithelium was obviously stratified, and cells showed loss of basement membrane dependency. Tubular cell adenomas, adenocarcinomas, and adenomas or adenocarcinomas (combined) in male rats occurred with significant positive trends; the incidences of tubular cell adenomas and adenomas or adenocarcinomas (combined) in dosed male rats were significantly greater than those in vehicle controls. Tubular cell adenomas generally were distinguished from hyperplasia by loss of tubular structure and larger size. These adenomas consisted of circumscribed masses of polyhedral epithelial cells arranged in solid masses, in small clusters separated by scant fibrovascular stroma, or in papillary formations. The adenocarcinomas were less well circumscribed and exhibited greater cellular atypia.

Parathyroids and Bone: Parathyroid hyperplasia was increased in dosed male rats (vehicle control, 2/49; low dose, 22/47; high dose, 18/48). Fibrous osteodystrophy of bone was also increased in dosed male rats (2/50; 10/50; 12/49) and is considered to be secondary to the renal disease and parathyroid hyperplasia (renal secondary hyperparathyroidism).

Zymbal Gland: The incidences of carcinomas or squamous cell carcinomas (combined) in dosed males were increased relative to that in vehicle controls (Table 13). (The Zymbal gland is a modified sebaceous gland located adjacent to the external ear canal.) These neoplasms consisted of interconnecting masses or cords of stratified epithelial cells exhibiting glandular or squamous differentiation and invading the adjacent connective tissue.

Subcutaneous Tissue: Fibromas in male rats occurred with a significant positive trend; the incidences in dosed males were significantly greater than that in vehicle controls (Table 14).

TABLE 12. RENAL LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (a)

	Vehicle Control	37.5 mg/kg	75 mg/kg
Mineralization of Renal Papilla			
Overall Rates	0/50 (0%)	0/50 (0%)	31/49 (63%)
Focal Hyperplasia of Renal Tubule			
Overall Rates	0/50 (0%)	8/50 (16%)	8/49 (16%)
Nephropathy (b)			
Overall Rates	48/50 (96%)	49/50 (98%)	47/49 (96%)
Grade 1	10/50 (20%)	10/50 (20%)	7/49 (14%)
Grade 2	28/50 (56%)	13/50 (26%)	9/49 (18%)
Grade 3	9/50 (18%)	13/50 (26%)	16/49 (33%)
Grade 4	1/50 (2%)	13/50 (26%)	15/49 (31%)
Tubular Cell Adenoma			
Overall Rates	1/50 (2%)	11/50 (22%)	8/49 (16%)
Adjusted Rates	3.3%	45.0%	30.5%
Terminal Rates	1/30 (3%)	4/16 (25%)	2/16 (13%)
Week of First Observation	106	95	80
Life Table Tests	P=0.003	P<0.001	P=0.004
Incidental Tumor Tests	P=0.031	P=0.004	P=0.026
Logistic Regression Analysis	P=0.008	P<0.001	P=0.009
Tubular Cell Adenocarcinoma			
Overall Rates	0/50 (0%)	1/50 (2%)	3/49 (6%)
Adjusted Rates	0.0%	6.2%	15.2%
Terminal Rates	0/30 (0%)	1/16 (6%)	2/16 (13%)
Week of First Observation		105	92
Life Table Tests	P=0.024	P=0.375	P=0.053
Incidental Tumor Tests	P=0.024	P=0.375	P=0.055
Logistic Regression Analysis	P=0.034	P=0.375	P=0.078
Tubular Cell Adenoma or Adenocarcinoma (c)			
Overall Rates	1/50 (2%)	12/50 (24%)	11/49 (22%)
Adjusted Rates	3.3%	49.6%	42.3%
Terminal Rates	1/30 (3%)	5/16 (31%)	4/16 (25%)
Week of First Observation	106	95	80
Life Table Tests	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests	P=0.003	P=0.001	P=0.002
Logistic Regression Analysis	P=0.001	P<0.001	P=0.001

(a) The statistical analyses used are discussed in Section II (Statistical Methods) and Table A3 (footnotes).
(b) Grades of severity: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
(c) Historical incidence in NTP studies (mean ± SD): 10/1,943 (0.5% ± 0.9%)

TABLE 13. ZYMBAL GLAND CARCINOMAS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN

	Vehicle Control	37.5 mg/kg	75 mg/kg
Carcinoma or Squamous Cell Carcinoma (a)			
Overall Rates	1/50 (2%)	7/50 (14%)	4/49 (8%)
Adjusted Rates	3.3%	29.1%	13.1%
Terminal Rates	1/30 (3%)	2/16 (13%)	0/16 (0%)
Week of First Observation	106	83	78
Life Table Tests	P=0.063	P=0.008	P=0.104
Incidental Tumor Tests	P=0.229	P=0.051	P=0.233
Logistic Regression Analysis	P=0.125	P=0.018	P=0.160

(a) Historical incidence of Zymbal gland tumors in NTP studies (mean ± SD): 16/1,949 (0.8% ± 1.3%)

TABLE 14. SUBCUTANEOUS TISSUE TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN

	Vehicle Control	37.5 mg/kg	75 mg/kg
Fibroma			
Overall Rates	1/50 (2%)	5/50 (10%)	7/49 (14%)
Adjusted Rates	3.3%	24.1%	30.9%
Terminal Rates	1/30 (3%)	2/16 (13%)	4/16 (25%)
Week of First Observation	106	100	57
Life Table Tests	P=0.004	P=0.029	P=0.006
Incidental Tumor Tests	P=0.012	P=0.115	P=0.009
Logistic Regression Analysis	P=0.012	P=0.040	P=0.022
Sarcoma			
Overall Rates	0/50 (0%)	0/50 (0%)	1/49 (2%)
Fibroma or Sarcoma (a)			
Overall Rates	1/50 (2%)	5/50 (10%)	8/49 (16%)
Adjusted Rates	3.3%	24.1%	32.3%
Terminal Rates	1/30 (3%)	2/16 (13%)	4/16 (25%)
Week of First Observation	106	100	13
Life Table Tests	P=0.002	P=0.029	P=0.003
Incidental Tumor Tests	P=0.009	P=0.115	P=0.008
Logistic Regression Analysis	P=0.011	P=0.040	P=0.024

(a) Historical incidence of integumentary system fibromas, neurofibromas, neurofibrosarcomas, sarcomas, or fibrosarcomas (combined) in NTP studies (mean ± SD): 171/1,949 (9% ± 4%)

Lung: Alveolar epithelial hyperplasia occurred in 5/50 vehicle control, 7/50 low dose, and 9/49 high dose male rats. Hyperplasia of the alveolar epithelium consisted of alveoli lined by increased numbers of cuboidal or columnar epithelial cells. Alveolar structure was generally intact but often distorted by the increased number of cells. Alveolar/bronchiolar adenomas occurred in 4/50 vehicle control, 9/50 low dose, and 9/49 high dose male rats; an alveolar/bronchiolar carcinoma occurred in one low dose male rat with an adenoma (Table 15). Hyperplasia, adenomas, and carcinomas are part of a morphologic continuum. The adenomas were distinguished from hyperplasia primarily on the basis of a greater degree and extent of the loss of normal alveolar structure. Alveoli were effaced by irregular branching or papillary formations consisting of columnar cells overlying a scant fibrovascular stroma. The carcinoma exhibited greater cellular atypia.

Oral Cavity: Squamous cell papillomas were observed in the palate or tongue of 1/50 low dose and 3/50 high dose female rats. None was found in controls. These papillomas were less than 1 mm in size and were not considered to be related to chemical administration. The mean historical incidence of squamous cell neoplasms of the oral cavity in corn oil vehicle control female F344/N rats is 6/1,950 (0.3%); the highest observed incidence is 2/50.

Forestomach: Chronic inflammation, ulcers, and epithelial hyperplasia were observed at increased incidences in dosed male rats (Table 16). Two squamous cell papillomas were observed in low dose male rats. The mean historical incidence of squamous cell neoplasms of the stomach in corn oil vehicle control male F344/N rats is 7/1,924 (0.4%); the highest observed incidence is 1/49.

TABLE 15. ALVEOLAR/BRONCHIOLAR ADENOMAS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (a)

	Vehicle Control	37.5 mg/kg	75 mg/kg
Overall Rates	4/50 (8%)	(b) 9/50 (18%)	9/49 (18%)
Adjusted Rates	12.0%	37.4%	35.8%
Terminal Rates	3/30 (10%)	4/16 (25%)	3/16 (19%)
Week of First Observation	84	87	68
Life Table Tests	P=0.015	P=0.022	P=0.022
Incidental Tumor Tests	P=0.075	P=0.077	P=0.131
Logistic Regression Analysis	P=0.048	P=0.075	P=0.069

(a) Historical incidence of adenomas or carcinomas (combined) in NTP studies (mean ± SD): 68/1,944 (3% ± 3%)
(b) An alveolar/bronchiolar carcinoma was observed in an animal with an adenoma.

TABLE 16. NUMBER OF RATS WITH SELECTED FORESTOMACH LESIONS IN THE TWO-YEAR GAVAGE STUDIES OF 8-METHOXYPSORALEN

Lesion	Male			Female		
	Vehicle Control	37.5 mg/kg	75 mg/kg	Vehicle Control	37.5 mg/kg	75 mg/kg
No. examined	50	50	49	50	50	50
Chronic inflammation	1	6	5	1	0	0
Ulcers	5	13	11	1	3	0
Epithelial hyperplasia	4	19	20	1	0	3
Squamous cell papilloma (a)	0	2	0	0	0	0

(a) Historical incidence of stomach squamous cell papillomas or carcinomas (combined) in male F344/N rats in NTP studies (mean ± SD): 7/1,924 (0.4% ± 0.8%)

Thyroid Gland: Diffuse hypertrophy was observed at increased incidences in dosed male rats (male: vehicle control, 2/50; low dose, 31/50; high dose, 39/49; female: none observed). Follicular cell adenomas or carcinomas (combined) were seen in 1/50 vehicle control, 3/50 low dose, and 3/49 high dose male rats; because of the low incidences, these neoplasms were not considered to be related to chemical administration.

Preputial Gland: Cysts were observed at an increased incidence in high dose male rats (vehicle control, 6/50; low dose, 4/50; high dose, 20/49).

Eye: Hemorrhage was observed at increased incidences in dosed male rats (male: vehicle control, 1/50; low dose, 14/50; high dose, 9/49; female: 1/50; 1/50; 2/50). Cataracts were seen in all groups (male: 44/50; 40/50; 36/49; female: 47/50; 41/50; 47/50); this lesion may have been related to the yellow fluorescent lighting used in the animal rooms.

Anterior Pituitary Gland: Adenomas in male rats occurred with a significant negative trend; the incidences in the dosed groups were significantly lower than that in vehicle controls by the incidental tumor test (Table 17). Adenomas were seen in 24/49 vehicle control, 24/49 low dose, and 15/49 high dose female rats.

Testis: Interstitial cell tumors occurred with a significant positive trend; the incidences in the dosed groups were significantly greater than that in vehicle controls (Table 18).

TABLE 17. ANTERIOR PITUITARY GLAND LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN

	Vehicle Control	37.5 mg/kg	75 mg/kg
Hyperplasia			
Overall Rates	7/49 (14%)	7/50 (14%)	5/48 (10%)
Adenoma (a)			
Overall Rates	24/49 (49%)	12/50 (24%)	12/48 (25%)
Adjusted Rates	59.0%	39.0%	45.2%
Terminal Rates	14/30 (47%)	2/16 (13%)	4/16 (25%)
Week of First Observation	62	70	84
Life Table Tests	P=0.231N	P=0.242N	P=0.298N
Incidental Tumor Tests	P=0.007N	P=0.003N	P=0.018N

(a) Historical incidence of anterior pituitary gland tumors in NTP studies (mean ± SD): 556/1,898 (29% ± 10%)

TABLE 18. TESTICULAR INTERSTITIAL CELL LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN

	Vehicle Control	37.5 mg/kg	75 mg/kg
Hyperplasia			
Overall Rates	7/50 (14%)	1/48 (2%)	3/49 (6%)
Tumor (a)			
Overall Rates	38/50 (76%)	44/48 (92%)	43/49 (88%)
Adjusted Rates	90.2%	95.6%	97.7%
Terminal Rates	26/30 (87%)	14/16 (88%)	15/16 (94%)
Week of First Observation	62	49	54
Life Table Tests	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests	P=0.006	P=0.017	P=0.007

(a) Historical incidence in NTP studies (mean ± SD): 1,674/1,944 (86% ± 9%)

8-Methoxypsoralen was mutagenic in four of five strains of *Salmonella typhimurium* when tested in a preincubation protocol with doses up to 3,333 µg/plate in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 (Table 19). A clearly positive response was obtained in strain TA104 in the presence of S9, and a weaker positive response occurred in the absence of activation; mutagenic responses were also recorded for strains TA98, TA102, and TA100 in the presence, but not in the absence, of S9. 8-Methoxypsoralen was not mutagenic in TA1535 with or without S9. 8-Methoxypsoralen was tested for chromosomal effects in cultured Chinese hamster ovary (CHO) cells in both the presence and the absence of Aroclor 1254-induced male Sprague Dawley rat liver S9. A highly significant, dose-related increase in sister-chromatid exchanges (SCEs) was observed over a concentration range of 3.3-100 µg/ml 8-methoxypsoralen in the absence of S9; a significant increase in SCEs was also observed in the presence of S9 at concentrations of 33-333 µg/ml of the study chemical (Table 20). In the test for chromosomal aberration induction in the absence of S9, an extended incubation protocol was used to offset chemical-induced cell cycle delay; treatment with up to 250 µg/ml 8-methoxypsoralen produced a significant increase in aberrations (Table 21). In the aberration test with S9, CHO cell cycle time was not delayed, and no significant increase in aberrations was observed with doses up to a maximum of 600 µg/ml 8-methoxypsoralen. This lack of an effect may have been due to the shorter exposure time (2 hours with S9 compared with 10 hours without S9) of the cells to the study chemical.

TABLE 19. MUTAGENICITY OF 8-METHOXYPSORALEN IN *SALMONELLA TYPHIMURIUM* (a)

Strain	Dose (µg/plate)	Revertants/Plate (b) –S9	+S9 (hamster) 5%	+S9 (hamster) 10%	+S9 (hamster) 30%	+S9 (rat) 5%	+S9 (rat) 10%	+S9 (rat) 30%
TA102	0	284 ± 16.0	450 ± 10.6	622 ± 40.2	387 ± 21.9	405 ± 13.5	489 ± 11.1	373 ± 39.0
	0.3	--	--	--	468 ± 6.4	--	--	327 ± 14.3
	1	--	--	--	445 ± 3.2	--	--	341 ± 23.1
	3	--	512 ± 6.0	665 ± 5.5	427 ± 20.3	--	--	316 ± 13.6
	10	313 ± 7.0	517 ± 16.7	623 ± 31.0	427 ± 14.6	478 ± 13.7	585 ± 24.8	352 ± 22.2
	33	304 ± 2.6	592 ± 22.5	687 ± 34.0	453 ± 33.7	530 ± 6.4	601 ± 8.8	406 ± 31.1
	66	237 ± 13.3	--	--	575 ± 3.2	510 ± 14.0	595 ± 19.4	522 ± 11.7
	100	307 ± 15.6	744 ± 35.2	699 ± 14.1	682 ± 15.3	659 ± 30.3	755 ± 28.8	590 ± 23.2
	166	283 ± 6.7	1,002 ± 28.4	820 ± 30.4	653 ± 27.9	831 ± 25.8	595 ± 73.1	716 ± 42.8
Trial summary		Negative	Positive	Equivocal	Positive	Positive	Weakly positive	Positive
Positive control (c)		586 ± 43.9	1,677 ± 103.0	1,990 ± 48.3	600 ± 9.2	971 ± 34.5	1,324 ± 59.1	1,487 ± 26.6
TA104	0	316 ± 18.5	539 ± 14.8	538 ± 3.8	376 ± 1.5	450 ± 15.2	515 ± 12.4	359 ± 20.5
	0.3	--	--	--	414 ± 8.5	--	--	369 ± 7.0
	1	--	--	--	447 ± 11.4	--	--	428 ± 9.2
	3	--	605 ± 8.0	633 ± 28.6	457 ± 4.7	--	--	424 ± 17.5
	10	378 ± 12.0	662 ± 21.4	767 ± 14.7	459 ± 9.5	725 ± 26.1	843 ± 20.5	539 ± 21.8
	33	424 ± 16.0	834 ± 35.2	842 ± 54.5	522 ± 19.5	865 ± 20.6	974 ± 24.9	615 ± 12.0
	66	405 ± 33.1	--	--	667 ± 21.8	989 ± 18.6	1,087 ± 8.7	833 ± 16.7
	100	474 ± 42.4	976 ± 46.4	909 ± 26.6	769 ± 3.5	1,061 ± 23.2	1,206 ± 31.7	972 ± 9.3
	166	389 ± 48.8	1,059 ± 59.6	1,046 ± 83.3	807 ± 18.7	1,157 ± 59.6	1,242 ± 13.0	911 ± 63.5
Trial summary		Weakly positive	Positive	Positive	Positive	Positive	Positive	Positive
Positive control (c)		(d) 308 ± 9.5	Toxic	Toxic	661 ± 26.4	690 ± 35.5	827 ± 16.0	808 ± 34.2
TA100	0	137 ± 4.9	133 ± 8.6	122 ± 6.6	95 ± 3.5	128 ± 11.5	137 ± 10.9	131 ± 13.9
	10	--	--	--	--	186 ± 7.0	145 ± 9.8	163 ± 7.4
	16	123 ± 6.6	--	--	--	--	--	--
	33	127 ± 13.7	141 ± 5.8	138 ± 5.8	114 ± 3.7	154 ± 12.1	163 ± 4.4	191 ± 27.7
	66	145 ± 9.0	--	----	----	134 ± 9.3	167 ± 10.3	213 ± 4.8
	100	125 ± 8.7	162 ± 15.0	125 ± 16.7	122 ± 10.6	141 ± 9.7	154 ± 4.4	229 ± 9.7
	166	120 ± 8.1	--	--	--	--	--	--
	333	--	249 ± 13.4	179 ± 10.4	168 ± 11.4	138 ± 13.0	147 ± 8.8	256 ± 4.3
	666	--	363 ± 3.3	284 ± 13.5	184 ± 14.7	--	--	--
	1,000	--	321 ± 19.6	317 ± 13.9	190 ± 30.0	--	--	--
Trial summary		Negative	Positive	Positive	Positive	Equivocal	Negative	Positive
Positive control (c)		376 ± 5.9	645 ± 26.0	466 ± 23.8	642 ± 12.3	396 ± 20.2	324 ± 6.2	263 ± 5.8

TABLE 19. MUTAGENICITY OF 8-METHOXYPSORALEN IN *SALMONELLA TYPHIMURIUM* (Continued)

	Dose (µg/plate)	Revertants/Plate (b) -S9	+S9 (hamster) 30%	+S9 (rat) 30%
TA1535	0	20 ± 1.2	8 ± 1.2	25 ± 5.0
	10	14 ± 0.9	11 ± 2.7	28 ± 3.2
	33	17 ± 2.9	8 ± 0.3	25 ± 2.6
	66	17 ± 4.4	8 ± 0.0	21 ± 2.3
	100	21 ± 2.3	10 ± 1.2	12 ± 1.5
	166	15 ± 2.0	7 ± 1.5	13 ± 1.9
	333	14 ± 3.6	8 ± 1.2	12 ± 2.3
	1,000	12 ± 2.6	9 ± 0.7	11 ± 2.0
	3,333	--	--	--
Trial summary		Negative	Negative	Negative
Positive control (c)		349 ± 2.6	356 ± 11.8	87 ± 6.1
TA98	0	15 ± 2.6	29 ± 4.4	46 ± 3.2
	10	32 ± 3.6	--	40 ± 2.6
	33	13 ± 1.3	29 ± 6.6	46 ± 1.2
	66	--	--	65 ± 1.9
	100	18 ± 3.5	31 ± 3.6	65 ± 0.9
	166	--	--	75 ± 0.6
	333	20 ± 1.5	35 ± 6.8	60 ± 3.2
	1,000	17 ± 1.8	34 ± 3.3	(d) 21 ± 2.6
	3,333	--	(e) 14 ± 1.0	--
Trial summary		Negative	Negative	Weakly positive
Positive control (c)		619 ± 16.5	487 ± 27.5	166 ± 3.2

(a) Study performed at SRI International. The detailed protocol is presented by Haworth et al. (1983). Cells and study compound or solvent (dimethyl sulfoxide) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague Dawley rat liver. High dose was limited by toxicity or solubility but did not exceed 10 mg/plate; 0 µg/plate dose is the solvent control.
(b) Revertants are presented as mean ± standard error from three plates.
(c) Positive control. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was used with TA98, sodium azide was used with TA100 and TA1535, and mitomycin C was used with TA102 and TA104. In the presence of S9, 2-aminoanthracene was used with TA100, TA1535, and TA98 and sterigmatocystin was used with TA102 and TA104.
(d) Slight toxicity
(e) Precipitate on plate

TABLE 20. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY 8-METHOXYPSORALEN (a)

Compound	Dose (µg/ml)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hours in BrdU	Relative SCEs/Cell (percent) (b)
--S9 (c)								
Trial 1--Summary: Positive								
Dimethyl sulfoxide		50	1,046	413	0.39	8.3	25.7	--
8-Methoxypsoralen	3.3	50	1,048	593	0.57	11.9	25.7	143.4
	10	50	1,034	581	0.56	11.6	25.7	139.8
	33.3	50	1,028	1,207	1.17	24.1	25.7	290.4
	100	9	189	259	1.37	28.8	25.7	347.0
	333.3	0						
Mitomycin C	0.001	50	1,046	877	0.84	17.5	25.7	210.8
	0.01	5	106	230	2.17	46.0	25.7	554.2
Trial 2--Summary: Positive								
Dimethyl sulfoxide		25	526	167	0.32	6.7	25.7	--
8-Methoxypsoralen	20.2	25	515	387	0.75	15.5	25.7	231.3
	50.5	25	515	410	0.80	16.4	25.7	244.8
	100.5	5	103	101	0.98	20.2	25.7	301.5
	150	0						
Mitomycin C	0.001	25	519	284	0.55	11.4	25.7	170.1
	0.01	5	104	198	1.90	39.6	25.7	591.0
+S9 (d)								
Summary: Positive								
Dimethyl sulfoxide		50	1,047	391	0.37	7.8	25.7	--
8-Methoxypsoralen	33.3	50	1,044	553	0.53	11.1	25.7	142.3
	100	50	1,047	611	0.58	12.2	25.7	156.4
	(e) 333.3	50	1,046	910	0.87	18.2	25.7	233.3
	1,000	0						
Cyclophosphamide	0.4	50	1,036	644	0.62	12.9	25.7	165.4
	2	5	104	180	1.73	36.0	25.7	461.5

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

TABLE 21. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY 8-METHOXYPSORALEN (a)

Trial 1					Trial 2				
Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
−S9 (b)--Harvest time 20.0 h (c)					−S9 (b)--Harvest time 20.2 h (c)				
Dimethyl sulfoxide					Dimethyl sulfoxide				
	200	3	0.02	1.5		100	1	0.01	1.0
8-Methoxypsoralen					8-Methoxypsoralen				
100	200	21	0.11	9.5	200	100	19	0.19	15.0
150	200	22	0.11	10.5	225	100	15	0.15	15.0
200	100	42	0.42	35.0	250	100	33	0.33	29.0
Summary: Positive					Summary: Positive				
Mitomycin C					Mitomycin C				
0.05	200	39	0.20	14.5	0.05	100	34	0.34	26.0
0.08	25	20	0.80	52.0	0.08	25	29	1.16	52.0
+S9 (d)--Harvest time 12.0 h (c)					+S9 (d)--Harvest time 12.0 h (c)				
Dimethyl sulfoxide					Dimethyl sulfoxide				
	200	5	0.03	2.5		100	2	0.02	2.0
8-Methoxypsoralen					8-Methoxypsoralen				
101	200	6	0.03	3.0	498	100	9	0.09	8.0
252	200	8	0.04	3.5	552	100	7	0.07	7.0
502.5	200	14	0.07	6.5	600	100	9	0.09	9.0
Summary: Negative					Summary: Negative				
Cyclophosphamide					Cyclophosphamide				
7.5	200	26	0.13	10.5	7.5	100	11	0.11	11.0
37.5	25	14	0.56	44.0	37.5	25	11	0.44	32.0

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

IV. DISCUSSION AND CONCLUSIONS

8-Methoxypsoralen, administered orally and followed by ultraviolet A radiation, is used in the treatment of psoriasis and vitiligo. This compound is also found in a variety of vegetables as a natural product. Previously, no long-term rodent studies had been performed to characterize the toxicity and carcinogenicity of orally administered 8-methoxypsoralen without ultraviolet radiation (IARC, 1987). Thus, single-administration, 16-day, 13-week, and 2-year studies were conducted by administering 8-methoxypsoralen to F344/N rats by gavage. In addition, short-term in vitro genetic toxicology studies were performed.

In the single-administration studies, 8-methoxypsoralen was administered at doses up to 1,000 mg/kg. Male and female rats that received 1,000 mg/kg died, but rats at the next lower dose (500 mg/kg) survived administration of the chemical. In the 16-day studies, rats received doses up to 800 mg/kg. All rats at 800 mg/kg and one male and one female at 400 mg/kg died.

In the 13-week studies, 8-methoxypsoralen was administered at doses up to 400 mg/kg; most rats that received 400 mg/kg died before the end of the studies; no deaths were seen at lower doses. Mean body weights of male rats at 100 and 200 mg/kg and female rats at 200 mg/kg were more than 10% lower than those of vehicle controls. Fatty changes in the liver were seen in males and females at 200 and 400 mg/kg. Because of the liver lesions, effects on weight gain, and survival, doses selected for the 2-year studies were 0, 37.5, and 75 mg/kg (or 0, 195, and 390 mg/m^2 body surface area, based on calculations of Freireich et al., 1966).

The average therapeutic dose of 8-methoxypsoralen for humans is up to 1 mg/kg per day (37 mg/m^2 per day body surface area) given three times per week, depending on size and skin type. The doses used in the current studies in rats (37 and 75 mg/kg per day or 195 and 390 mg/m^2 per day) were therefore up to approximately 37-75 times the dose for humans per day, when compared on a milligram per kilogram basis, and 5.3-10.5 times the dose for humans per day, when compared on a milligram per square meter surface area basis. An estimate of human consumption of 8-methoxypsoralen can also be made. If, for example, humans were to consume 100 mg of vegetables per day containing 8-methoxypsoralen at a concentration of 100 ppm (100 mg/kg vegetable), the intake of 8-methoxypsoralen for a 70-kg man would be 0.1 mg 8-methoxypsoralen/kg body weight.

In the 2-year studies, survival of low and high dose male rats was reduced toward the end of the study, but survival was greater than 70% in all groups at week 88. The decreased survival in dosed male rats was probably a result of kidney toxicity, an effect of compound administration which was apparently absent in the 13-week studies. Survival of vehicle control and dosed female rats was comparable; no kidney toxicity was seen in females.

The kidney was the principal target organ in dosed male rats; the incidences of both nonneoplastic lesions and neoplastic lesions were increased in the 2-year studies. Mineralization of the kidney papilla and increased severity of nephropathy were observed in dosed males, and the incidences of tubular cell hyperplasia, adenomas, and adenomas or adenocarcinomas (combined) were increased in dosed male rats. Compound-related nonneoplastic and neoplastic lesions of the kidney were seen only in males, which suggests that these effects may be a sex-influenced phenomenon, as has been observed with other chemicals such as dimethyl methylphosphonate (NTP, 1987a), petroleum hydrocarbons (Short et al., 1987), and 1,4-dichlorobenzene (NTP, 1987b). In contrast to these studies, however, an increase in hyaline droplets and associated changes were not observed in the kidney of male rats in the 13-week studies of 8-methoxypsoralen. Further studies are necessary to elucidate the cause or causes of sex-influenced toxicity of the kidney in F344 rats. In humans, males also develop a higher incidence of kidney neoplasms than do females (Page and Asire, 1985; Pickle et al., 1987).

Secondary effects of nephropathy seen in kidneys were parathyroid gland hyperplasia and fibrous osteodystrophy of bone, a response seen in other 2-year studies. Renal disease may lead to a decreased plasma calcium level; to compensate for this imbalance, the parathyroid gland responds by secreting parathyroid hormone, and parathyroid hyperplasia may result from prolonged stimulation by low concentrations of serum calcium ions. Mobilization of calcium from the bone and increases in urinary phosphate excretion and calcium ion reabsorption may help restore the plasma concentrations of calcium and phosphate to normal levels. In the current studies, calcium levels were not measured, although mineralization was observed in the kidney.

A dose-related carcinogenic effect was seen in the Zymbal gland of male rats, where an increase in carcinomas or squamous cell carcinomas occurred (vehicle control, 1/50; low dose, 7/50; high dose, 4/49); the incidences of these neoplasms were above the mean historical incidence of 16/1,949 (0.8%). Increased incidences of subcutaneous tissue fibromas or sarcomas (combined) in dosed male rats also occurred (1/50; 5/50; 8/49) and, because the incidences in dosed animals were somewhat greater than the historical incidence of 171/1,949 (9%), these neoplasms may have been related to chemical administration. Similarly, the increased incidences of alveolar/bronchiolar adenomas seen in dosed male rats (4/50; 9/50; 9/49) were above the historical incidence of 68/1,944 (3%), and thus this neoplasm may have been related to chemical administration.

No increased incidences of neoplasms in dosed female rats were observed; the different carcinogenic response in the kidney, Zymbal gland, subcutaneous tissue, and lung indicates a sex difference in response to 8-methoxypsoralen exposure. No toxicity was seen in these organs in the 13-week studies.

8-Methoxypsoralen has been shown to be a mutagen in Salmonella (see below), and, in a review of 222 National Cancer Institute/National Toxicology Program rodent carcinogenicity studies (Ashby and Tennant, 1988), positive results in Salmonella have been shown to correlate with the presence of tumors in the Zymbal gland, lung, and subcutaneous tissue of rats.

In contrast to the results seen when 8-methoxypsoralen is given alone, when 8-methoxypsoralen is administered in combination with ultraviolet A radiation in HRA/Skh mice for 13 weeks, the primary target organ is the skin. This toxicity to the skin is the limiting factor in selecting doses of 8-methoxypsoralen for longer term studies. Eye lesions were associated with 8-methoxypsoralen/ultraviolet A radiation, but no other target organs were seen (Dunnick et al., 1987; Appendix E).

8-Methoxypsoralen has been shown to intercalate with DNA in vitro (Isaacs et al., 1984), and this intercalation has been proposed as a possible mechanism for mutagenicity of 8-methoxypsoralen (Dall'Acqua et al., 1978). In NTP bacterial mutagenicity tests conducted under yellow light to eliminate interaction of the chemical with ambient ultraviolet A radiation, 8-methoxypsoralen produced a positive response in four strains of *Salmonella typhimurium* in the presence of S9 (see Table 19). Results of NTP-sponsored cytogenetics tests in cultured Chinese hamster ovary cells showed induction of both chromosomal aberrations and sister chromatid exchanges (see Tables 20 and 21). Other reports in the literature indicate no mutagenic activity by 8-methoxypsoralen in the absence of ultraviolet radiation. 8-Methoxypsoralen without ultraviolet A radiation has not been tested adequately for in vivo mutagenic effects. 8-Methoxypsoralen alone generally has a much lower level of genetic toxicity than 8-methoxypsoralen given in combination with ultraviolet radiation.

Psoralens have been found to bind to mammalian cells, and psoralens in combination with ultraviolet A radiation have been found to inhibit epidermal growth factor activity (Laskin et al., 1985, 1986). Psoralens alone also are reported to affect the activity of epidermal growth factor and may stimulate cell growth (personal communication from M. Gallo, UMDMJ-Rutgers Medical School to J. Dunnick, NTP).

The experimental and tabulated data for the NTP Technical Report on 8-methoxypsoralen were examined for accuracy, consistency, completeness, and compliance with Good Laboratory Practice regulations. As summarized in Appendix F, the audit revealed no major problems with the conduct of the studies or with collection and documentation of the experimental data. No discrepancies were found that influenced the final interpretation of the results of these studies.

IV. DISCUSSION AND CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity** of 8-methoxypsoralen (without ultraviolet radiation) for male F344/N rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney and carcinomas of the Zymbal gland. Subcutaneous tissue fibromas and alveolar/bronchiolar adenomas of the lung in male F344/N rats may have been related to chemical administration. Dose-related nonneoplastic lesions in male F344/N rats included increased severity of nephropathy and mineralization of the kidney and forestomach lesions. There was *no evidence of carcinogenic activity* of 8-methoxypsoralen for female F344/N rats given the chemical at 37.5 or 75 mg/kg per day for 2 years.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 6.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 9.

V. REFERENCES

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APPENDIX A

SUMMARY OF LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN

	Vehicle Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals removed	50	50	49
Animals examined histopathologically	50	50	49
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(49)
Squamous cell papilloma	2 (4%)	2 (4%)	
Squamous cell carcinoma		1 (2%)	1 (2%)
Basal cell tumor	1 (2%)	2 (4%)	1 (2%)
Basal cell carcinoma	1 (2%)		
Sebaceous adenoma	2 (4%)		
Keratoacanthoma	3 (6%)	3 (6%)	
Malignant melanoma	1 (2%)		
*Subcutaneous tissue	(50)	(50)	(49)
Sarcoma, NOS			1 (2%)
Fibroma	1 (2%)	5 (10%)	7 (14%)
Lipoma	1 (2%)		
RESPIRATORY SYSTEM			
#Lung	(50)	(50)	(49)
Carcinoma, NOS, metastatic		3 (6%)	1 (2%)
Squamous cell carcinoma		1 (2%)	
Alveolar/bronchiolar adenoma	4 (8%)	9 (18%)	9 (18%)
Alveolar/bronchiolar carcinoma		1 (2%)	
Tubular cell adenocarcinoma, metastatic			2 (4%)
Pheochromocytoma, metastatic	1 (2%)	1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(49)
Malignant lymphoma, lymphocytic type		1 (2%)	
Leukemia, mononuclear cell	12 (24%)	7 (14%)	5 (10%)
#Spleen	(50)	(50)	(49)
Tubular cell adenocarcinoma, metastatic			1 (2%)
Sarcoma, NOS		1 (2%)	
Lipoma			1 (2%)
#Lymph node	(50)	(50)	(49)
Tubular cell adenocarcinoma, metastatic			1 (2%)
CIRCULATORY SYSTEM			
*Skin	(50)	(50)	(49)
Hemangiopericytoma, NOS		1 (2%)	
#Heart	(50)	(50)	(49)
Carcinoma, NOS, metastatic		1 (2%)	
DIGESTIVE SYSTEM			
*Oral cavity	(50)	(50)	(49)
Carcinoma, NOS, metastatic		1 (2%)	
*Palate	(50)	(50)	(49)
Squamous cell papilloma	1 (2%)		
*Lip	(50)	(50)	(49)
Squamous cell papilloma	1 (2%)		
*Tongue	(50)	(50)	(49)
Squamous cell papilloma		1 (2%)	2 (4%)
Squamous cell carcinoma			1 (2%)
#Pancreas	(50)	(50)	(49)
Carcinoma, NOS, metastatic		1 (2%)	
Acinar cell adenoma	2 (4%)	3 (6%)	4 (8%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Forestomach	(50)	(50)	(49)
Squamous cell papilloma		2 (4%)	
#Duodenum	(50)	(50)	(49)
Mucinous adenocarcinoma		1 (2%)	
URINARY SYSTEM			
#Kidney	(50)	(50)	(49)
Carcinoma, NOS, metastatic		1 (2%)	
Tubular cell adenoma	1 (2%)	11 (22%)	8 (16%)
Tubular cell adenocarcinoma		1 (2%)	3 (6%)
Sarcoma, NOS		1 (2%)	
#Urinary bladder	(50)	(48)	(49)
Leiomyosarcoma			1 (2%)
ENDOCRINE SYSTEM			
#Anterior pituitary	(49)	(50)	(48)
Adenoma, NOS	24 (49%)	12 (24%)	12 (25%)
#Adrenal	(50)	(50)	(49)
Carcinoma, NOS, metastatic		1 (2%)	
#Adrenal cortex	(50)	(50)	(49)
Adenoma, NOS	1 (2%)		
#Adrenal medulla	(50)	(50)	(49)
Pheochromocytoma	13 (26%)	11 (22%)	9 (18%)
Pheochromocytoma, malignant	1 (2%)	3 (6%)	1 (2%)
#Thyroid	(50)	(50)	(49)
Tubular cell adenocarcinoma, metastatic			1 (2%)
Follicular cell adenoma		1 (2%)	2 (4%)
Follicular cell carcinoma	1 (2%)	2 (4%)	1 (2%)
C-cell adenoma	5 (10%)	6 (12%)	5 (10%)
C-cell carcinoma	3 (6%)		1 (2%)
#Pancreatic islets	(50)	(50)	(49)
Islet cell adenoma	4 (8%)	1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(49)
Papillary carcinoma	1 (2%)		
Adenoma, NOS		1 (2%)	
Fibroadenoma	2 (4%)		4 (8%)
*Preputial gland	(50)	(50)	(49)
Carcinoma, NOS	1 (2%)	1 (2%)	
Squamous cell carcinoma	1 (2%)		
Adenoma, NOS	1 (2%)	4 (8%)	1 (2%)
#Testis	(50)	(48)	(49)
Interstitial cell tumor	38 (76%)	44 (92%)	43 (88%)
NERVOUS SYSTEM			
#Brain	(50)	(50)	(49)
Carcinoma, NOS, metastatic		1 (2%)	1 (2%)
Glioma, NOS	1 (2%)	1 (2%)	
Astrocytoma		1 (2%)	
Neurilemoma	1 (2%)		
SPECIAL SENSE ORGANS			
*Zymbal gland	(50)	(50)	(49)
Carcinoma, NOS		7 (14%)	4 (8%)
Squamous cell carcinoma	1 (2%)		

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	Low Dose	High Dose
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
*Peritoneum	(50)	(50)	(49)
Mesothelioma, metastatic			1 (2%)
*Pericardium	(50)	(50)	(49)
Alveolar/bronchiolar carcinoma, metastatic		1 (2%)	
*Tunica vaginalis	(50)	(50)	(49)
Mesothelioma, NOS	1 (2%)		1 (2%)
Mesothelioma, malignant			1 (2%)
ALL OTHER SYSTEMS			
Orbital region			
Carcinoma, NOS, metastatic		1	
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	5	6	9
Moribund sacrifice	11	27	20
Terminal sacrifice	30	14	16
Dosing accident	4	2	4
Accidentally killed, NOS		1	1
TUMOR SUMMARY			
Total animals with primary tumors**	48	47	45
Total primary tumors	133	149	130
Total animals with benign tumors	48	47	45
Total benign tumors	108	118	109
Total animals with malignant tumors	19	23	18
Total malignant tumors	24	30	20
Total animals with secondary tumors##	1	6	5
Total secondary tumors	1	12	9
Total animals with tumors-- uncertain benign or malignant	1	1	1
Total uncertain tumors	1	1	1

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

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN: VEHICLE CONTROL

ANIMAL NUMBER	043	042	002	016	006	047	035	046	029	033	039	040	001	038	026	003	012	024	004	005	007	008	009	010	011
WEEKS ON STUDY	019	062	064	082	084	084	087	088	090	090	090	090	092	092	097	098	098	101	102	102	106	106	106	106	106
INTEGUMENTARY SYSTEM																									
Skin	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Basal cell tumor																									
Basal cell carcinoma																X									
Sebaceous adenoma																									
Keratoacanthoma																								X	
Malignant melanoma																									
Subcutaneous tissue	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																								X	
Lipoma																							X		
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X															X				
Pheochromocytoma, metastatic																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	−	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell papilloma																					X				
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell adenoma																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS		X				X		X		X	X	X				X	X		X	X					
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Pheochromocytoma																			X		X	X	X		X
Pheochromocytoma, malignant																									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell carcinoma																									
C-cell adenoma																					X			X	X
C-cell carcinoma																				X	X				
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma						X										X									X
REPRODUCTIVE SYSTEM																									
Mammary gland	N	+	+	+	+	+	+	N	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papillary carcinoma																									
Fibroadenoma																								X	
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor		X		X	X		X	X	X				X	X	X	X	X	X			X	X	X	X	X
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
Squamous cell carcinoma																				X					
Adenoma, NOS																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma, NOS				X																					
Neurilemoma																									
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell carcinoma																									
BODY CAVITIES																									
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma, NOS																						X			
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell								X	X				X		X			X		X					

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

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

ANIMAL NUMBER	013	014	015	017	018	019	020	021	022	023	025	027	028	030	031	032	034	036	037	041	044	045	048	049	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell papilloma											X			X												2
Basal cell tumor																							X			1
Basal cell carcinoma																										1
Sebaceous adenoma					X			X																		2
Keratoacanthoma							X																	X		3
Malignant melanoma								X																		1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma																										1
Lipoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma									X					X												4
Pheochromocytoma, metastatic													X													1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	-	+	+	+	+	-	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	41
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell papilloma																							X			2
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinar cell adenoma								X			X															2
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenoma																				X						1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS	X	X			X	X	X	X	X	X	X				X		X			X	X		X			24
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS																					X					1
Pheochromocytoma	X		X		X					X		X				X			X						X	13
Pheochromocytoma, malignant													X													1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell carcinoma								X																		1
C-cell adenoma			X										X													5
C-cell carcinoma																	X									3
Parathyroid	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma						X																				4
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	*50
Papillary carcinoma														X												1
Fibroadenoma										X																2
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell tumor		X	X	X	X	X	X	X		X	X	X		X	X	X	X	X	X		X	X	X	X	X	38
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS										X																1
Squamous cell carcinoma																										1
Adenoma, NOS																					X					1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Glioma, NOS																										1
Neurilemoma																								X		1
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell carcinoma				X																						1
BODY CAVITIES																										
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Mesothelioma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell					X				X	X		X		X								X				12

* Animals necropsied

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN: LOW DOSE

ANIMAL NUMBER	029	031	033	004	035	043	011	019	045	046	023	040	020	039	034	044	017	049	026	024	008	028	038	022	006
WEEKS ON STUDY	009	049	058	060	070	078	079	080	083	083	084	084	087	087	089	089	090	091	092	094	095	095	095	096	097
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	N	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Squamous cell carcinoma																									
Basal cell tumor																									
Keratoacanthoma																		X							
Hemangiopericytoma, NOS																X									
Subcutaneous tissue	+	+	+	+	+	+	N	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic										X										X					
Squamous cell carcinoma																					X				
Alveolar/bronchiolar adenoma														X					X		X				X
Alveolar/bronchiolar carcinoma																									
Pheochromocytoma, metastatic																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS									X																
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	-	-	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic																									
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS, metastatic																									
Squamous cell papilloma																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic																									
Acinar cell adenoma																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma									X													X			
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mucinous adenocarcinoma																					X				
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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

ANIMAL NUMBER	027	007	014	018	010	016	021	032	041	036	001	002	003	005	009	012	013	015	025	030	037	042	047	048	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	098	100	100	100	103	103	103	103	103	104	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell papilloma					X																			X		2
Squamous cell carcinoma		X																								1
Basal cell tumor			X																X							2
Keratoacanthoma		X		X																						3
Hemangiopericytoma, NOS																										1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma			X			X			X									X		X						5
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, metastatic																						X				3
Squamous cell carcinoma																										1
Alveolar/bronchiolar adenoma									X		X						X			X		X				9
Alveolar/bronchiolar carcinoma											X															1
Pheochromocytoma, metastatic																								X		1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	48
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, NOS																										1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	−	+	−	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	39
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, metastatic																						X				1
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS, metastatic							X																			1
Squamous cell papilloma				X																						1
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, metastatic																						X				1
Acinar cell adenoma					X											X		X								3
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										2
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mucinous adenocarcinoma																										1
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

* Animals necropsied

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

ANIMAL NUMBER	029	031	033	004	035	043	011	019	045	046	023	040	020	039	034	044	017	049	026	024	008	028	038	022	006
WEEKS ON STUDY	009	049	058	060	070	078	079	080	083	083	084	084	087	087	089	089	090	091	092	094	095	095	095	096	097
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic																									
Tubular cell adenoma																						X			X
Tubular cell adenocarcinoma																									
Sarcoma, NOS																									
Urinary bladder	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS					X			X				X								X			X		
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic																									
Pheochromocytoma														X									X		X
Pheochromocytoma, malignant												X													
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																									
Follicular cell carcinoma																					X				
C-cell adenoma						X								X				X							
Parathyroid	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																						X			
Testis	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Interstitial cell tumor		X		X	X	X	X		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
Prostate	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
Adenoma, NOS					X																				
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic																				X					
Glioma, NOS																		X							
Astrocytoma																		X							
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS										X										X					
BODY CAVITIES																									
Pericardium	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Alveolar/bronchiolar carcinoma, metastatic																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, lymphocytic type																									
Leukemia, mononuclear cell						X					X	X				X			X						
Orbital region																									
Carcinoma, NOS, metastatic																									

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

ANIMAL NUMBER	027	007	014	018	010	016	021	032	041	036	001	002	003	005	009	012	013	015	025	030	037	042	047	048	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	098	100	100	100	103	103	103	103	103	104	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, metastatic																						X				1
Tubular cell adenoma	X			X	X			X	X				X		X				X						X	11
Tubular cell adenocarcinoma												X														1
Sarcoma, NOS																							X			1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS	X	X	X	X				X										X							X	12
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, metastatic																						X				1
Pheochromocytoma	X				X		X				X	X		X					X						X	11
Pheochromocytoma, malignant									X															X		3
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma					X																					1
Follicular cell carcinoma														X												2
C-cell adenoma				X						X	X															6
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma																					X					1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Adenoma, NOS																										1
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Interstitial cell tumor	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X		X	44
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS													X													1
Adenoma, NOS									X	X				X												4
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, metastatic																										1
Glioma, NOS																										1
Astrocytoma																										1
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS							X	X	X				X									X				7
BODY CAVITIES																										
Pericardium	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Alveolar/bronchiolar carcinoma, meta.											X															1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, lymphocytic type														X												1
Leukemia, mononuclear cell										X								X								7
Orbital region																										
Carcinoma, NOS, metastatic							X																			1

* Animals necropsied

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN: HIGH DOSE

ANIMAL NUMBER	029	010	045	021	022	033	040	038	028	031	030	043	002	012	008	036	039	048	001	020	026	015	025	027	006
WEEKS ON STUDY	013	023	024	048	054	054	057	068	070	078	080	083	084	086	087	090	091	091	092	092	092	093	093	093	094
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	N	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Basal cell tumor																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	N	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS	X																								
Fibroma							X									X	X								
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic																									
Alveolar/bronchiolar adenoma								X																X	X
Tubular cell adenocarcinoma, metastatic																			X						
Pheochromocytoma, metastatic																									X
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	–	–	–	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Tubular cell adenocarcinoma, metastatic																			X						
Lipoma																									
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Tubular cell adenocarcinoma, metastatic																			X						
Thymus	+	+	+	+	–	–	+	–	+	+	+	+	+	+	A	+	+	–	+	–	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N
Squamous cell papilloma																		X							
Squamous cell carcinoma																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Acinar cell adenoma																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma											X											X	X		X
Tubular cell adenocarcinoma																			X						
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																									
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	–	+	+	+
Adenoma, NOS													X			X							X	X	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																X		X							
Pheochromocytoma, malignant																									X
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Tubular cell adenocarcinoma, metastatic																			X						
Follicular cell adenoma																									X
Follicular cell carcinoma																									
C-cell adenoma														X											
C-cell carcinoma																									
Parathyroid	+	+	–	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	N	+	+	+	+	+
Fibroadenoma	X											X													
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor						X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																			X						
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic																									
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS										X							X			X					
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N
Mesothelioma, metastatic														X											
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Mesothelioma, NOS																									
Mesothelioma, malignant														X											
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell											X						X								

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

ANIMAL NUMBER	007	016	034	023	003	018	014	042	049	004	005	009	011	013	017	019	024	032	035	037	041	044	046	047	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	097	097	097	100	101	101	102	102	102	104	104	104	104	104	104	104	104	104	104	105	105	105	105	105	105	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Squamous cell carcinoma					X																					1
Basal cell tumor											X															1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Sarcoma, NOS																										1
Fibroma												X		X					X					X		7
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, NOS, metastatic							X																			1
Alveolar/bronchiolar adenoma	X		X					X									X			X	X					9
Tubular cell adenocarcinoma, metastatic																	X									2
Pheochromocytoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	45
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tubular cell adenocarcinoma, metastatic																										1
Lipoma		X																								1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tubular cell adenocarcinoma, metastatic																										1
Thymus	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	41
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Squamous cell papilloma	X																									2
Squamous cell carcinoma			X																							1
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Acinar cell adenoma									X		X								X	X						4
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tubular cell adenoma		X					X																X	X		8
Tubular cell adenocarcinoma																	X			X						3
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leiomyosarcoma																									X	1
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma, NOS		X	X	X				X								X			X	X				X		12
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma					X				X				X	X	X							X			X	9
Pheochromocytoma, malignant																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tubular cell adenocarcinoma, metastatic																										1
Follicular cell adenoma						X																				2
Follicular cell carcinoma				X																						1
C-cell adenoma						X		X		X						X										5
C-cell carcinoma						X																				1
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islet cell adenoma																X										1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	*49
Fibroadenoma				X								X														4
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Interstitial cell tumor	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	43
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	48
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Adenoma, NOS																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, NOS, metastatic							X																			1
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Carcinoma, NOS							X																			4
BODY CAVITIES																										
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Mesothelioma, metastatic																										1
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Mesothelioma, NOS					X																					1
Mesothelioma, malignant																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Leukemia, mononuclear cell						X			X												X					5

* Animals necropsied

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN

	Vehicle Control	37.5 mg/kg	75 mg/kg
Skin: Keratoacanthoma			
Overall Rates (a)	3/50 (6%)	3/50 (6%)	0/49 (0%)
Adjusted Rates (b)	10.0%	11.1%	0.0%
Terminal Rates (c)	3/30 (10%)	0/16 (0%)	0/16 (0%)
Week of First Observation	104	91	
Life Table Tests (d)	P=0.226N	P=0.449	P=0.250N
Incidental Tumor Tests (d)	P=0.123N	P=0.666N	P=0.250N
Cochran-Armitage Trend Test (d)	P=0.104N		
Fisher Exact Test (d)		P=0.661	P=0.125N
Skin: Squamous Cell Papilloma or Carcinoma			
Overall Rates (a)	2/50 (4%)	3/50 (6%)	1/49 (2%)
Adjusted Rates (b)	6.7%	14.4%	4.8%
Terminal Rates (c)	2/30 (7%)	1/16 (6%)	0/16 (0%)
Week of First Observation	104	100	101
Life Table Tests (d)	P=0.562	P=0.279	P=0.693N
Incidental Tumor Tests (d)	P=0.406N	P=0.489	P=0.559N
Cochran-Armitage Trend Test (d)	P=0.407N		
Fisher Exact Test (d)		P=0.500	P=0.508N
Skin: Basal Cell Tumor or Sebaceous Adenoma			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	1/49 (2%)
Adjusted Rates (b)	10.0%	10.2%	6.3%
Terminal Rates (c)	3/30 (10%)	1/16 (6%)	1/16 (6%)
Week of First Observation	104	100	104
Life Table Tests (d)	P=0.453N	P=0.618N	P=0.547N
Incidental Tumor Tests (d)	P=0.383N	P=0.650N	P=0.547N
Cochran-Armitage Trend Test (d)	P=0.228N		
Fisher Exact Test (d)		P=0.500N	P=0.316N
Skin: Basal Cell Tumor, Sebaceous Adenoma, or Basal Cell Carcinoma			
Overall Rates (a)	4/50 (8%)	2/50 (4%)	1/49 (2%)
Adjusted Rates (b)	12.6%	10.2%	6.3%
Terminal Rates (c)	3/30 (10%)	1/16 (6%)	1/16 (6%)
Week of First Observation	98	100	104
Life Table Tests (d)	P=0.308N	P=0.593N	P=0.395N
Incidental Tumor Tests (d)	P=0.193N	P=0.387N	P=0.288N
Cochran-Armitage Trend Test (d)	P=0.122N		
Fisher Exact Test (d)		P=0.399N	P=0.187N
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	1/50 (2%)	5/50 (10%)	7/49 (14%)
Adjusted Rates (b)	3.3%	24.1%	30.9%
Terminal Rates (c)	1/30 (3%)	2/16 (13%)	4/16 (25%)
Week of First Observation	104	100	57
Life Table Tests (d)	P=0.004	P=0.029	P=0.006
Incidental Tumor Tests (d)	P=0.012	P=0.115	P=0.009
Cochran-Armitage Trend Test (d)	P=0.023		
Fisher Exact Test (d)		P=0.102	P=0.028
Subcutaneous Tissue: Fibroma or Sarcoma			
Overall Rates (a)	1/50 (2%)	5/50 (10%)	8/49 (16%)
Adjusted Rates (b)	3.3%	24.1%	32.3%
Terminal Rates (c)	1/30 (3%)	2/16 (13%)	4/16 (25%)
Week of First Observation	104	100	13
Life Table Tests (d)	P=0.002	P=0.029	P=0.003
Incidental Tumor Tests (d)	P=0.009	P=0.115	P=0.008
Cochran-Armitage Trend Test (d)	P=0.012		
Fisher Exact Test (d)		P=0.102	P=0.014

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	37.5 mg/kg	75 mg/kg
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	4/50 (8%)	(e) 9/50 (18%)	9/49 (18%)
Adjusted Rates (b)	12.0%	37.4%	35.8%
Terminal Rates (c)	3/30 (10%)	4/16 (25%)	3/16 (19%)
Week of First Observation	84	87	68
Life Table Tests (d)	P=0.015	P=0.022	P=0.022
Incidental Tumor Tests (d)	P=0.075	P=0.077	P=0.131
Cochran-Armitage Trend Test (d)	P=0.094		
Fisher Exact Test (d)		P=0.117	P=0.109
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	12/50 (24%)	7/50 (14%)	5/49 (10%)
Adjusted Rates (b)	32.2%	23.4%	20.0%
Terminal Rates (c)	6/30 (20%)	2/16 (13%)	1/16 (6%)
Week of First Observation	88	78	80
Life Table Tests (d)	P=0.217N	P=0.464N	P=0.275N
Incidental Tumor Tests (d)	P=0.057N	P=0.160N	P=0.063N
Cochran-Armitage Trend Test (d)	P=0.042N		
Fisher Exact Test (d)		P=0.154N	P=0.059N
Oral Cavity: Squamous Cell Papilloma or Carcinoma			
Overall Rates (a)	2/50 (4%)	1/50 (2%)	3/49 (6%)
Adjusted Rates (b)	6.7%	4.2%	10.7%
Terminal Rates (c)	2/30 (7%)	0/16 (0%)	0/16 (0%)
Week of First Observation	106	100	91
Life Table Tests (d)	P=0.247	P=0.678N	P=0.318
Incidental Tumor Tests (d)	P=0.422	P=0.545N	P=0.515
Cochran-Armitage Trend Test (d)	P=0.391		
Fisher Exact Test (d)		P=0.500N	P=0.490
Pancreas: Acinar Cell Adenoma			
Overall Rates (a)	2/50 (4%)	3/50 (6%)	4/49 (8%)
Adjusted Rates (b)	6.7%	16.7%	23.0%
Terminal Rates (c)	2/30 (7%)	2/16 (13%)	3/16 (19%)
Week of First Observation	106	103	102
Life Table Tests (d)	P=0.073	P=0.252	P=0.109
Incidental Tumor Tests (d)	P=0.125	P=0.350	P=0.168
Cochran-Armitage Trend Test (d)	P=0.255		
Fisher Exact Test (d)		P=0.500	P=0.329
Kidney: Tubular Cell Adenoma			
Overall Rates (a)	1/50 (2%)	11/50 (22%)	8/49 (16%)
Adjusted Rates (b)	3.3%	45.0%	30.5%
Terminal Rates (c)	1/30 (3%)	4/16 (25%)	2/16 (13%)
Week of First Observation	106	95	80
Life Table Tests (d)	P=0.003	P<0.001	P=0.004
Incidental Tumor Tests (d)	P=0.031	P=0.004	P=0.026
Cochran-Armitage Trend Test (d)	P=0.025		
Fisher Exact Test (d)		P=0.002	P=0.014
Kidney: Tubular Cell Adenocarcinoma			
Overall Rates (a)	0/50 (0%)	1/50 (2%)	3/49 (6%)
Adjusted Rates (b)	0.0%	6.2%	15.2%
Terminal Rates (c)	0/30 (0%)	1/16 (6%)	2/16 (13%)
Week of First Observation		105	92
Life Table Tests (d)	P=0.024	P=0.375	P=0.053
Incidental Tumor Tests (d)	P=0.024	P=0.375	P=0.055
Cochran-Armitage Trend Test (d)	P=0.058		
Fisher Exact Test (d)		P=0.500	P=0.117

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	37.5 mg/kg	75 mg/kg
Kidney: Tubular Cell Adenoma or Adenocarcinoma			
Overall Rates (a)	1/50 (2%)	12/50 (24%)	11/49 (22%)
Adjusted Rates (b)	3.3%	49.6%	42.3%
Terminal Rates (c)	1/30 (3%)	5/16 (31%)	4/16 (25%)
Week of First Observation	106	95	80
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests (d)	P=0.003	P=0.001	P=0.002
Cochran-Armitage Trend Test (d)	P=0.004		
Fisher Exact Test (d)		P<0.001	P=0.002
Anterior Pituitary Gland: Adenoma			
Overall Rates (a)	24/49 (49%)	12/50 (24%)	12/48 (25%)
Adjusted Rates (b)	59.0%	39.0%	45.2%
Terminal Rates (c)	14/30 (47%)	2/16 (13%)	4/16 (25%)
Week of First Observation	62	70	84
Life Table Tests (d)	P=0.231N	P=0.242N	P=0.298N
Incidental Tumor Tests (d)	P=0.007N	P=0.003N	P=0.018N
Cochran-Armitage Trend Test (d)	P=0.008N		
Fisher Exact Test (d)		P=0.009N	P=0.013N
Adrenal Medulla: Pheochromocytoma			
Overall Rates (a)	13/50 (26%)	11/50 (22%)	9/49 (18%)
Adjusted Rates (b)	41.9%	46.0%	41.5%
Terminal Rates (c)	12/30 (40%)	5/16 (31%)	5/16 (31%)
Week of First Observation	102	87	90
Life Table Tests (d)	P=0.316	P=0.215	P=0.392
Incidental Tumor Tests (d)	P=0.491N	P=0.550	P=0.588
Cochran-Armitage Trend Test (d)	P=0.214N		
Fisher Exact Test (d)		P=0.408N	P=0.251N
Adrenal Medulla: Malignant Pheochromocytoma			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	1/49 (2%)
Adjusted Rates (b)	3.3%	12.9%	3.8%
Terminal Rates (c)	1/30 (3%)	1/16 (6%)	0/16 (0%)
Week of First Observation	106	84	94
Life Table Tests (d)	P=0.428	P=0.169	P=0.652
Incidental Tumor Tests (d)	P=0.599	P=0.281	P=0.759N
Cochran-Armitage Trend Test (d)	P=0.603		
Fisher Exact Test (d)		P=0.309	P=0.747
Adrenal Medulla: Pheochromocytoma or Malignant Pheochromocytoma			
Overall Rates (a)	14/50 (28%)	14/50 (28%)	10/49 (20%)
Adjusted Rates (b)	45.1%	54.6%	43.8%
Terminal Rates (c)	13/30 (43%)	6/16 (38%)	5/16 (31%)
Week of First Observation	102	84	90
Life Table Tests (d)	P=0.262	P=0.082	P=0.352
Incidental Tumor Tests (d)	P=0.498N	P=0.344	P=0.592
Cochran-Armitage Trend Test (d)	P=0.227N		
Fisher Exact Test (d)		P=0.588	P=0.259N
Thyroid Gland: Follicular Cell Adenoma or Carcinoma			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	3/49 (6%)
Adjusted Rates (b)	3.3%	13.7%	12.6%
Terminal Rates (c)	1/30 (3%)	1/16 (6%)	0/16 (0%)
Week of First Observation	106	95	94
Life Table Tests (d)	P=0.107	P=0.166	P=0.166
Incidental Tumor Tests (d)	P=0.314	P=0.340	P=0.456
Cochran-Armitage Trend Test (d)	P=0.231		
Fisher Exact Test (d)		P=0.309	P=0.301

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	37.5 mg/kg	75 mg/kg
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	5/50 (10%)	6/50 (12%)	5/49 (10%)
Adjusted Rates (b)	16.7%	22.6%	23.2%
Terminal Rates (c)	5/30 (17%)	2/16 (13%)	2/16 (13%)
Week of First Observation	106	78	86
Life Table Tests (d)	P=0.242	P=0.213	P=0.279
Incidental Tumor Tests (d)	P=0.412	P=0.345	P=0.439
Cochran-Armitage Trend Test (d)	P=0.551		
Fisher Exact Test (d)		P=0.500	P=0.617
Thyroid Gland: C-Cell Carcinoma			
Overall Rates (a)	3/50 (6%)	0/50 (0%)	1/49 (2%)
Adjusted Rates (b)	9.6%	0.0%	4.8%
Terminal Rates (c)	2/30 (7%)	0/16 (0%)	0/16 (0%)
Week of First Observation	102		101
Life Table Tests (d)	P=0.334N	P=0.237N	P=0.518N
Incidental Tumor Tests (d)	P=0.171N	P=0.129N	P=0.271N
Cochran-Armitage Trend Test (d)	P=0.180N		
Fisher Exact Test (d)		P=0.121N	P=0.316N
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	7/50 (14%)	6/50 (12%)	5/49 (10%)
Adjusted Rates (b)	22.5%	22.6%	23.2%
Terminal Rates (c)	6/30 (20%)	2/16 (13%)	2/16 (13%)
Week of First Observation	102	78	86
Life Table Tests (d)	P=0.425	P=0.386	P=0.468
Incidental Tumor Tests (d)	P=0.461N	P=0.612	P=0.549N
Cochran-Armitage Trend Test (d)	P=0.335N		
Fisher Exact Test (d)		P=0.500N	P=0.394N
Pancreatic Islets: Islet Cell Adenoma			
Overall Rates (a)	4/50 (8%)	1/50 (2%)	1/49 (2%)
Adjusted Rates (b)	11.3%	6.3%	6.3%
Terminal Rates (c)	2/30 (7%)	1/16 (6%)	1/16 (6%)
Week of First Observation	84	104	104
Life Table Tests (d)	P=0.245N	P=0.345N	P=0.360N
Incidental Tumor Tests (d)	P=0.180N	P=0.223N	P=0.253N
Cochran-Armitage Trend Test (d)	P=0.104N		
Fisher Exact Test (d)		P=0.181N	P=0.188N
Mammary Gland: Fibroadenoma			
Overall Rates (a)	2/50 (4%)	0/50 (0%)	4/49 (8%)
Adjusted Rates (b)	6.7%	0.0%	14.5%
Terminal Rates (c)	2/30 (7%)	0/16 (0%)	1/16 (6%)
Week of First Observation	106		13
Life Table Tests (d)	P=0.118	P=0.384N	P=0.176
Incidental Tumor Tests (d)	P=0.206	P=0.384N	P=0.330
Cochran-Armitage Trend Test (d)	P=0.216		
Fisher Exact Test (d)		P=0.247N	P=0.329
Mammary Gland: Adenoma or Fibroadenoma			
Overall Rates (a)	2/50 (4%)	1/50 (2%)	4/49 (8%)
Adjusted Rates (b)	6.7%	3.3%	14.5%
Terminal Rates (c)	2/30 (7%)	0/16 (0%)	1/16 (6%)
Week of First Observation	106	95	13
Life Table Tests (d)	P=0.124	P=0.663N	P=0.176
Incidental Tumor Tests (d)	P=0.251	P=0.545N	P=0.330
Cochran-Armitage Trend Test (d)	P=0.231		
Fisher Exact Test (d)		P=0.500N	P=0.329

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	37.5 mg/kg	75 mg/kg
Mammary Gland: Adenoma, Fibroadenoma, or Papillary Carcinoma			
Overall Rates (a)	3/50 (6%)	1/50 (2%)	4/49 (8%)
Adjusted Rates (b)	10.0%	3.3%	14.5%
Terminal Rates (c)	3/30 (10%)	0/16 (0%)	1/16 (6%)
Week of First Observation	106	95	13
Life Table Tests (d)	P=0.228	P=0.501N	P=0.276
Incidental Tumor Tests (d)	P=0.393	P=0.390N	P=0.451
Cochran-Armitage Trend Test (d)	P=0.402		
Fisher Exact Test (d)		P=0.309N	P=0.489
Preputial Gland: Adenoma			
Overall Rates (a)	1/50 (2%)	4/50 (8%)	1/49 (2%)
Adjusted Rates (b)	3.3%	18.5%	3.1%
Terminal Rates (c)	1/30 (3%)	2/16 (13%)	0/16 (0%)
Week of First Observation	106	70	92
Life Table Tests (d)	P=0.405	P=0.073	P=0.671
Incidental Tumor Tests (d)	P=0.569	P=0.150	P=0.681
Cochran-Armitage Trend Test (d)	P=0.593		
Fisher Exact Test (d)		P=0.181	P=0.747
Preputial Gland: Adenoma, Carcinoma, or Squamous Cell Carcinoma			
Overall Rates (a)	3/50 (6%)	5/50 (10%)	1/49 (2%)
Adjusted Rates (b)	9.6%	24.3%	3.1%
Terminal Rates (c)	2/30 (7%)	3/16 (19%)	0/16 (0%)
Week of First Observation	102	70	92
Life Table Tests (d)	P=0.519N	P=0.136	P=0.489N
Incidental Tumor Tests (d)	P=0.320N	P=0.302	P=0.354N
Cochran-Armitage Trend Test (d)	P=0.272N		
Fisher Exact Test (d)		P=0.357	P=0.316N
Testis: Interstitial Cell Tumor			
Overall Rates (a)	38/50 (76%)	44/48 (92%)	43/49 (88%)
Adjusted Rates (b)	90.2%	95.6%	97.7%
Terminal Rates (c)	26/30 (87%)	14/16 (88%)	15/16 (94%)
Week of First Observation	62	49	54
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests (d)	P=0.006	P=0.017	P=0.007
Cochran-Armitage Trend Test (d)	P=0.066		
Fisher Exact Test (d)		P=0.033	P=0.104
Zymbal Gland: Carcinoma or Squamous Cell Carcinoma			
Overall Rates (a)	1/50 (2%)	7/50 (14%)	4/49 (8%)
Adjusted Rates (b)	3.3%	29.1%	13.1%
Terminal Rates (c)	1/30 (3%)	2/16 (13%)	0/16 (0%)
Week of First Observation	106	83	78
Life Table Tests (d)	P=0.063	P=0.008	P=0.104
Incidental Tumor Tests (d)	P=0.229	P=0.051	P=0.233
Cochran-Armitage Trend Test (d)	P=0.170		
Fisher Exact Test (d)		P=0.030	P=0.175
All Sites: Benign Tumors			
Overall Rates (a)	48/50 (96%)	47/50 (94%)	45/49 (92%)
Adjusted Rates (b)	100.0%	100.0%	100.0%
Terminal Rates (c)	30/30 (100%)	16/16 (100%)	16/16 (100%)
Week of First Observation	62	49	13
Life Table Tests (d)	P=0.006	P=0.006	P=0.008
Incidental Tumor Tests (d)	P=0.359	P=0.626	P=0.387
Cochran-Armitage Trend Test (d)	P=0.255N		
Fisher Exact Test (d)		P=0.500N	P=0.329N

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	37.5 mg/kg	75 mg/kg
All Sites: Malignant Tumors			
Overall Rates (a)	19/50 (38%)	23/50 (46%)	18/49 (37%)
Adjusted Rates (b)	48.9%	72.8%	55.8%
Terminal Rates (c)	11/30 (37%)	9/16 (56%)	4/16 (25%)
Week of First Observation	82	78	13
Life Table Tests (d)	P=0.097	P=0.021	P=0.147
Incidental Tumor Tests (d)	P=0.518	P=0.225	P=0.486N
Cochran-Armitage Trend Test (d)	P=0.492N		
Fisher Exact Test (d)		P=0.272	P=0.531N
All Sites: All Tumors			
Overall Rates (a)	48/50 (96%)	47/50 (94%)	45/49 (92%)
Adjusted Rates (b)	100.0%	100.0%	100.0%
Terminal Rates (c)	30/30 (100%)	16/16 (100%)	16/16 (100%)
Week of First Observation	62	49	13
Life Table Tests (d)	P=0.006	P=0.006	P=0.008
Incidental Tumor Tests (d)	P=0.359	P=0.626	P=0.387
Cochran-Armitage Trend Test (d)	P=0.255N		
Fisher Exact Test (d)		P=0.500N	P=0.329N

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

TABLE A4a. HISTORICAL INCIDENCE OF RENAL TUBULAR CELL TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

	Incidence of Adenomas or Adenocarcinomas in Vehicle Controls
No 2-year studies by SRI International are included in the historical data base.	
Overall Historical Incidence	
TOTAL	(b) 10/1,943 (0.5%)
SD (c)	0.89%
Range (d)	
High	1/48
Low	0/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Includes three tubular cell adenomas, two adenocarcinomas, NOS, and five tubular cell adenocarcinomas
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE A4b. HISTORICAL INCIDENCE OF ZYMBAL GLAND TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

	Incidence of Adenomas or Carcinomas in Vehicle Controls
No 2-year studies by SRI International are included in the historical data base.	
Overall Historical Incidence	
TOTAL	(b) 16/1,949 (0.8%)
SD (c)	1.28%
Range (d)	
High	(e) 2/49
Low	0/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Includes two adenomas, NOS, three squamous cell carcinomas, one sebaceous adenocarcinoma, and one ceruminous carcinoma
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) Carcinomas, NOS

TABLE A4c. HISTORICAL INCIDENCE OF INTEGUMENTARY SYSTEM TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

	Incidence in Vehicle Controls		
	Fibroma	Fibrosarcoma	Fibroma or Fibrosarcoma
No 2-year studies by SRI International are included in the historical data base.			
Overall Historical Incidence			
TOTAL	(b) 126/1,949 (6.5%)	(c) 47/1,949 (2.4%)	(b,c) 171/1,949 (8.8%)
SD (d)	3.35%	2.65%	4.15%
Range (e)			
High	8/50	6/50	9/50
Low	0/50	0/50	1/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Includes five neurofibromas
(c) Includes 15 sarcomas, NOS, and 5 neurofibrosarcomas
(d) Standard deviation
(e) Range and SD are presented for groups of 35 or more animals.

TABLE A4d. HISTORICAL INCIDENCE OF ALVEOLAR/BRONCHIOLAR TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

	Incidence in Vehicle Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
No 2-year studies by SRI International are included in the historical data base.			
Overall Historical Incidence			
TOTAL	45/1,944 (2.3%)	25/1,944 (1.3%)	68/1,944 (3.5%)
SD (b)	2.38%	1.62%	2.94%
Range (c)			
High	4/50	3/50	5/50
Low	0/50	0/50	0/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE A4e. HISTORICAL INCIDENCE OF STOMACH SQUAMOUS CELL TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

	Incidence of Papillomas or Carcinomas in Vehicle Controls
No 2-year studies by SRI International are included in the historical data base.	
Overall Historical Incidence	
TOTAL	(b) 7/1,924 (0.4%)
SD (c)	0.78%
Range (d)	
High	1/49
Low	0/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Includes one papilloma, NOS, five squamous cell papillomas, and one squamous cell carcinoma
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE A4f. HISTORICAL INCIDENCE OF THYROID GLAND FOLLICULAR CELL TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

	Incidence in Vehicle Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
No 2-year studies by SRI International are included in the historical data base.			
Overall Historical Incidence			
TOTAL	(b) 18/1,909 (0.9%)	28/1,909 (1.5%)	(b) 46/1,909 (2.4%)
SD (c)	1.73%	1.85%	2.44%
Range (d)			
High	2/50	4/50	5/50
Low	0/50	0/50	0/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Includes one cystadenoma, NOS
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE A4g. HISTORICAL INCIDENCE OF ANTERIOR PITUITARY GLAND TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

	Incidence in Vehicle Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
No 2-year studies by SRI International are included in the historical data base.			
Overall Historical Incidence			
TOTAL	(b) 519/1,898 (27.3%)	(c) 38/1,898 (2.0%)	(b,c) 556/1,898 (29.3%)
SD (d)	10.31%	2.61%	10.48%
Range (e)			
High	26/48	4/47	26/48
Low	5/50	0/50	6/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Includes 34 chromophobe adenomas and 1 acidophil adenoma
(c) Includes three adenocarcinomas, NOS, and four chromophobe carcinomas
(d) Standard deviation
(e) Range and SD are presented for groups of 35 or more animals.

TABLE A4h. HISTORICAL INCIDENCE OF TESTICULAR INTERSTITIAL CELL TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

	Incidence in Vehicle Controls
No 2-year studies by SRI International are included in the historical data base.	
Overall Historical Incidence	
TOTAL	(b) 1,674/1,944 (86.1%)
SD (c)	9.42%
Range (d)	
High	48/50
Low	31/49

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Includes two malignant interstitial cell tumors
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN

	Vehicle Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals removed	50	50	49
Animals examined histopathologically	50	50	49
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(49)
Epidermal inclusion cyst	1 (2%)	4 (8%)	2 (4%)
Hyperkeratosis	2 (4%)		
RESPIRATORY SYSTEM			
#Nasal cavity	(48)	(48)	(45)
Hemorrhage	1 (2%)		1 (2%)
Inflammation, acute	2 (4%)	1 (2%)	
Inflammation, chronic	6 (13%)		4 (9%)
Reaction, foreign body		1 (2%)	
Infection, fungal	1 (2%)		
Metaplasia, squamous		1 (2%)	2 (4%)
#Accessory sinus	(48)	(48)	(45)
Abscess, NOS		1 (2%)	
#Lung/bronchus	(50)	(50)	(49)
Inflammation, NOS		1 (2%)	1 (2%)
#Lung	(50)	(50)	(49)
Atelectasis	1 (2%)		1 (2%)
Congestion, NOS	5 (10%)	8 (16%)	12 (24%)
Edema, NOS			1 (2%)
Hemorrhage	1 (2%)	1 (2%)	2 (4%)
Inflammation, NOS	7 (14%)	5 (10%)	1 (2%)
Inflammation, acute	1 (2%)	2 (4%)	
Abscess, NOS	1 (2%)		1 (2%)
Inflammation, chronic			1 (2%)
Granuloma, NOS	37 (74%)	25 (50%)	25 (51%)
Hyperplasia, alveolar epithelium	5 (10%)	7 (14%)	9 (18%)
Metaplasia, osseous	1 (2%)	2 (4%)	
Histiocytosis		3 (6%)	
#Lung/alveoli	(50)	(50)	(49)
Granuloma, NOS	1 (2%)		1 (2%)
Histiocytosis	1 (2%)		
HEMATOPOIETIC SYSTEM			
#Bone marrow	(50)	(50)	(49)
Hypoplasia, NOS	4 (8%)		1 (2%)
Hyperplasia, NOS		1 (2%)	1 (2%)
#Spleen	(50)	(50)	(49)
Congenital malformation, NOS		1 (2%)	
Congestion, NOS	3 (6%)	3 (6%)	6 (12%)
Fibrosis	4 (8%)	7 (14%)	5 (10%)
Fibrosis, focal		1 (2%)	
Infarct, NOS	4 (8%)	1 (2%)	1 (2%)
Hemosiderosis	1 (2%)	3 (6%)	
Metaplasia, myeloid	3 (6%)	3 (6%)	3 (6%)
#Lymph node	(50)	(50)	(49)
Congestion, NOS	1 (2%)		3 (6%)
Hemorrhage		1 (2%)	
Inflammation, chronic		1 (2%)	
Hemosiderosis		1 (2%)	
Hyperplasia, NOS	3 (6%)	5 (10%)	8 (16%)
Histiocytosis		9 (18%)	
Plasmacytosis	8 (16%)	7 (14%)	2 (4%)
#Mandibular lymph node	(50)	(50)	(49)
Plasmacytosis	1 (2%)		

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Mesenteric lymph node	(50)	(50)	(49)
Hyperplasia, NOS			1 (2%)
#Lung	(50)	(50)	(49)
Leukocytosis, NOS		1 (2%)	
#Thymus	(41)	(39)	(41)
Necrosis, focal		1 (3%)	
CIRCULATORY SYSTEM			
#Lymph node	(50)	(50)	(49)
Lymphangiectasis		1 (2%)	
#Lung	(50)	(50)	(49)
Thrombosis, NOS			1 (2%)
#Heart	(50)	(50)	(49)
Abscess, NOS	1 (2%)		
Inflammation, chronic focal	1 (2%)		
Fibrosis	17 (34%)	10 (20%)	14 (29%)
Fibrosis, focal	11 (22%)	1 (2%)	1 (2%)
Endocardiosis	1 (2%)		
Necrosis, focal			1 (2%)
Infarct, NOS		1 (2%)	1 (2%)
Calcification, NOS		2 (4%)	1 (2%)
#Heart/atrium	(50)	(50)	(49)
Thrombus, mural	2 (4%)		
*Blood vessel	(50)	(50)	(49)
Calcification, NOS		1 (2%)	
*Artery	(50)	(50)	(49)
Inflammation, NOS		1 (2%)	
Polyangiitis		1 (2%)	
*Aorta	(50)	(50)	(49)
Calcification, NOS		2 (4%)	
*Coronary artery	(50)	(50)	(49)
Inflammation, necrotizing			1 (2%)
*Hepatic artery	(50)	(50)	(49)
Aneurysm		1 (2%)	
*Mesenteric artery	(50)	(50)	(49)
Aneurysm		1 (2%)	
*Vein	(50)	(50)	(49)
Thrombosis, NOS		1 (2%)	
*Vena cava	(50)	(50)	(49)
Calcification, NOS		1 (2%)	
*Portal vein	(50)	(50)	(49)
Thrombus, organized	1 (2%)		
#Testis	(50)	(48)	(49)
Periarteritis	1 (2%)		
DIGESTIVE SYSTEM			
*Tongue	(50)	(50)	(49)
Hyperkeratosis			1 (2%)
#Salivary gland	(50)	(50)	(49)
Edema, NOS	1 (2%)		
Inflammation, acute	1 (2%)		
Fibrosis		1 (2%)	
Atrophy, NOS		1 (2%)	
#Liver	(50)	(50)	(49)
Hernia, NOS	3 (6%)		3 (6%)
Congestion, NOS	4 (8%)	2 (4%)	5 (10%)
Abscess, NOS		1 (2%)	
Granuloma, NOS			2 (4%)
Fibrosis, focal	1 (2%)		
Peliosis hepatis		5 (10%)	3 (6%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM			
#Liver (Continued)	(50)	(50)	(49)
Degeneration, lipoid	1 (2%)		
Necrosis, NOS	1 (2%)	1 (2%)	
Infarct, NOS	1 (2%)	1 (2%)	1 (2%)
Metamorphosis, fatty	11 (22%)	17 (34%)	17 (35%)
Basophilic cyto change	4 (8%)	11 (22%)	10 (20%)
Ground glass cyto change			1 (2%)
Clear cell change	1 (2%)		3 (6%)
Hyperplasia, focal		1 (2%)	
#Bile duct	(50)	(50)	(49)
Hyperplasia, NOS	24 (48%)	14 (28%)	8 (16%)
#Pancreas	(50)	(50)	(49)
Hematoma, NOS		1 (2%)	
#Pancreatic acinus	(50)	(50)	(49)
Atrophy, NOS	4 (8%)	6 (12%)	1 (2%)
Atrophy, focal	1 (2%)	1 (2%)	7 (14%)
Hyperplasia, NOS	1 (2%)	4 (8%)	3 (6%)
#Esophagus	(50)	(50)	(49)
Inflammation, acute focal	1 (2%)		
#Stomach	(50)	(50)	(49)
Calcification, NOS		1 (2%)	
#Glandular stomach	(50)	(50)	(49)
Ulcer, NOS	2 (4%)		
Inflammation, acute			1 (2%)
Erosion			1 (2%)
Calcification, NOS	1 (2%)	2 (4%)	
Hyperplasia, epithelial	9 (18%)	3 (6%)	6 (12%)
#Forestomach	(50)	(50)	(49)
Edema, NOS			4 (8%)
Ulcer, NOS	5 (10%)	13 (26%)	11 (22%)
Inflammation, acute			1 (2%)
Inflammation, active chronic			1 (2%)
Inflammation, acute/chronic		1 (2%)	1 (2%)
Inflammation, chronic	1 (2%)	5 (10%)	3 (6%)
Erosion		2 (4%)	2 (4%)
Fibrosis, focal	1 (2%)		
Degeneration, NOS		1 (2%)	
Calcification, NOS		2 (4%)	
Hyperplasia, epithelial	4 (8%)	19 (38%)	20 (41%)
#Colon	(50)	(50)	(49)
Parasitism			1 (2%)
#Cecum	(50)	(50)	(49)
Edema, NOS	1 (2%)		
*Rectum	(50)	(50)	(49)
Parasitism			1 (2%)
Necrosis, NOS			1 (2%)
URINARY SYSTEM			
#Kidney	(50)	(50)	(49)
Hydronephrosis			1 (2%)
Cyst, NOS	1 (2%)	3 (6%)	2 (4%)
Abscess, NOS	1 (2%)		
Nephropathy	48 (96%)	49 (98%)	47 (96%)
Calcification, NOS		1 (2%)	1 (2%)
#Kidney/cortex	(50)	(50)	(49)
Cyst, NOS			1 (2%)
#Renal papilla	(50)	(50)	(49)
Mineralization			31 (63%)
#Kidney/tubule	(50)	(50)	(49)
Hyperplasia, focal		8 (16%)	8 (16%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM			
#Anterior pituitary	(49)	(50)	(48)
Cyst, NOS	6 (12%)	2 (4%)	2 (4%)
Hyperplasia, NOS	7 (14%)	7 (14%)	5 (10%)
Angiectasis	1 (2%)		
#Adrenal cortex	(50)	(50)	(49)
Congestion, NOS			2 (4%)
Degeneration, lipoid	11 (22%)	9 (18%)	12 (24%)
Metamorphosis, fatty	11 (22%)	16 (32%)	17 (35%)
Hypertrophy, focal	1 (2%)		
Hyperplasia, focal	2 (4%)	1 (2%)	3 (6%)
Hyperplasia, diffuse	3 (6%)	2 (4%)	3 (6%)
#Adrenal medulla	(50)	(50)	(49)
Hyperplasia, NOS	10 (20%)	10 (20%)	8 (16%)
#Thyroid	(50)	(50)	(49)
Cyst, NOS			1 (2%)
Abscess, NOS	1 (2%)		
Hypertrophy, diffuse	2 (4%)	31 (62%)	39 (80%)
Hyperplasia, C-cell	7 (14%)	6 (12%)	3 (6%)
Hyperplasia, follicular cell			1 (2%)
#Parathyroid	(49)	(47)	(48)
Hyperplasia, secondary	2 (4%)	22 (47%)	18 (38%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(49)
Hyperplasia, NOS	2 (4%)	1 (2%)	1 (2%)
Lactation	6 (12%)	1 (2%)	2 (4%)
*Preputial gland	(50)	(50)	(49)
Cyst, NOS	6 (12%)	4 (8%)	20 (41%)
Abscess, NOS			1 (2%)
Hyperplasia, epithelial			1 (2%)
#Prostate	(50)	(48)	(48)
Edema, NOS		1 (2%)	
Inflammation, acute	5 (10%)		3 (6%)
Hyperplasia, NOS			1 (2%)
*Seminal vesicle	(50)	(50)	(49)
Degeneration, NOS	1 (2%)		
Atrophy, NOS	5 (10%)	14 (28%)	12 (24%)
#Testis	(50)	(48)	(49)
Granuloma, foreign body	1 (2%)		
Atrophy, NOS	4 (8%)	5 (10%)	5 (10%)
Hyperplasia, interstitial cell	7 (14%)	1 (2%)	3 (6%)
*Epididymis	(50)	(50)	(49)
Degeneration, NOS	8 (16%)		11 (22%)
*Spermatic cord	(50)	(50)	(49)
Hematoma, organized			1 (2%)
NERVOUS SYSTEM			
#Brain	(50)	(50)	(49)
Hydrocephalus, NOS	2 (4%)		1 (2%)
#Cerebral hemisphere	(50)	(50)	(49)
Atrophy, NOS			1 (2%)
SPECIAL SENSE ORGANS			
*Eye	(50)	(50)	(49)
Hemorrhage	1 (2%)	14 (28%)	9 (18%)
Cataract	44 (88%)	40 (80%)	36 (73%)
Phthisis bulbi		1 (2%)	

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	Low Dose	High Dose
SPECIAL SENSE ORGANS (Continued)			
*Eye/anterior chamber	(50)	(50)	(49)
Hemorrhage			1 (2%)
Inflammation, acute suppurative		1 (2%)	
*Eye/cornea	(50)	(50)	(49)
Inflammation, acute			2 (4%)
*Eye/retina	(50)	(50)	(49)
Degeneration, NOS	46 (92%)	42 (84%)	40 (82%)
*Harderian gland	(50)	(50)	(49)
Abscess, NOS		1 (2%)	
Atrophy, focal			1 (2%)
Hyperplasia, NOS	1 (2%)		
*Ear canal	(50)	(50)	(49)
Inflammation, active chronic		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*Bone	(50)	(50)	(49)
Inflammation, acute/chronic	1 (2%)		
Fibrous osteodystrophy	2 (4%)	10 (20%)	12 (24%)
Atrophy, NOS	3 (6%)		
*Intervertebral disc	(50)	(50)	(49)
Degeneration, NOS	1 (2%)		
BODY CAVITIES			
*Mediastinum	(50)	(50)	(49)
Hematoma, NOS			1 (2%)
Inflammation, acute	1 (2%)		
Abscess, NOS	1 (2%)		
Granuloma, foreign body			1 (2%)
*Pericardium	(50)	(50)	(49)
Inflammation, acute	1 (2%)		
Inflammation, chronic			1 (2%)
Granuloma, foreign body			1 (2%)
ALL OTHER SYSTEMS			
Neck			
Abscess, NOS	1	1	
Adipose tissue			
Necrosis, fat	8	1	1
SPECIAL MORPHOLOGY SUMMARY			
Autolysis/no necropsy			1

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

APPENDIX B

SUMMARY OF LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN

	Vehicle Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)		
Basal cell tumor		1 (2%)	
Trichoepithelioma			1 (2%)
Keratoacanthoma			1 (2%)
*Subcutaneous tissue	(50)	(50)	(50)
Fibroma	2 (4%)		1 (2%)
Lipoma			1 (2%)
RESPIRATORY SYSTEM			
#Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	5 (10%)	2 (4%)	2 (4%)
C-cell carcinoma, metastatic	1 (2%)		
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Malignant lymphoma, NOS			1 (2%)
Leukemia, mononuclear cell	10 (20%)	8 (16%)	11 (22%)
#Spleen	(50)	(50)	(50)
Leukemia, mononuclear cell	4 (8%)	1 (2%)	
#Liver	(50)	(50)	(50)
Leukemia, mononuclear cell	2 (4%)		
#Thymus	(43)	(43)	(41)
Thymoma, benign	1 (2%)		
CIRCULATORY SYSTEM			
None			
DIGESTIVE SYSTEM			
*Palate	(50)	(50)	(50)
Squamous cell papilloma		1 (2%)	2 (4%)
*Tongue	(50)	(50)	(50)
Squamous cell papilloma			1 (2%)
#Liver	(50)	(50)	(50)
Neoplastic nodule	1 (2%)		1 (2%)
#Pancreas	(50)	(50)	(50)
Acinar cell adenoma	2 (4%)		1 (2%)
URINARY SYSTEM			
None			
ENDOCRINE SYSTEM			
#Anterior pituitary	(49)	(49)	(49)
Adenoma, NOS	24 (49%)	24 (49%)	15 (31%)
#Adrenal cortex	(49)	(50)	(50)
Adenoma, NOS	2 (4%)		
#Adrenal medulla	(49)	(50)	(50)
Pheochromocytoma	2 (4%)	1 (2%)	

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Thyroid	(50)	(50)	(50)
Follicular cell adenoma	1 (2%)		
C-cell adenoma	8 (16%)	6 (12%)	2 (4%)
C-cell carcinoma	1 (2%)	1 (2%)	1 (2%)
#Pancreatic islets	(50)	(50)	(50)
Islet cell adenoma		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Adenocarcinoma, NOS	1 (2%)	1 (2%)	1 (2%)
Fibroadenoma	15 (30%)	19 (38%)	10 (20%)
*Clitoral gland	(50)	(50)	(50)
Squamous cell carcinoma			1 (2%)
Adenoma, NOS	4 (8%)	3 (6%)	
#Uterus	(50)	(50)	(49)
Endometrial stromal polyp	18 (36%)	14 (28%)	14 (29%)
Endometrial stromal sarcoma	1 (2%)		
#Cervix uteri	(50)	(50)	(49)
Endometrial stromal polyp			1 (2%)
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Zymbal gland	(50)	(50)	(50)
Carcinoma, NOS	2 (4%)	1 (2%)	
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
None			
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	6	3	5
Moribund sacrifice	5	12	8
Terminal sacrifice	38	33	36
Dosing accident	1	2	1

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	46	43	37
Total primary tumors	107	84	69
Total animals with benign tumors	42	41	31
Total benign tumors	85	72	53
Total animals with malignant tumors	20	11	15
Total malignant tumors	21	12	15
Total animals with secondary tumors##	1		
Total secondary tumors	1		
Total animals with tumors-- uncertain benign or malignant	1		1
Total uncertain tumors	1		1

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

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN: VEHICLE CONTROL

ANIMAL NUMBER	021	030	008	013	003	049	002	044	004	018	026	046	042	001	005	006	007	009	010	011	012	014	015	016	017
WEEKS ON STUDY	073	074	075	091	095	095	096	096	097	098	102	104	105	106	106	106	106	106	106	106	106	106	106	106	106
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma								X																	
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma					X																				
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma		X																					X		X
C-cell carcinoma, metastatic									X																
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia, mononuclear cell																									
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma, benign														X											
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule														X											
Leukemia, mononuclear cell			X																						
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell adenoma																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS		X			X		X		X	X		X	X			X				X	X		X		
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Pheochromocytoma													X								X				
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma								X																	
C-cell adenoma							X									X								X	
C-cell carcinoma									X																
Parathyroid	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS													X												
Fibroadenoma											X	X	X	X	X	X	X								
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS							X																		
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp		X		X	X		X			X					X	X		X						X	
Endometrial stromal sarcoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS				X												X									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell						X		X					X	X											

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed

: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

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

ANIMAL NUMBER	019	020	022	023	024	025	027	028	029	031	032	033	034	035	036	037	038	039	040	041	043	045	047	048	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell papilloma																										1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma																	X									2
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X				X																			5
C-cell carcinoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia, mononuclear cell		X				X											X							X		4
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	+	+	-	-	+	-	+	+	43
Thymoma, benign																										1
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule																										1
Leukemia, mononuclear cell														X												2
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinar cell adenoma																		X			X					2
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS	X	X	X	X				X			X		X	X		X			X		X		X		X	24
Adrenal	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS									X												X					2
Pheochromocytoma																										2
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma																										1
C-cell adenoma			X							X							X		X						X	8
C-cell carcinoma																										1
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	47
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	*50
Adenocarcinoma, NOS																										1
Fibroadenoma		X									X		X			X			X		X		X	X		15
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS				X							X								X							4
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrial stromal polyp					X		X				X		X		X				X	X				X	X	18
Endometrial stromal sarcoma											X															1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										2
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell			X				X		X	X									X			X				10

* Animals necropsied

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN: LOW DOSE

ANIMAL NUMBER	011	050	045	005	020	002	029	027	037	031	035	023	018	038	024	014	033	001	003	004	006	007	008	009	010
WEEKS ON STUDY	061	065	074	076	076	077	083	087	087	088	091	093	095	096	099	102	102	105	105	105	105	105	105	105	105
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell tumor																									X
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																								X	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia, mononuclear cell																									
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	−	+	+	+	−	−	+	+	−	+	+	+	−	+	+	+	+	+	+	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell papilloma																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS	X	X				X					X			X	X	X		X	X			X		X	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																		X							
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma														X		X			X						
C-cell carcinoma																									
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																									
Fibroadenoma								X			X	X					X	X	X		X	X		X	
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS							X																		
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp			X	X					X					X	X				X				X	X	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS											X														
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell			X		X											X								X	

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

ANIMAL NUMBER	012	013	015	016	017	019	021	022	025	026	028	030	032	034	036	039	040	041	042	043	044	046	047	048	049	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Basal cell tumor																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																			X							2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nasal cavity	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia, mononuclear cell	X																									1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	43
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell papilloma											X															1
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	49
Adenoma, NOS		X				X		X	X	X				X	X				X	X		X	X	X	X	24
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell adenoma						X							X											X		6
C-cell carcinoma										X																1
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma																							X			1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Adenocarcinoma, NOS																								X		1
Fibroadenoma			X	X			X	X			X			X	X					X		X	X			19
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS										X					X											3
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrial stromal polyp					X				X		X	X											X		X	14
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell			X		X	X																		X		8

* Animals necropsied

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN: HIGH DOSE

ANIMAL NUMBER	013	020	038	045	007	024	014	016	048	022	027	042	002	012	001	003	004	005	006	008	009	010	011	015	017
WEEKS ON STUDY	060	076	087	087	091	091	092	092	094	096	098	098	101	102	104	104	104	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	N	+	+
Trichoepithelioma																									
Keratoacanthoma																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	N	+	+
Fibroma																					X				
Lipoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X																					
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	−	+	+	+	+	+	+	+	−	−	+	−	+	+	+	−	+	+	+	+	+	−	−	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell papilloma									X																X
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule					X																				
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell adenoma																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS					X			X	X		X	X				X	X							X	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma											X	X													
C-cell carcinoma																									
Parathyroid	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma													X												
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS											X														
Fibroadenoma					X																			X	X
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell carcinoma																				X					
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp				X											X		X	X		X	X			X	X
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, NOS										X															
Leukemia, mononuclear cell			X			X							X	X		X	X						X		

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

ANIMAL NUMBER	018	019	021	023	025	026	028	029	030	031	032	033	034	035	036	037	039	040	041	043	044	046	047	049	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	105	105	105	105	105	105	105	105	105	105	105	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Trichoepithelioma						X																				1
Keratoacanthoma																	X									1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma																										1
Lipoma					X																					1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																	X									2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	41
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell papilloma																								X		3
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinar cell adenoma	X																									1
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS	X				X				X								X	X					X	X		15
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell adenoma																										2
C-cell carcinoma										X																1
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	48
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma																										1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Adenocarcinoma, NOS																										1
Fibroadenoma		X						X	X	X											X	X		X		10
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell carcinoma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endometrial stromal polyp						X			X				X					X		X				X	X	15
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, NOS																										1
Leukemia, mononuclear cell														X				X		X		X				11

* Animals necropsied

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN

	Vehicle Control	37.5 mg/kg	75 mg/kg
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	5/50 (10%)	2/50 (4%)	2/50 (4%)
Adjusted Rates (b)	12.1%	6.1%	4.8%
Terminal Rates (c)	4/39 (10%)	2/33 (6%)	1/36 (3%)
Week of First Observation	74	105	87
Life Table Tests (d)	P=0.171N	P=0.280N	P=0.242N
Incidental Tumor Tests (d)	P=0.130N	P=0.235N	P=0.176N
Cochran-Armitage Trend Test (d)	P=0.146N		
Fisher Exact Test (d)		P=0.218N	P=0.218N
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	16/50 (32%)	9/50 (18%)	11/50 (22%)
Adjusted Rates (b)	37.6%	23.8%	26.9%
Terminal Rates (c)	13/39 (33%)	6/33 (18%)	7/36 (19%)
Week of First Observation	75	74	87
Life Table Tests (d)	P=0.210N	P=0.177N	P=0.257N
Incidental Tumor Tests (d)	P=0.170N	P=0.114N	P=0.176N
Cochran-Armitage Trend Test (d)	P=0.146N		
Fisher Exact Test (d)		P=0.083N	P=0.184N
Oral Cavity: Squamous Cell Papilloma			
Overall Rates (a)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted Rates (b)	0.0%	3.0%	7.8%
Terminal Rates (c)	0/39 (0%)	1/33 (3%)	2/36 (6%)
Week of First Observation		105	94
Life Table Tests (d)	P=0.057	P=0.467	P=0.109
Incidental Tumor Tests (d)	P=0.055	P=0.467	P=0.105
Cochran-Armitage Trend Test (d)	P=0.060		
Fisher Exact Test (d)		P=0.500	P=0.121
Anterior Pituitary Gland: Adenoma			
Overall Rates (a)	24/49 (49%)	24/49 (49%)	15/49 (31%)
Adjusted Rates (b)	54.3%	60.6%	36.0%
Terminal Rates (c)	19/39 (49%)	17/32 (53%)	10/36 (28%)
Week of First Observation	74	61	91
Life Table Tests (d)	P=0.103N	P=0.279	P=0.106N
Incidental Tumor Tests (d)	P=0.061N	P=0.455	P=0.059N
Cochran-Armitage Trend Test (d)	P=0.041N		
Fisher Exact Test (d)		P=0.580	P=0.049N
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	8/50 (16%)	6/50 (12%)	2/50 (4%)
Adjusted Rates (b)	19.8%	16.9%	5.0%
Terminal Rates (c)	7/39 (18%)	4/33 (12%)	0/36 (0%)
Week of First Observation	96	96	98
Life Table Tests (d)	P=0.056N	P=0.518N	P=0.064N
Incidental Tumor Tests (d)	P=0.055N	P=0.517N	P=0.066N
Cochran-Armitage Trend Test (d)	P=0.037N		
Fisher Exact Test (d)		P=0.387N	P=0.046N
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	9/50 (18%)	7/50 (14%)	3/50 (6%)
Adjusted Rates (b)	21.7%	19.8%	7.6%
Terminal Rates (c)	7/39 (18%)	5/33 (15%)	1/36 (3%)
Week of First Observation	96	96	98
Life Table Tests (d)	P=0.074N	P=0.534N	P=0.085N
Incidental Tumor Tests (d)	P=0.073N	P=0.534N	P=0.086N
Cochran-Armitage Trend Test (d)	P=0.049N		
Fisher Exact Test (d)		P=0.393N	P=0.061N

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	37.5 mg/kg	75 mg/kg
Mammary Gland: Fibroadenoma			
Overall Rates (a)	15/50 (30%)	19/50 (38%)	10/50 (20%)
Adjusted Rates (b)	37.5%	50.8%	26.6%
Terminal Rates (c)	14/39 (36%)	15/33 (45%)	9/36 (25%)
Week of First Observation	102	87	91
Life Table Tests (d)	P=0.234N	P=0.112	P=0.238N
Incidental Tumor Tests (d)	P=0.156N	P=0.172	P=0.188N
Cochran-Armitage Trend Test (d)	P=0.161N		
Fisher Exact Test (d)		P=0.263	P=0.178N
Mammary Gland: Fibroadenoma or Adenocarcinoma			
Overall Rates (a)	15/50 (30%)	20/50 (40%)	11/50 (22%)
Adjusted Rates (b)	37.5%	53.6%	28.5%
Terminal Rates (c)	14/39 (36%)	16/33 (48%)	9/36 (25%)
Week of First Observation	102	87	91
Life Table Tests (d)	P=0.312N	P=0.075	P=0.322N
Incidental Tumor Tests (d)	P=0.224N	P=0.119	P=0.269N
Cochran-Armitage Trend Test (d)	P=0.224N		
Fisher Exact Test (d)		P=0.201	P=0.247N
Clitoral Gland: Adenoma			
Overall Rates (a)	4/50 (8%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	9.8%	8.2%	0.0%
Terminal Rates (c)	3/39 (8%)	2/33 (6%)	0/36 (0%)
Week of First Observation	96	83	
Life Table Tests (d)	P=0.062N	P=0.582N	P=0.075N
Incidental Tumor Tests (d)	P=0.038N	P=0.472N	P=0.078N
Cochran-Armitage Trend Test (d)	P=0.049N		
Fisher Exact Test (d)		P=0.500N	P=0.059N
Clitoral Gland: Adenoma or Squamous Cell Carcinoma			
Overall Rates (a)	4/50 (8%)	3/50 (6%)	1/50 (2%)
Adjusted Rates (b)	9.8%	8.2%	2.8%
Terminal Rates (c)	3/39 (8%)	2/33 (6%)	1/36 (3%)
Week of First Observation	96	83	104
Life Table Tests (d)	P=0.158N	P=0.582N	P=0.206N
Incidental Tumor Tests (d)	P=0.115N	P=0.472N	P=0.211N
Cochran-Armitage Trend Test (d)	P=0.133N		
Fisher Exact Test (d)		P=0.500N	P=0.181N
Uterus: Endometrial Stromal Polyp			
Overall Rates (a)	18/50 (36%)	14/50 (28%)	15/49 (31%)
Adjusted Rates (b)	40.4%	35.6%	41.3%
Terminal Rates (c)	13/39 (33%)	9/33 (27%)	14/35 (40%)
Week of First Observation	74	74	87
Life Table Tests (d)	P=0.415N	P=0.450N	P=0.464N
Incidental Tumor Tests (d)	P=0.343N	P=0.251N	P=0.370N
Cochran-Armitage Trend Test (d)	P=0.318N		
Fisher Exact Test (d)		P=0.260N	P=0.361N
All Sites: Benign Tumors			
Overall Rates (a)	42/50 (84%)	41/50 (82%)	31/50 (62%)
Adjusted Rates (b)	89.3%	87.1%	71.8%
Terminal Rates (c)	34/39 (87%)	27/33 (82%)	24/36 (67%)
Week of First Observation	74	61	87
Life Table Tests (d)	P=0.085N	P=0.223	P=0.083N
Incidental Tumor Tests (d)	P=0.008N	P=0.564N	P=0.012N
Cochran-Armitage Trend Test (d)	P=0.007N		
Fisher Exact Test (d)		P=0.500N	P=0.012N

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	37.5 mg/kg	75 mg/kg
All Sites: Malignant Tumors			
Overall Rates (a)	20/50 (40%)	11/50 (22%)	15/50 (30%)
Adjusted Rates (b)	45.0%	28.5%	35.3%
Terminal Rates (c)	15/39 (38%)	7/33 (21%)	9/36 (25%)
Week of First Observation	75	74	87
Life Table Tests (d)	P=0.248N	P=0.126N	P=0.296N
Incidental Tumor Tests (d)	P=0.158N	P=0.038N	P=0.171N
Cochran-Armitage Trend Test (d)	P=0.165N		
Fisher Exact Test (d)		P=0.042N	P=0.201N
All Sites: All Tumors			
Overall Rates (a)	46/50 (92%)	43/50 (86%)	37/50 (74%)
Adjusted Rates (b)	93.9%	89.5%	78.7%
Terminal Rates (c)	36/39 (92%)	28/33 (85%)	26/36 (72%)
Week of First Observation	74	61	87
Life Table Tests (d)	P=0.172N	P=0.322	P=0.184N
Incidental Tumor Tests (d)	P=0.009N	P=0.287N	P=0.009N
Cochran-Armitage Trend Test (d)	P=0.010N		
Fisher Exact Test (d)		P=0.263N	P=0.016N

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

TABLE B4. HISTORICAL INCIDENCE OF ORAL CAVITY SQUAMOUS CELL TUMORS IN FEMALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE

	Incidence of Papillomas in Vehicle Controls (a)
No 2-year studies by SRI International are included in the historical data base.	
Overall Historical Incidence	
TOTAL	6/1,950 (0.3%)
SD (b)	0.86%
Range (c)	
High	2/50
Low	0/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks; no malignant tumors have been observed.
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN

	Vehicle Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Epidermal inclusion cyst		1 (2%)	
Erosion	1 (2%)		
RESPIRATORY SYSTEM			
#Nasal cavity	(50)	(49)	(49)
Hemorrhage		1 (2%)	
Inflammation, acute			1 (2%)
Inflammation, chronic	2 (4%)		1 (2%)
Infection, fungal	1 (2%)		
Metaplasia, squamous			7 (14%)
#Lung/bronchus	(50)	(50)	(50)
Foreign body, NOS			1 (2%)
#Lung	(50)	(50)	(50)
Atelectasis	1 (2%)	1 (2%)	
Congestion, NOS	2 (4%)	1 (2%)	2 (4%)
Hemorrhage		1 (2%)	1 (2%)
Inflammation, acute			1 (2%)
Pneumonia, interstitial chronic	1 (2%)		
Granuloma, NOS	13 (26%)	4 (8%)	3 (6%)
Hyperplasia, alveolar epithelium	2 (4%)		
Metaplasia, osseous	1 (2%)		
Histiocytosis	4 (8%)	3 (6%)	
HEMATOPOIETIC SYSTEM			
#Bone marrow	(50)	(50)	(49)
Granuloma, NOS			2 (4%)
Hypoplasia, NOS	5 (10%)		
Atrophy, NOS			1 (2%)
#Spleen	(50)	(50)	(50)
Congestion, NOS		1 (2%)	3 (6%)
Hematoma, NOS		1 (2%)	
Granuloma, NOS		1 (2%)	
Fibrosis	1 (2%)		1 (2%)
Hemosiderosis	21 (42%)	18 (36%)	13 (26%)
Myeloproliferative disorder	1 (2%)		
Metaplasia, myeloid	12 (24%)	9 (18%)	3 (6%)
#Lymph node	(50)	(50)	(50)
Hemorrhage			1 (2%)
Granuloma, NOS	1 (2%)	2 (4%)	
Hemosiderosis	1 (2%)	2 (4%)	3 (6%)
Hyperplasia, NOS	2 (4%)	9 (18%)	7 (14%)
Angiectasis	1 (2%)		
Histiocytosis		4 (8%)	1 (2%)
Plasmacytosis	4 (8%)	3 (6%)	3 (6%)
#Submandibular lymph node	(50)	(50)	(50)
Atrophy, NOS	1 (2%)		
Plasmacytosis	2 (4%)		
#Mesenteric lymph node	(50)	(50)	(50)
Hyperplasia, NOS	1 (2%)		
#Renal lymph node	(50)	(50)	(50)
Granuloma, NOS	1 (2%)		
#Liver	(50)	(50)	(50)
Leukocytosis, NOS	1 (2%)		
Metaplasia, myeloid	1 (2%)	1 (2%)	

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Thymus	(43)	(43)	(41)
Cyst, NOS			1 (2%)
CIRCULATORY SYSTEM			
#Heart	(50)	(50)	(50)
Inflammation, chronic			1 (2%)
Fibrosis	7 (14%)		6 (12%)
Fibrosis, focal	2 (4%)		
Infarct, acute	1 (2%)		
*Artery	(50)	(50)	(50)
Inflammation, NOS			1 (2%)
*Coronary artery	(50)	(50)	(50)
Inflammation, focal			1 (2%)
*Uterine vein	(50)	(50)	(50)
Thrombosis, NOS		1 (2%)	
DIGESTIVE SYSTEM			
*Palate	(50)	(50)	(50)
Inflammation, active chronic			1 (2%)
*Tongue	(50)	(50)	(50)
Hyperplasia, epithelial	1 (2%)	1 (2%)	2 (4%)
#Salivary gland	(50)	(50)	(50)
Atrophy, NOS			1 (2%)
Metaplasia, squamous			1 (2%)
#Liver	(50)	(50)	(50)
Hernia, NOS	10 (20%)	5 (10%)	2 (4%)
Congestion, NOS	1 (2%)		
Inflammation, chronic focal			1 (2%)
Granuloma, NOS	9 (18%)	14 (28%)	11 (22%)
Peliosis hepatis	2 (4%)		1 (2%)
Necrosis, NOS	1 (2%)	1 (2%)	
Infarct, focal	1 (2%)		
Metamorphosis, fatty	3 (6%)	7 (14%)	7 (14%)
Basophilic cyto change	4 (8%)	6 (12%)	
Clear cell change			1 (2%)
Hyperplasia, NOS	1 (2%)		
Angiectasis			1 (2%)
#Bile duct	(50)	(50)	(50)
Dilatation, NOS		1 (2%)	
Inflammation, acute		1 (2%)	
Hyperplasia, NOS	25 (50%)	4 (8%)	10 (20%)
#Pancreas	(50)	(50)	(50)
Edema, NOS	1 (2%)		
#Pancreatic acinus	(50)	(50)	(50)
Atrophy, NOS	6 (12%)	2 (4%)	4 (8%)
Hyperplasia, NOS			1 (2%)
#Esophagus	(50)	(50)	(50)
Perforation, inflammatory			1 (2%)
#Periesophageal tissu	(50)	(50)	(50)
Abscess, chronic			1 (2%)
#Stomach	(50)	(50)	(50)
Edema, NOS	1 (2%)		
#Forestomach	(50)	(50)	(50)
Ulcer, NOS	1 (2%)	3 (6%)	
Inflammation, chronic	1 (2%)		
Hyperplasia, epithelial	1 (2%)		3 (6%)
#Small intestine	(50)	(50)	(50)
Ulcer, NOS			1 (2%)
#Colon	(49)	(50)	(50)
Parasitism	1 (2%)		

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Cecum	(49)	(50)	(50)
Edema, NOS	2 (4%)		1 (2%)
Hematoma, NOS	1 (2%)		
Erosion			1 (2%)
Necrosis, NOS	1 (2%)		
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Pyelonephritis, chronic		1 (2%)	
Nephropathy	35 (70%)	28 (56%)	35 (70%)
Glomerulosclerosis, NOS			1 (2%)
Calcification, NOS	2 (4%)		2 (4%)
#Urinary bladder	(48)	(49)	(50)
Edema, NOS	2 (4%)		
ENDOCRINE SYSTEM			
#Anterior pituitary	(49)	(49)	(49)
Cyst, NOS	9 (18%)	12 (24%)	10 (20%)
Hemorrhage	1 (2%)	1 (2%)	1 (2%)
Hyperplasia, NOS	5 (10%)	2 (4%)	7 (14%)
#Adrenal	(49)	(50)	(50)
Congestion, NOS	1 (2%)		
Infarct, NOS	1 (2%)		
#Adrenal cortex	(49)	(50)	(50)
Metamorphosis, fatty	6 (12%)	3 (6%)	5 (10%)
#Thyroid	(50)	(50)	(50)
Cyst, NOS			1 (2%)
Hyperplasia, C-cell	12 (24%)	7 (14%)	7 (14%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Cyst, NOS			2 (4%)
Hyperplasia, NOS	22 (44%)	12 (24%)	9 (18%)
Lactation			1 (2%)
*Clitoral gland	(50)	(50)	(50)
Cyst, NOS	13 (26%)	8 (16%)	16 (32%)
Inflammation, acute	1 (2%)		
Abscess, NOS	1 (2%)		
Metaplasia, squamous			1 (2%)
*Vagina	(50)	(50)	(50)
Cyst, NOS			1 (2%)
Epidermal inclusion cyst			1 (2%)
#Uterus	(50)	(50)	(49)
Prolapse	1 (2%)		
Dilatation, NOS		1 (2%)	
Hydrometra	1 (2%)		
Cyst, NOS	5 (10%)		
Pyometra	1 (2%)		
#Cervix uteri	(50)	(50)	(49)
Cyst, NOS	1 (2%)		
Epidermal inclusion cyst	1 (2%)	3 (6%)	2 (4%)
Abscess, NOS		1 (2%)	
#Uterus/endometrium	(50)	(50)	(49)
Cyst, NOS	1 (2%)		
Inflammation, chronic			1 (2%)
#Ovary	(50)	(50)	(49)
Cyst, NOS	6 (12%)	5 (10%)	4 (8%)

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF 8-METHOXYPSORALEN (Continued)

	Vehicle Control	Low Dose	High Dose
NERVOUS SYSTEM			
#Brain	(50)	(50)	(49)
Hemorrhage	1 (2%)		
SPECIAL SENSE ORGANS			
*Eye	(50)	(50)	(50)
Hemorrhage	1 (2%)	1 (2%)	2 (4%)
Cataract	47 (94%)	41 (82%)	47 (94%)
Phthisis bulbi			1 (2%)
*Eye/retina	(50)	(50)	(50)
Degeneration, NOS	48 (96%)	42 (84%)	46 (92%)
Atrophy, NOS		1 (2%)	
*Harderian gland	(50)	(50)	(50)
Dilatation, NOS			1 (2%)
Cyst, NOS			1 (2%)
Inflammation, chronic focal			1 (2%)
Granuloma, NOS		1 (2%)	2 (4%)
Hyperplasia, NOS		1 (2%)	
*Ear	(50)	(50)	(50)
Abscess, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*Bone	(50)	(50)	(50)
Osteosclerosis	4 (8%)		3 (6%)
BODY CAVITIES			
*Mesentery	(50)	(50)	(50)
Necrosis, fat			1 (2%)
ALL OTHER SYSTEMS			
Adipose tissue			
Inflammation, chronic	1		
Necrosis, fat	15	11	13
SPECIAL MORPHOLOGY SUMMARY			
None			

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

APPENDIX C

SENTINEL ANIMAL PROGRAM

APPENDIX C. SENTINEL ANIMAL PROGRAM

I. Methods

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

Fifteen 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. Data from animals surviving 24 months were collected from 5/50 randomly selected vehicle control animals of each sex. The blood from each animal was collected and clotted, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates' Comprehensive Animal Diagnostic Service for determination of the viral antibody titers. The following tests were performed:

Hemagglutination Inhibition	Complement Fixation	ELISA
PVM KRV (Kilham rat virus) H-1 (Toolan's H-1 virus) Sendai	RCV (rat coronavirus) (6,12,18 mo)	RCV/SDA (sialodacryoadenitis virus) (24 mo)

II. Results

No positive titers were observed in any rats at 6, 12, 18, or 24 months.

APPENDIX D

INGREDIENTS, NUTRIENT COMPOSITION, AND

CONTAMINANT LEVELS IN

NIH 07 RAT AND MOUSE RATION

Pelleted Diet: April 1981 to April 1983

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

TABLE D1. 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 D2. 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 D3. NUTRIENT COMPOSITION OF NIH 07 RAT AND MOUSE RATION (a)

Nutrients	Mean ± Standard Deviation	Range	Number of Samples
Crude protein (percent by weight)	23.8 ± 0.87	22.2-25.3	24
Crude fat (percent by weight)	5.0 ± 0.45	4.2-5.7	24
Crude fiber (percent by weight)	3.3 ± 0.23	2.9-3.8	24
Ash (percent by weight)	6.4 ± 0.37	5.7-7.1	24
Amino Acids (percent of total diet) (a)			
Arginine	1.323 ± 0.830	1.21-1.39	4
Cystine	0.310 ± 0.099	0.218-0.400	4
Glycine	1.155 ± 0.069	1.06-1.21	4
Histidine	0.572 ± 0.030	0.530-0.603	4
Isoleucine	0.910 ± 0.033	0.881-0.944	4
Leucine	1.949 ± 0.065	1.85-1.99	4
Lysine	1.279 ± 0.075	1.20-1.37	4
Methionine	0.422 ± 0.187	0.306-0.699	4
Phenylalanine	0.909 ± 0.167	0.665-1.04	4
Threonine	0.844 ± 0.029	0.824-0.886	4
Tryptophan	0.187	0.171-0.211	3
Tyrosine	0.631 ± 0.094	0.566-0.769	4
Valine	1.11 ± 0.050	1.05-1.17	4
Essential Fatty Acids (percent of total diet) (a)			
Linoleic	2.44	2.37-2.52	3
Linolenic	0.274	0.256-0.308	3
Arachidonic	0.008		1
Vitamins (a)			
Vitamin A (IU/kg)	11,183 ± 2,211	8,400-18,000	24
Vitamin D (IU/kg)	4,650	3,000-6,300	2
α-Tocopherol (ppm)	41.53 ± 7.52	31.1-48.9	4
Thiamine (ppm)	16.4 ± 2.17	13.0-21.0	(b) 23
Riboflavin (ppm)	7.5 ± 0.96	6.1-8.2	4
Niacin (ppm)	85.0 ± 14.20	65.0-97.0	4
Pantothenic acid (ppm)	29.3 ± 4.6	23.0-34.0	4
Pyridoxine (ppm)	7.6 ± 1.5	5.6-8.8	4
Folic acid (ppm)	2.8 ± 0.88	1.8-3.7	4
Biotin (ppm)	0.27 ± 0.05	0.21-0.32	4
Vitamin B_{12} (ppb)	21.0 ± 11.9	11.0-38.0	4
Choline (ppm)	3,302.0 ± 120.0	3,200-3,430	4
Minerals (a)			
Calcium (percent)	1.22 ± 0.11	1.08-1.53	24
Phosphorus (percent)	0.97 ± 0.04	0.88-1.1	24
Potassium (percent)	0.862 ± 0.10	0.772-0.970	3
Chloride (percent)	0.546 ± 0.10	0.442-0.635	4
Sodium (percent)	0.311 ± 0.038	0.258-0.350	4
Magnesium (percent)	0.169 ± 0.133	0.151-0.181	4
Sulfur (percent)	0.316 ± 0.070	0.270-0.420	4
Iron (ppm)	447.0 ± 57.3	409-523	4
Manganese (ppm)	90.6 ± 8.20	81.7-99.4	4
Zinc (ppm)	53.6 ± 5.27	46.1-58.6	4
Copper (ppm)	10.77 ± 3.19	8.09-15.39	4
Iodine (ppm)	2.95 ± 1.05	1.52-3.82	4
Chromium (ppm)	1.81 ± 0.28	1.44-2.09	4
Cobalt (ppm)	0.68 ± 0.14	0.49-0.80	4

(a) One to four batches of feed analyzed for nutrients reported in this table were manufactured during 1983-85.
(b) One batch (7/22/81) was not analyzed for thiamine.

TABLE D4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Contaminants	Mean ± Standard Deviation	Range	Number of Samples
Arsenic (ppm)	0.46 ± 0.10	<0.29-0.70	24
Cadmium (ppm) (a)	<0.1		24
Lead (ppm)	0.95 ± 0.76	0.33-3.37	24
Mercury (ppm) (a)	< 0.05		24
Selenium (ppm)	0.29 ± 0.07	0.13-0.40	24
Aflatoxins (ppb) (b)	<10	<5.0-<10.0	24
Nitrate nitrogen (ppm) (c)	10.24 ± 4.1	3.8-22.0	24
Nitrite nitrogen (ppm) (c)	2.0 ± 1.6	<0.4-6.9	24
BHA (ppm) (d)	6.1 ± 4.9	<0.4-17.0	24
BHT (ppm) (d)	3.3 ± 2.6	0.9-12.0	24
Aerobic plate count (CFU/g) (e)	39,879 ± 27,920	4,900-88,000	24
Coliform (MPN/g) (f)	15.5 ± 22.7	<3-93	23
Coliform (MPN/g) (g)	34.0 ± 93.4	<3-460	24
E. coli (MPN/g) (h)	<3		24
Total nitrosamines (ppb) (i, j)	3.7 ± 2.7	0.8-9.3	23
Total nitrosamines (ppb) (k, j)	15.2 ± 56.4	0.8-279.5	24
N-Nitrosodimethylamine (ppb) (l, j)	2.7 ± 2.5	0.8-8.3	23
N-Nitrosodimethylamine (ppb) (m, j)	14.1 ± 56.3	0.8-278.0	24
N-Nitrosopyrrolidine (ppb)	1.2 ± 0.5	<0.9-2.9	24
Pesticides (ppm)			
α-BHC (a,n)	<0.01		24
β-BHC (a)	<0.02		24
γ-BHC-Lindane (a)	<0.01		24
δ-BHC (a)	<0.01		24
Heptachlor (a)	<0.01		24
Aldrin (a)	<0.01		24
Heptachlor epoxide (a)	<0.01		24
DDE (a)	<0.01		24
DDD (a)	<0.01		24
DDT (a)	<0.01		24
HCB (a)	<0.01		24
Mirex (a)	<0.01		24
Methoxychlor (o)	<0.05	0.09 (8/26/81)	24
Dieldrin (a)	<0.01		24
Endrin (a)	<0.01		24
Telodrin (a)	<0.01		24
Chlordane (a)	<0.05		24
Toxaphene (a)	<0.1		24
Estimated PCB's (a)	<0.2		24
Ronnel (a)	<0.01		24
Ethion (a)	<0.02		24
Trithion (a)	<0.05		24
Diazinon (a)	<0.1		24
Methyl parathion (a)	<0.02		24
Ethyl parathion (a)	<0.02		24
Malathion (p)	0.09 ± 0.06	<0.05-0.27	24
Endosulfan I (a,q)	<0.01		18
Endosulfan II (a,q)	<0.01		18
Endosulfan sulfate (a,q)	<0.03		18

TABLE D4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION (Continued)

(a) All values were less than the detection limit, given in the table as the mean.
(b) The detection limit was reduced from 10 ppb to 5 ppb after 7/81.
(c) Source of contamination: alfalfa, grains, and fish meal
(d) Source of contamination: soy oil and fish meal
(e) CFU = colony-forming unit
(f) Mean, standard deviation, and range exclude one very high value of 460 obtained for the batch produced on 9/23/82; MPN = most probable number.
(g) Mean, standard deviation, and range include the very high value given in footnote (f).
(h) All values were less than 3 MPN/g.
(i) Mean, standard deviation, and range exclude one very high value of 279.5 obtained for the batch produced on 4/27/81.
(j) All values were corrected for percent recovery.
(k) Mean, standard deviation, and range include the high value given in footnote (i).
(l) Mean, standard deviation, and range exclude one very high value of 278 obtained for the batch produced on 4/27/81.
(m) Mean, standard deviation, and range include the high value listed in footnote (l).
(n) BHC = hexachlorocyclohexane or benzene hexachloride
(o) One observation was above the detection limit. The value and the date it was obtained are listed under the range.
(p) Ten batches contained more than 0.05 ppm.
(q) Six batches were not analyzed for endosulfan I, endosulfan II, or endosulfan sulfate.

APPENDIX E

TOXICITY OF 8-METHOXYPSORALEN, 5-METHOXYPSORALEN, 3-CARBETHOXYPSORALEN, OR 5-METHYLISOPSORALEN WITH ULTRAVIOLET RADIATION IN THE HAIRLESS (HRA/Skh) MOUSE

Dunnick, J.K.; Forbes, P.D.; Davies, R.E.; Iverson, W.O. (1987) Toxicol. Appl. Pharmacol. 89:73-80
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Toxicity of 8-Methoxypsoralen, 5-Methoxypsoralen, 3-Carbethoxypsoralen, or 5-Methylisopsoralen with Ultraviolet Radiation in the Hairless (HRA/Skh) Mouse

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Toxicity of 8-Methoxypsoralen, 5-Methoxypsoralen, 3-Carbethoxypsoralen, or 5-Methylisopsoralen with Ultraviolet Radiation in the Hairless (HRA/Skh) Mouse. DUNNICK, J. K., FORBES, P. D., DAVIES, R. E., AND IVERSON, W. O. (1987). *Toxicol. Appl. Pharmacol.* 89, 73–80. An experimental design to simulate PUVA therapy (oral 8-methoxypsoralen followed by uv radiation) has been tested in a 13-week subchronic study to determine the relative toxicities of 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), 5-methylisopsoralen (5-MIP), and 3-carbethoxypsoralen (3-CEP) in inbred hairless mice (HRA/Skh). Drug was administered by 1-hr pulse feedings three times a week after mice were fasted overnight; individually housed animals were then exposed to uv radiation (320–400 nm; less than 2% output < 320 nm). 8-MOP or 5-MOP administered orally (at doses of approximately 240 or 480 mg/m^2 body surface area per week) followed one-half hour later with uv radiation of 2 J/cm^2 for 13 weeks were found to cause skin toxicity including inflammation, hyperplasia, ulceration, and cellular atypia. Dose-related toxicity was not seen in other organ systems. Corresponding levels of 5-MIP or 3-CEP with uv radiation did not produce skin toxicity. These studies show that the psoralens with two potential DNA-binding sites (8-MOP and 5-MOP) were more toxic than psoralens with only one photoreactive site (5-MIP and 3-CEP).

Oral administration of 8-methoxypsoralen (8-MOP) followed by exposure to ultraviolet radiation (primarily UVA, 320–400 nm), referred to as PUVA therapy, is used in the treatment of vitiligo and psoriasis (Kraning and Odland, 1979). Exposure to psoralen derivatives also occurs through ingestion of common vegetables such as parsnips, carrots, and parsley (Pathak *et al.*, 1962; Ivie *et al.*, 1981). Clinical trials have reported an increased incidence in cutaneous squamous cell carcinoma in patients receiving PUVA therapy (Stern *et al.*, 1979, 1984). Other psoralen or isopsoralen derivatives have been used in preliminary clinical trials in Europe for the treatment of psoriasis, including 3-carbethoxypsoralen (3-CEP; Dubertret *et al.*, 1978), 5-methoxypsoralen (5-MOP; Honigsmann *et al.*, 1979), and 5-methylisopsoralen (5-MIP; Bordin *et al.*, 1981). This paper describes comparative toxicity from psoralen/isopsoralen derivatives with and without uv radiation.

Previous rodent studies have reported on the toxicity of topically applied psoralens in mice. 8-MOP with uv radiation (Grube *et al.*, 1977; Ljunggren *et al.*, 1981; Young *et al.*, 1983), 5-MOP with uv radiation (Zajdela and

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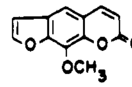
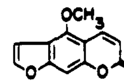
Bisagni, 1981), and 5-MIP with uv radiation (Mullen *et al.*, 1984) have been reported to be carcinogenic in mice after topical application, while 3-CEP with uv radiation (Dubertret *et al.*, 1978 and Mullen *et al.*, 1984) was not carcinogenic in mice after topical application. The toxicity and carcinogenicity of psoralen, administered orally, followed by uv radiation has not been thoroughly studied in rodent systems (Langner *et al.*, 1977; Griffin *et al.*, 1958).

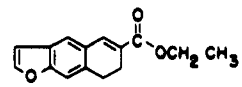
The psoralen/isopsoralen and uv radiation treatments described in this report were designed to mimic the clinical treatment of psoriatic patients in which the drug is administered orally. The studies were conducted in the HRA/Skh mouse, an inbred hairless mouse strain developed at Temple University for the study of ultraviolet light effects (Mann, 1971a,b; Forbes, 1981, 1982; Smith *et al.*, 1982). The four psoralen/isopsoralen drugs were given three times a week (at equimolar concentrations) after overnight fasting in a 1-hr oral pulse feed dose followed 1/2 hr later by uv radiation.



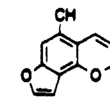
8-METHOXYPSORALEN
$C_{12}H_8O_4$ M.W. 216.18



5-METHOXYPSORALEN
$C_{12}H_8O_4$ M.W. 216.81



3-CARBETHOXYPSORALEN
$C_{14}H_{10}O_5$ M.W. 258.23



5-METHYLISOPSORALEN
$C_{12}H_8O_3$ M.W. 200:20

FIGURE 1

MATERIALS AND METHODS

Chemicals. 8-Methoxypsoralen (CAS NO. 298-81-7; lot 21900) was obtained from Elder Pharmaceuticals (Bryan, OH); 5-methoxypsoralen (CAS NO. 484-20-8; lot TO 32681) was prepared by Memphis Chemical Company (Zeitoun, Egypt); 3-carbethoxypsoralen (CAS NO. 20073-24-9; lot TO 32681) was obtained from Professor R. Latarjet, Fondation Curie, Institute du Radium (Paris, France); and 5-methylisopsoralen (CAS NO. 15912-88-6; lot H110381) was synthesized by HRI Associates, (Emeryville, CA) (Fig. 1). Chemical analyses of the psoralens were performed using a variety of techniques including thin-layer chromatography, gas chromatography, and infrared and nuclear magnetic resonance. The purity of 8-MOP, 5-MOP, 3-CEP, and 5-MIP was 99, 93, 97, and 99%, respectively (Jameson *et al.*, 1984).

8-MOP, 5-MOP, 3-CEP, and 5-MIP were mixed in NIH-07 feed (Zeigler Bros. Inc., Gardners, PA) using a Hobart C-100 mixer and half-inch diameter pellets were made for each drug (Dyets Inc., Bethlehem, PA) to give the following concentrations: 8-MOP: 50, 100, 625, and 1250 ppm; 5-MOP: 50, 100, 625, and 1250 ppm; 3-CEP: 60, 120, 750, and 1500 ppm; and 5-MIP: 46, 92, 575, and 1150 ppm. These concentrations yielded equimolar concentrations of drug in feed.

Animals. Male and female HRA/Skh mice were obtained from the Animal Services Division at the Skin and Cancer Hospital, Temple University Health Sciences Center (Philadelphia, PA). The animals were housed individually in stainless steel wire mesh cages (3 × 3 × 3½ in. Harford Mfg. Co., Aberdeen, MD) 72 mice per rack (Forbes, 1982). Animal cages were rotated one position clockwise on the rack each week.

A 12-hr room light cycle was provided using gold fluorescent lamps. Temperature was maintained at 76–80°F, and humidity was maintained at 50–70%. Tap water and NIH-07 chow (Zeigler Bros. Inc.) were available *ad libitum* except during the treatments of mice described under the experimental design. All animals were checked twice daily for morbidity and mortality. Moribund animals were killed and necropsied. Clinical signs, skin appearance, and body weights were recorded weekly.

Experimental groups. Animals were randomized into 1 of 27 experimental groups; each experimental group contained 12 male and 12 female HRA/Skh mice; each

TABLE I

SURVIVAL AND MEAN BODY WEIGHTS OF MALE AND FEMALE HRA/Skh MICE ADMINISTERED PSORALEN/ISOPSORALEN FOR 13 WEEKS WITH AND WITHOUT uv

			Male mice mean body weights (g)				Female mice mean body weights (g)		
Psoralen dose (mmol/kg feed)	uv (J/cm²)	Survival[a]	Initial (week 1)	Final (week 12)	Change (+g)	Survival[a]	Initial (week 1)	Final (week 12)	Change (+g)
Common controls									
0	0	12/12	31.0 ± 1.5	34.8 ± 2.5	+3.8	12/12	28.2 ± 2.3	31.1 ± 2.6	+2.9
0	2	12/12	31.7 ± 1.0	34.7 ± 2.5	+3.5	12/12	27.5 ± 1.3	32.0 ± 0.8	+3.5
0	48	12/12	31.5 ± 3.0	33.3 ± 3.5	+1.8	12/12	27.6 ± 1.6	30.8 ± 1.6	+3.2
8-Methoxypsoralen									
0.46	0	12/12	31.6 ± 1.7	34.8 ± 1.7	+3.2	12/12	27.0 ± 2.0	30.8 ± 2.2	+3.8
5.8	0	12/12	32.3 ± 2.3	35.7 ± 1.8	+3.4	12/12	28.3 ± 1.5	32.3 ± 1.3	+4.0
2.9	2	10/12	31.4 ± 1.8	32.8 ± 4.4	+1.4	11/12	27.5 ± 2.6	29.5 ± 2.3	+2.0
5.8	2	12/12	31.3 ± 1.7	31.0 ± 2.6	−0.3	12/12	28.1 ± 2.4	27.3 ± 2.5	−0.8
0.23	48	12/12	31.8 ± 2.4	35.5 ± 1.6	+3.7	12/12	27.2 ± 3.0	30.4 ± 2.8	+3.2
0.46	48	11/12	30.8 ± 1.8	34.1 ± 1.4	+3.3	11/12	26.7 ± 1.8	30.6 ± 2.2	+3.9
5-Methoxypsoralen									
0.46	0	12/12	31.5 ± 2.2	35.1 ± 1.9	+3.6	12/12	27.2 ± 1.6	31.4 ± 1.7	+4.2
5.8	0	12/12	28.1 ± 1.2	32.1 ± 1.3	+4.0	12/12	31.8 ± 2.3	35.0 ± 1.7	+3.2
2.9	2	12/12	31.1 ± 3.7	36.1 ± 2.5	+5.0	12/12	28.0 ± 2.3	31.5 ± 1.7	+3.5
5.8	2	12/12	31.4 ± 3.5	32.9 ± 3.3	+1.5	11/12	26.6 ± 4.8	27.3 ± 2.4	+0.7
0.23	48	12/12	31.1 ± 2.3	34.4 ± 3.0	+3.3	12/12	27.7 ± 2.1	31.5 ± 2.3	+3.8
0.46	48	12/12	31.1 ± 2.0	34.8 ± 2.5	+3.7	12/12	27.3 ± 2.8	31.5 ± 2.2	+4.2
3-Carbethoxypsoralen									
0.46	0	11/12	30.8 ± 2.0	34.8 ± 1.4	+4.0	11/12	27.4 ± 1.4	31.1 ± 1.6	+3.7
5.8	0	11/12	31.6 ± 2.7	35.2 ± 2.2	+3.6	11/12	26.8 ± 2.0	31.4 ± 1.8	+4.8
2.9	2	12/12	31.4 ± 2.8	34.1 ± 2.6	+2.7	12/12	26.0 ± 2.3	30.4 ± 1.9	+4.4
5.8	2	11/12	31.6 ± 2.1	35.6 ± 2.4	+4.0	12/12	27.4 ± 1.4	31.0 ± 2.2	+3.6
0.23	48	12/12	30.7 ± 2.1	34.2 ± 1.8	+3.5	12/12	27.5 ± 1.2	31.4 ± 1.9	+3.9
0.46	48	12/12	31.8 ± 1.7	35.0 ± 0.8	+3.2	12/12	26.9 ± 1.6	29.1 ± 1.7	+2.3
5-Methylisopsoralen									
0.46	0	12/12	31.7 ± 2.5	34.9 ± 2.2	+3.2	12/12	27.7 ± 1.7	31.7 ± 2.1	+4.0
5.8	0	12/12	31.9 ± 1.6	35.4 ± 1.9	+3.5	12/12	26.7 ± 1.7	30.8 ± 4.1	+4.1
2.9	2	12/12	31.4 ± 2.2	34.2 ± 2.7	+2.8	12/12	26.3 ± 2.8	30.6 ± 2.1	+4.3
5.8	2	12/12	31.4 ± 2.8	34.1 ± 2.6	+2.7	12/12	26.5 ± 1.7	30.5 ± 1.9	+4.0
0.23	48	12/12	30.1 ± 2.1	33.8 ± 2.3	+3.7	12/12	26.7 ± 2.1	30.6 ± 1.8	+3.8
0.46	48	12/12	31.2 ± 2.2	34.4 ± 2.2	+3.2	12/12	25.9 ± 2.4	29.2 ± 1.3	+3.3

[a] Number surviving/number per group at necropsy.

experimental group had four replicates of 3 males and 3 females that started on the study 2 weeks apart. The experimental groups were as follows: (a) common control groups (1–3): no psoralen, no uv; no psoralen, uv 2 J/cm²; no psoralen, uv 48 J/cm²; (b) drug treatment groups (4–11) at uv 0 J/cm²; psoralen/isopsoralen levels of 0.46 and 5.8 mmol/kg/feed; (c) drug treatment groups (12–19) at uv 2 J/cm²: psoralen/isopsoralen levels of 2.8 and 5.8 mmol/kg/feed; (d) drug treatment groups (20–27) at 48 J/cm²: psoralen/isopsoralen levels of 0.23 and 0.46 mmol/kg/feed.

The study involved a total of 648 HRA/Skh mice: 162 mice were in each of the four replicates. Every 2 weeks animals from the Temple breeding facility, 10–12 weeks of age, were randomized into 1 of the 27 treatment groups. Replicates were necessitated because the maximum colony output was approximately 200 animals every 2 weeks.

Treatment of animals. For the first 2 weeks of study animals received food pellets with or without the test article three times a week (Monday, Wednesday, and Friday). Animals were fasted overnight for 16 hr and then allowed access to a preweighed food pellet for 1 hr. For treatment weeks 3–13 the animals were "pulse" fed in the same manner followed by 2 or 48 J/cm² per exposure and beginning 1/2 hr later exposed to 0 and 2 J/cm² (5-min exposure) or 48 J/cm² (120-min exposure) uv radiation.

TABLE 2

RESPONSE OF HRA/Skh MICE ADMINISTERED PSORALENS OR ISOPSORALENS FOR 13 WEEKS WITH AND WITHOUT uv[a]

	Psoralen dose (mmol/kg feed)	uv (J/cm²)	Average skin response by clinical observation[b]				Frequency of histopathologic findings[c]							
			Week 3		Week 13		Hyperplasia		Inflammation		Ulceration		Atypical nuclei	
			M	F	M	F	M	F	M	F	M	F	M	F
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	2	0	0.1	0	0.04	0	0	0	2	0	0	0	0
	0	48	0.1	1.6	0	2.1	1	0	0	0	0	0	0	0
8-MOP	0.46	0	0	0	0	0	0	0	0	0	0	0	0	0
5-MOP	0.46	0	0	0	0	0	0	0	0	1	0	0	0	0
3-CEP	0.46	0	0	0	0	0	0	0	0	1	0	0	0	0
5-MIP	0.46	0	0	0	0	0	0	0	0	0	0	0	0	0
8-MOP	5.8	0	0	0	0	0	0	0	0	1	0	0	0	0
5-MOP	5.8	0	0	0	0	0	0	0	0	0	0	0	0	0
3-CEP	5.8	0	0	0	0	0	0	0	0	0	0	0	0	0
5-MIP	5.8	0	0	0	0	0	0	0	0	0	0	0	0	0
8-MOP	2.9	2	1.0	1.8	0.6	3.6	1	8	0	1	0	0	0	0
5-MOP	2.9	2	0.9	1.2	0.7	1.4	0	4	1	0	0	0	0	2
3-CEP	2.9	2	0	0	0	0	0	0	0	0	0	0	0	0
5-MIP	2.9	2	0	0	0	0	0	0	0	1	0	0	0	0
8-MOP	5.8	2	1.8	4.1	1.8	4.0	0	11	5	12	1	6	3	7
5-MOP	5.8	2	1.1	3.2	1.1	3.6	7	7	1	5	2	4	6	9
3-CEP	5.8	2	0	0	0	0	0	0	0	1	0	0	0	0
5-MIP	5.8	2	0	0	0	0	0	0	0	2	0	0	0	0
8-MOP	0.23	48	0.3	1.8	0	2.0	1	1	1	2	0	0	0	0
5-MOP	0.23	48	0	1.5	0	1.9	0	0	0	0	0	0	0	0
3-CEP	0.23	48	0	1.4	0	1.8	0	0	0	0	0	0	0	0
5-MIP	0.23	48	0	1.3	0	0.9	0	0	0	0	0	0	0	0
8-MOP	0.46	48	0.2	1.4	0.1	2.6	1	5	0	4	0	2	0	1
5-MOP	0.46	48	0	1.6	0	1.8	0	1	0	0	0	0	0	0
3-CEP	0.46	48	0	1.2	0	1.5	0	0	0	0	0	0	0	0
5-MIP	0.46	48	0	1.1	0	1.4	0	0	0	0	0	0	0	0

[a] Twelve animals in each group.

[b] Average clinical response on the skin (back) graded as 0, no response; 1, slight edema, dry flaking; 2, edema, erythema, mild hyperplasia, and mild desquamation; 3, edema, erythema, moderate hyperplasia, and moderate desquamatis; 4, extensive edema, erythema, chronic inflammation, and severe hyperplasia; 5, severe edema, erythema, widespread inflammation and desquamation, generalized hyperplasia, and ulceration.

[c] Histopathologic analysis of skin (back); results given as number of animals out of 12 with histopathologic finding.

Radiation treatment. Phototherapy lamps (blacklight fluorescent light, code No. FR74T12 PUVA) were obtained from GTE Sylvania (Danvers, MA). The spectrum output was characteristic of near ultraviolet light, primarily 320–400 nm (UVA) with a peak at 354 nm, and less than 2% radiation below 320 nm (Forbes *et al.*, 1976 and Cole *et al.*, 1984). Exposures were controlled by the Mu3 Dosimetry Systems (Model 2A, Solar Light Co., Philadelphia, PA). During the uv exposure period, a rack of animal cages was placed in front of a vertical bank of 36 lamps; animals were allowed to move freely during the radiation period. After uv treatment, animals were returned to food and water *ad libitum*. Treatment consisted of a total of 38 pulse feedings and 32 uv radiations over a 13-week period.

Pathology. Moribund animals and animals surviving to the end of the study were killed with carbon dioxide gas and then necropsied. Examinations for grossly visible

TABLE 3

COMPARISON OF ANIMAL AND HUMAN DOSE OF 8-METHOXYPSORALEN (8-MOP) PER KILOGRAM BODY WEIGHT OR SQUARE METER SURFACE AREA

	8-MOP dose	
Animal dose (mmol/Kg feed)	mg/kg body wt/week	mg/m^2 body surface area/week[a]
0.23	6	18
0.46	12	36
2.91	80	240
5.8	160	480
Average human dose[b]	1.8	67.0

[a] Calculations for body surface area based on Freireich *et al.*, 1966.

[b] Physician's Desk Reference, 1983.

lesions were performed on major tissues and organs. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: gross lesions and tissue masses, skin, mandibular and mesenteric lymph nodes, salivary glands, sternebrae and marrow, thyroids, parathyroids, small intestine, cecum, colon and rectum, liver, gallbladder, prostate, testes, epididymis, ovaries, lungs and mainstem bronchi, nasal cavity and turbinates, heart, esophagus, stomach, uterus, brain (three sections), thymus, trachea, pancreas, spleen, kidneys, adrenals, urinary bladder, pituitary, eyes, and mammary gland.

Complete histopathology was performed on the three common control groups, drug treatment groups at 5.8 mmol/kg and 0 uv radiation, 5.8 mmol/kg and 2 J/cm^2 uv light, 0.46 mmol/kg and 48 J/cm^2 uv radiation, and on all animals dying during the course of the experiment. Histopathologic examination of skin alone was performed on all other animals.

Body and organ weights (brain, liver, thymus, right kidney, right testis, heart, lung, and brain) were taken at the time of sacrifice and the body–organ weight ratios were calculated.

RESULTS

Survival, Body Weight, and Food Consumption

No dose-related mortality was seen in any treatment group. Dose-related body weight effects were seen only in the high-dose 8-MOP and 5-MOP groups receiving uv radiation at 2 J/cm^2 (Table 1). No differences were seen in organ/body weight ratios (liver, thymus, kidney, testis, heart, lung, or brain) in treated and control groups. Treated and control groups consumed similar amounts of feed during the pulse feedings, averaging 4 g of feed per animal per week during the three weekly pulse feedings. An estimate of 6–160 mg psoralen/kg body wt was consumed per week (or 18–480 mg psoralen/m^2 body surface area per week calculated according to procedures outlined by Freireich *et al.*, 1966) for the 0.23–5.8 mmol/kg feed dose groups, respectively.

Toxicity

Skin toxicity was estimated by clinical observation and histopathologic analyses of the tissues (Table 2). Both types of analyses showed similar results with skin toxicity being most severe in the high dose 8-MOP and 5-MOP groups with uv radiation (2 J/cm^2). Skin toxicity was not seen after 3-CEP and 5-MIP dosing followed by uv radiation at 2 J/cm^2.

No skin toxicity was seen in any group when the psoralen/isopsoralen was administered without uv radiation. Ultraviolet radiation with low-level 8-MOP or 5-MOP at 48 J/cm^2 produced some skin toxicity but this toxicity was milder than that seen with the higher level 8-MOP or 5-MOP plus uv radiation (2 J/cm^2). Ultraviolet radiation at 2 J/cm^2 (no drug) did not produce skin toxicity. Treatment with psoralen with or without uv radiation produced no signs of toxicity to the internal organs.

Characterization of Skin Toxicity

The skin toxicity in the 8-MOP and 5-MOP groups with uv radiation was characterized by clinical observations as erythema, widespread inflammation with desquamation, parched-looking body skin, scarred ears, inflammed eyelids, generalized hyperplasia, and scattered small ulcerations.

Pathologically the skin lesions were characterized by hyperplasia: increased thickness of the epidermis (up to 8–10 layers) over a background of 2–3 cell layers thick; inflammation: the presence of inflammatory cells including neutrophils, lymphocytes, and macrophages; ulceration: loss of epithelial cells with disruption of the adjacent basement membrane; hyperplasia in which there was a proliferative response with a loss of regular differentiation including cellular atypia and disorderly arrangement of cells; and atypical nuclei: large nuclei within hypertrophic squamous epithelial cells.

DISCUSSION

In these subchronic toxicity studies the primary toxic response was in the skin, and this response was dependent on the type of psoralen used and dose of uv radiation. Toxicity to internal organ systems was not seen. Survival was comparable among treated and control groups of animals. Each dose group consumed approximately the same amount of psoralen (mmol/kg body wt) on treatment days.

Skin toxicity was measured by clinical observations and histopathologic responses. Both measurements showed that skin toxicity was most frequent in mice receiving high doses of 8-MOP and 5-MOP (2.9 and 5.8 mmol/kg feed) and ultraviolet radiation at 2 J/cm^2. Skin lesions were characterized histologically as hyperplasia, inflammation, ulceration, and atypical nuclei. 3-CEP and 5-MIP given at the same dose levels with ultraviolet radiation did not cause skin toxicity. Ocular damage consisting of dense central corneal opacification after exposure to 8-MOP and 5-MOP and uv radiation was observed in these HRA/Skh mouse studies (Barker *et al.*, 1986).

Psoralens alone did not cause any evidence of toxicity to the skin or other organ systems. Ultraviolet radiation alone at 2 J/cm^2 produced no sign of skin toxicity, but after ultraviolet radiation at 48 J/cm^2 there was a skin toxic response characterized as edema or erythema. Ultraviolet radiation at 48 J with 8-MOP (0.46 mmol/kg feed) produced skin toxicity (hyperplasia, inflammation, and ulceration) that was more extensive in female mice than in male mice. Ultraviolet radiation at 48 J/cm^2 with 5-MOP, 3-CEP, or 5-MIP (0.46 mmol/kg feed) produced little or no increase in skin toxicity over the level of skin toxicity seen with radiation alone. The 8-MOP data indicate that in general female mice had a more severe skin toxic response than did male mice at corresponding dose levels.

8-MOP and 5-MOP have two photoreactive sites, at the 3,4 and 4',5' double bonds, which allow formation of monoadducts which can crosslink with DNA. In contrast, the angular structure of 5-MIP creates steric constraints on interaction with DNA, and the substitution at the C-3 position in 3-CEP blocks the 3,4 reactive site, thereby preventing the potential for crosslinking with DNA (Song and Tapley, 1979). Thus, in this study, skin toxicity correlated with the ability of the psoralen molecule to form DNA crosslinks. It is possible that at higher concentrations of 3-CEP and 5-MIP skin toxicity might be observed. In other studies (Lowe *et al.*, 1984) 5-MOP and 8-MOP with uv radiation have been shown to be good inducers of ornithine decarboxylase (ODC) levels. Increases in ODC levels have been shown to accompany the onset of proliferative events (Luk and Baylin, 1984). In this study 8-MOP and 5-MOP produced phototoxicity while 5-MIP and 3-CEP produced little phototoxicity at corresponding dose levels. Other workers have shown that the carcinogenic potential of psoralens cannot be directly related to phototoxic properties (Mullen *et al.*, 1984), and further studies are needed to determine potential carcinogenicity of these psoralen/isopsoralen derivatives after oral administration.

These studies used a "pulse feed" method to deliver psoralen to fasting animals, followed 1/2 hr later by uv radiation. This treatment schedule enabled a large number of animals to be treated on a carefully prescribed schedule that was designed to mimic PUVA therapy used in the treatment of psoriasis (Melski and Stern, 1982; PDR, 1983). Repeated exposure of the HRA/Skh mouse to 8-MOP or 5-MOP followed by uv radiation leads to skin toxicity. This skin toxicity occurred at a uv exposure level (2 J/cm^2) similar to that used in human therapy (Table 3) and at a psoralen dose level (240 mg psoralen/m^2 body surface area per week) approximately four times the weekly dose used in humans. A chronic study of 8-MOP and uv radiation is currently in progress in which the HRA/Skh mouse is being dosed three times per week (at 0, 0.46, 1.1, and 2.9 mmol psoralen/kg feed) followed by uv radiation at 2 J/cm^2. This range of psoralen doses spans human dose levels when compared on a basis of mg drug/m^2 body surface area.

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APPENDIX F

AUDIT SUMMARY

APPENDIX F. AUDIT SUMMARY

The pathology specimens, experimental data, study documents, and the NTP Technical Report No. 359 for the 2-year studies of 8-methoxypsoralen in rats were audited for the NIEHS at the NTP Archives during July 1986, December 1987, and January 1988 by quality assurance support contractors. Complete reports are on file at the NIEHS. The audit included review of:

(1) All records concerning animal receipt, quarantine, randomization, and disposition prior to study start.
(2) All inlife records including protocol, correspondence, animal husbandry, environmental conditions, dosing, external masses, mortality, animal identification, and serology.
(3) Body weight and clinical observation data for a random 10% sample of the study animals.
(4) All chemistry records.
(5) All postmortem records for individual animals concerning identification, condition codes, disposition codes, tissue accountability, correlation of masses or clinical signs recorded at the last inlife observation with gross observations and microscopic diagnoses, and correlation between gross observations and microscopic diagnoses.
(6) All wet tissue bags for inventory and wet tissues from a random 10% sample of rats in all study groups, plus other relevant cases to verify animal identification and to examine for untrimmed potential lesions.
(7) Blocks and slides of tissues from a random 20% sample of animals from each study group to examine for proper match and inventory.
(8) Correlation between original microscopic observations and tabulated pathology diagnoses for a random 10% of study animals to verify computer data entry.
(9) Correlation between the data, results, and procedures for the 2-year studies presented in the draft Technical Report and the records available at the NTP Archives.

The audit showed that inlife procedures and events were documented by the archival records with some exceptions: disposition of surplus animals, some standard operating procedures, frequency of cage and rack changes, balance calibration, and light cycle checks. The archival records indicated that doses were prepared and administered to animals according to protocols, that group body weight measurements were computed accurately, and that clinical observations were recorded consistently throughout the study. Of the external masses noted inlife, 123/129 in rats were correlated with necropsy observations. The inlife mode and date of death records for all early-death animals were correlated with necropsy records. The analytical chemistry records from the study laboratory were present and accurate and documented procedures adequately.

Inspection of residual wet tissues for individual animal identifiers (punched ears) showed that 12/75 rats were identified correctly and 15/75 had only one ear, correctly punched, present. Although ears for the other animals were documented as not saved, examination of other toxicology and pathology records gave no indication that individual animals had been exchanged between or within groups. The audit found 20 untrimmed lesions in 75 rats examined, including 7 of the forestomach in dosed male rats (most of which occurred in rats for which other forestomach lesions has been sectioned and examined). The residual segments of the intestinal tract (2-10 cm) were not completely opened, but no potential lesions were visible by external examination during the audit. All Individual Animal Data Record forms were reviewed, and there were seven gross observations (nontarget organs) that lacked a corresponding microscopic diagnosis.

In conclusion, the data and results presented in the Technical Report for the 2-year gavage studies of 8-methoxypsoralen are supported by the records at the NTP Archives.

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

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