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BIOASSAY OF SELSUN®

FOR POSSIBLE CARCINOGENICITY

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

BIOASSAY OF

SELSUN®

FOR POSSIBLE CARCINOGENICITY

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BIOASSAY OF
SELSUN®
FOR POSSIBLE CARCINOGENICITY
(Skin Painting Study)

Carcinogenesis Testing Program
National Cancer Institute/National Toxicology Program

FOREWORD

This report presents the results of the bioassay of Selsun® conducted for the Carcinogenesis Testing Program, National Cancer Institute (NCI)/ National Toxicology Program (NTP). This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. A negative result, in which the test animals do not have a greater incidence of cancer than control animals, does not necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. A positive result demonstrates that the test chemical is carcinogenic for animals under the conditions of the test and indicates that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from chemicals found to be carcinogenic in animals requires a wider analysis.

CONTRIBUTORS

This bioassay of Selsun® was conducted by Hazleton Laboratories America, Inc., Vienna, Virginia, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., Rockville, Maryland, prime contractor for the NCI Carcinogenesis Testing Program.

The persons responsible for selecting the protocols used in this bioassay were Drs. O. G. Fitzhugh (1,2), J. F. Robens (1,3), M. B. Powers (4,5), and C. Cueto (6,7). The principal investigators were Drs. M. B. Powers (4,5) and R. W. Voelker (4), and Mr. J. L. Gargus (4) was assistant investigator. Ms. K. J. Petrovics (4) was responsible for data management, and Mr. J. Everly (4) was the supervisor of animal care. Histopathologic examinations on the subchronic studies were performed by Drs. D. A. Banas (4) and R. W. Voelker (4). Histopathologic examinations on the chronic study were performed by Dr. D. S. Wyand (8). The pathology report and selected slides were evaluated by the NCI Pathology Working Group as described in Ward et al. (1978)

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (9). Statistical analyses were performed by Dr. J. R. Joiner (1) and Ms. S. Vatsan (1), using methods selected for the bioassay program by Dr. J. J. Gart (10).

Chemicals used in this bioassay were analyzed at Midwest Research Institute (11), and dose solutions containing the test chemical were analyzed at Hazleton Laboratories by Dr. C. L. Guyton (4) and Mr. E. Missaghi (4). The results of these analyses were reviewed by Ms. P. Wagner (1, 12).

This report was prepared at Tracor Jitco in collaboration with Hazleton Laboratories and NCI. Those responsible for the report at Tracor Jitco were Dr. L. A. Campbell, Acting Director of the Bioassay Program; Dr. S. S. Olin, Associate Director; Dr. R. L. Schueler, pathologist; Dr. D. J. Beach, reports manager; Dr. A. C. Jacobs, bioscience writer; and Dr. W. D. Theriault and Ms. M. W. Glasser, technical editors.

The following scientists at NCI (6) were responsible for evaluating the bioassay, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Michael P. Dieter, Dr. J. Fielding Douglas, Dr. Richard A. Griesemer, Dr. Charles K. Grieshaber, Dr. Thomas E. Hamm, Dr. William V. Hartwell, Dr. Y. Jack Lee, Dr. Harry Mahar, Dr. James McCoy, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Marcelina B. Powers, Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

A bioassay of Selsun® for possible carcinogenicity was conducted by applying this substance dermally to ICR Swiss mice. Selsun®, an antidandruff shampoo, contains 2.5% selenium sulfide.

Groups of 50 mice of each sex were exposed to 0.05 ml of 25% or 50% Selsun® in distilled water three times a week on a 2- x 3-cm clipped dorsal surface. Vehicle controls consisted of 50 mice of each sex that were clipped and treated with distilled water. Untreated controls consisted of 50 mice of each sex that were only clipped. Surviving mice were killed and necropsied at week 88.

Mean body weights of untreated control, vehicle control, low-dose, and high-dose groups were comparable throughout the bioassay. Amyloidosis was a factor in the deaths of most animals after 1 year. In male mice, alveolar/bronchiolar carcinomas or adenomas occurred with a dose-related trend that was significant (P=0.008). The result of the Fisher exact test comparing the incidence in the high-dose group with that in the vehicle controls is also significant, but the incidence of the high-dose group, when compared with that of the untreated controls, is not significant.

Under conditions of this bioassay, dermal application of Selsun® was not carcinogenic for ICR Swiss mice. The study was limited, however, by the relatively short lifespan of this strain of mouse.

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I. INTRODUCTION

Selsun®(NCI C54546) is a prescription antidandruff shampoo containing 2.5% selenium sulfide (SeS). Selsun Blue®, a nonprescription antidandruff shampoo (Physician's Desk Reference, 1978), and Seleen®, a dermal cleansing agent for dogs (Seigmund, 1967; Federal Register, 1978), both contain 1% selenium sulfide. Selenium sulfide may be present in other hair grooming products (Lehne, 1972). Two bioassays of selenium sulfides were conducted concurrently in the same laboratory as the Selsun® study: a gavage study (NCI, 1980) and a dermal study (NCI, 1980a).

Selenium shampoos are used in the treatment of seborrheic dermatitis, seborrheic sicca, and tinea versicolor (Rook, 1972; Swinyard, 1975; AMA Department of Drugs, 1977). The shampoos are generally applied once or twice a week and are left in contact with the skin for 2 to 3 minutes, rinsed, and reapplied a second time for a similar time period.

Although residues of selenium sulfide remain on the scalp after rinsing (AMA Department of Drugs, 1977), there is no substantial absorption of the chemical through intact skin from this type of use (Slinger and Hubbard, 1951; Cummins and Kimura, 1971). Absorption had been reported only in patients with open lesions on the scalp (Ransone et al., 1961) or in patients using a 1% cream on the back (Sternberg et al., 1964).

Sodium selenate and selenite have been used in animal feeds to prevent selenium deficiency diseases in livestock and poultry (Federal Register, 1974). Selenium is also essential for rats (National Academy of Sciences, 1976). In man, selenium is required for three enzyme-catalyzed oxidation-reduction reactions; excessive quantities of selenium, however, may interfere in cellular metabolism (Stadtman, 1974). In nuclear medicine, ^{75}Se-selenomethionine is used as a radioisotopic tracer and diagnostic aid for the detection of human liver cancer, pancreatic cancer, and placental insufficiency (Greig and Gillespie, 1975).

Other selenium compounds are used in the manufacture of glass; in electronic rectifiers; in photoelectric cells; as alloys in copper and steel; as vulcanizing agents in rubber; as oxidizing agents, solvents, and lubricants; and in the printing and photographic industries (Stone, 1973).

Production figures show that 200 kg of selenium sulfide are produced annually for use as an antidandruff agent (IARC, 1974). One hundred and sixty thousand kilograms of waste from the medicinal industry, containing 320 kg selenium sulfide, are generated annually (Environmental Protection Agency, 1976). The industrial production of selenium in the United States for the remaining uses is estimated at approximately 1 million kilograms (Stone, 1973).

The oral LD_{50} of selenium sulfide in male Sprague-Dawley rats is 138 mg/kg body weight, and the oral LD_{50} of sodium selenite in male Sprague-Dawley rats is 7 mg/kg body weight when tested under the same conditions (Cummins and Kimura, 1971). Henschler and Kirschner (1969) estimated the oral LD_{50} of selenium sulfide in female NMRI mice to be 3,700 mg/kg. It was suggested by Cummins and Kimura (1971) that this difference in toxicity might be due to the particle size, which differed in each test.

Shampoo formulations in which selenium sulfide is incorporated with wetting agents, sequestrants, a fungicide, and other ingredients (Physicians' Desk Reference, 1978) have oral LD_{50}'s in male Sprague-Dawley rats of 14.2 ml/kg (1% selenium sulfide) and 5.3 ml/kg (2.5% selenium sulfide) (Cummins and Kimura, 1971). In female Swiss Webster mice, the oral LD_{50}'s of selenium sulfide shampoos are 7.8 ml/kg (1% selenium sulfide) and 4.9 ml/kg (2.5% selenium sulfide) (Cummins and Kimura, 1971).

Sodium selenite has been widely used in media to culture Salmonella. The possibility of teratogenic effects among pregnant laboratory workers handling sodium selenite as an ingredient in culture media is presented by Robertson (1970).

Selsun® was selected for testing by a dermal route because of the human exposure from its use in dandruff shampoos. Another dermal test that was conducted concurrently under identical protocols using selenium sulfide, the active ingredient in these shampoos, will be reported separately (NCI, 1980a).

II. MATERIALS AND METHODS

A. Chemical

Selsun®, Selenium Sulfide Lotion N.F., was obtained in three batches (Lot No. 43-660AF, used in the subchronic studies and Lot No. 46-660AF and Lot No. 65-938AF used in the chronic studies). Selsun® is a product of Abbott Laboratories, Chicago, Illinois. X-ray diffraction patterns were run on the powdered yellow/orange crystalline needles extracted from each batch of Selsun® with carbon disulfide and were found to be consistent with the American Society for Testing Materials data (Smith, 1960) reported for "selenium monosulfide" powder (Appendix C). The diffraction patterns were also identical to each other and to that of the selenium sulfide Lot No. 47E204 obtained from City Chemical Corporation (New York, N.Y.) and tested concurrently (NCI, 1980a).

Analyses by atomic absorption with selenium sulfide as a standard confirmed that Selsun® contains 2.5% (weight per volume) selenium sulfide (Appendix D).

Selsun® was stored at 4°C.

B. Dosage Preparation and Administration

The Selsun® used in the subchronic and chronic tests was applied full strength or diluted to the proper concentrations with distilled water. Selsun® dilutions were prepared weekly and stored at 4°C. To ensure that the solutions were of the proper concentration, randomly selected samples were taken and analyzed as described in Appendix D. The amount of selenium sulfide found in each sample was within 5% or less of the theoretical concentrations.

Hair was removed from the interscapular area of all mice with a hair clipper at least once each week to expose a 2- x 3-cm dorsal skin. The test solutions were applied to the skin via an automatic pipette (Becton-Dickinson, Rutherford, N. J.) and spread evenly over the surface of the skin with a glass rod.

C. Animals

Male and female ICR Swiss mice used in this bioassay were obtained at 5 weeks of age from Charles River Breeding Laboratories, Wilmington, Massachusetts. Upon receipt, animals were isolated and observed for disease for 2 weeks before being placed on test.

D. Animal Maintenance

Mice were housed in a room with the temperature maintained at 22° to 24°C, and the relative humidity at 45% to 55%. A single-pass-through air-handling system provided 7 to 10 changes of room air per hour. Room vents were fitted with 2-inch-thick disposable fiberglass filters. Fluorescent lighting was provided 12 hours per day.

Mice were housed individually in stainless steel cages with perforated bottoms and fronts (Hoeltge, Cincinnati, Ohio), which were suspended from racks over stainless steel drop pans containing absorbent paper sheets. Wayne® Lab Blox nuggets (Allied Mills, Chicago, Ill.) and well water were provided ad libitum.

Cages, pans, and racks were washed once a week at 81°C in an industrial cage washer. Paper liners in the drop pans were replaced three times per week. Glass water bottles and stainless steel sipper tubes were replaced daily as needed and washed routinely twice a week in a tunnel washer at 81°C. Feed hoppers were washed weekly. Acclaim® detergent (Economics Laboratory, St. Paul, Minn.) was used to wash all equipment.

The dermal studies of Selsun® and selenium sulfide were conducted concurrently in the same room. Untreated controls, but not vehicle controls, were shared between the two tests.

E. Subchronic Studies

To establish the doses of Selsun® to be used in the chronic study, two 13-week subchronic studies were performed. In the first study, groups of 10 male and 10 female mice received one dermal application of 0.05, 0.1, or 0.2 ml of undiluted Selsun® per day, 5 days per week and 10 mice of each sex received two dermal applications of 0.2 ml undiluted Selsun® per day, 5 days

per week (Table 1). Ten mice of each sex served as vehicle controls, receiving applications of saline containing 0.5% sodium carboxymethylcellulose.

Mice were observed daily for mortality, toxic signs, and skin irritation and were weighed weekly. After 13 weeks, survivors were killed by cervical dislocation. Necropsies were performed on all animals, and certain tissues were taken for histopathologic analysis.

All animals in all test groups survived, although administration of the test chemical was discontinued in the group of mice treated with 0.2 ml either once or twice per day, because of the severe skin irritation that occurred in these animals.

Slight to moderate skin irritation and redness were evident in animals administered 0.05 or 0.1 ml of Selsun®.

Histologically, minimal to moderately severe acanthosis and slight hyperkeratosis occurred in mice treated daily with 0.05 or 0.1 ml of Selsun®. Following discontinuation of treatment in the higher exposure groups, some recovery from their skin lesions occurred, but 7/10 male and 7/10 female mice in the groups treated twice per day with 0.2 ml and 6/10 of the females dosed once per day with 0.2 ml of Selsun® still had some residual acanthosis and hyperkeratosis 11 weeks later.

In the liver, focal coagulation necrosis was found in one female mouse receiving 0.05 ml of Selsun®, and bile duct proliferation occurred in one male mouse receiving 0.1 ml of Selsun®. Occasionally, micro-granulomas and minimal nonsuppurative pericholangitis were seen in the livers of mice in control and dosed groups.

The incidence and severity of nephritis appeared to be increased at the higher discontinued doses. Hydronephrosis was found in two male mice treated with 0.2 ml of Selsun® twice a day. Spleen and bone marrow of controls and treated mice were normal.

Because histopathologic alterations of the skin occurred at all doses and histopathologic changes in the liver and kidney were suspected of being related to treatment, a second 13-week subchronic study was performed by administering 0.05 ml of 10%, 25%, or 50% solutions of Selsun® in distilled water in the same manner as before (Table 2).

Table 1. Doses, Survival, and Mean Body Weights of Mice in the First 13-Week Subchronic Study of Selsun®

Doses				Mean Body Weights (grams)			
Selsun® Dose (a) (ml/day)	Selenium Sulfide Equivalent (mg/day)	Total Exposure to Selenium Sulfide (mg)	Survival(b)	Initial Weight	Final Weight	Gain	Weight Change Relative to Controls (c) (percent)
MALES							
0 (d)	0	0	10/10	28.9	35.4	6.5	
0.05	1.25	81	10/10	30.6	39.6	9	+38
0.1	2.5	162	10/10	31.3	38.4	7.1	+9
0.2 (e)	5.0	45	10/10	30.4	38.4	8	+23
0.4 (f,g)	10.0	85	10/10	27.8	39.2	11.4	+75
FEMALES							
0(d)	0	0	10/10	24.1	30.8	6.7	
0.05	1.25	81	10/10	23.9	32.8	8.9	+33
0.1	2.5	162	10/10	24.5	33.6	9.1	+36
0.2 (e)	5.0	45	10/10	25.3	33.4	8.1	+21
0.4 (f,g)	10.0	85	10/10	24.4	32.1	7.7	+15

(a) Dosed animals received undiluted Selsun® containing 2.5% selenium sulfide, five times per week.
(b) Number survivors/number per group.
(c) Weight Change Relative to Controls = $\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$
(d) Controls received 0.2 ml of saline containing 0.5% sodium carboxymethylcellulose.
(e) Dosing was discontinued after nine applications.
(f) This was the total dose resulting from two daily applications.
(g) Dosing was discontinued after 17 applications.

Table 2. Doses, Survival, and Mean Body Weights of Mice in the Second 13-Week Subchronic Study of Selsun®

Doses				Mean Body Weights (grams)			
Selsun®(a) Dose	Selenium Sulfide Equivalent (mg/day	Total Exposure to Selenium Sulfide (mg)	Survival(b)	Initial Weight	Final Weight	Gain	Weight Change Relative to Controls (c) (percent)
MALES							
0 (d)	0	0	10/10	24.9	36.9	12	-
10	0.125	8	10/10	25.2	37.3	12.1	+1
25	0.31	20	10/10	24.5	36.3	11.8	-2
50	0.625	40	10/10	24.7	35.3	10.6	-12
FEMALES							
0 (d)	0	0	10/10	22.7	31.0	8.3	
10	0.125	8	10/10	21.7	30.6	8.9	+7
25	0.31	20	10/10	22.5	30.7	8.2	-1
50	0.625	40	10/10	22.5	31.7	9.2	+11

(a) Dosed animals received 0.05 ml of sterile distilled water containing either 10%, 25%, or 50% Selsun® five times per week. (Selsun® contained 2.5% selenium sulfide).

(b) Number survivors/number per group.

(c) Weight Change Relative to Controls = $\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$

(d) Controls received 0.05 ml of sterile distilled water.

All animals tested in the second subchronic study survived. Redness and irritation were observed in some mice receiving 25% Selsun® and in about one-fourth of the animals receiving 50% Selsun®.

Histologically, minimal to moderate acanthosis and hyperkeratosis were detected in skin sections from 8/10 male and 9/10 female mice treated with 10% Selsun® and in all mice treated with 25% or 50% Selsun®. Similar changes detected in three control females were attributed to trauma induced by the application procedure. Other lesions included increased extramedullary hematopoiesis in the spleen (one female), minimal chronic interstitial nephritis (in about 50% of the male mice and 50% of the female mice in all dosed groups and in the controls), minimal nonsuppurative pericholangitis (three females), and scattered microgranulomas in the liver (one male and one female).

Because of the intensity of dose-dependent skin irritation that was observed in the subchronic studies, aqueous solutions containing 25% and 50% Selsun® were selected for use in the 2-year dermal study.

F. Chronic Study

The test groups, doses administered, and durations of the chronic study are shown in Table 3. The frequency of dose application was reduced from 5 days per week (in the subchronic study) to 3 days per week (in the chronic study) to further reduce the possibility of skin irritation.

G. Clinical Examinations and Pathology

Animals were observed daily for mortality. Treatment sites were observed three times weekly, and the general appearance and behavior of the animals were recorded at that time. Body weights were recorded every 4 weeks.

Animals that were moribund and those that survived to the termination of the study were killed and necropsied following anesthetization by intraperitoneal injections containing 60 mg/kg of sodium pentobarbital (Diabutal®, Diamond Laboratories, Inc., Des Moines, Iowa).

Table 3. Experimental Design of the Selsun® Chronic Dermal Study in Mice

Sex and Test Group	Initial Number of Animals	Dose (a) Selsun® (percent)	Dose (a) Selenium Sulfide Equivalent (mg)	Time on Study Dosed(b) (weeks)	Time on Study Observed (weeks)
MALES					
Untreated-Control(c)	50	0	0	0	83-87
Vehicle-Control(d)	50	0	0	86	2
Low-Dose	50	25	0.31	86	2
High-Dose	50	50	0.625	86	2
FEMALES					
Untreated-Control(c)	50	0	0	0	83-87
Vehicle-Control(d)	50	0	0	86	2
Low-Dose	50	25	0.31	86	2
High-Dose	50	50	0.625	86	2

(a) Each animal received 0.05 ml of the test solution of distilled water containing 25 or 50% Selsun® , three times per week. (Selsun® contains 2.5% selenium sulfide.)
(b) Dosing was discontinued when survival in one group decreased to 10%.
(c) Untreated controls were shaved only.
(d) Vehicle controls were shaved and painted with distilled water.

Gross and microscopic examinations were performed on major tissues, major organs, and all gross lesions from killed animals and from animals found dead. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: skin (treated areas), lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, pancreas, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, and brain.

Necropsies were performed on all animals found dead unless precluded in whole or in part by autolysis. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically 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) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is reported only when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific

anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

The approximate 95% confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971).

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that, in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result has occurred (P less than 0.025 one-tailed test when the control incidence is not zero, P less than 0.050 when the control incidence is zero). When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper

limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS

A. Body Weights and Clinical Signs

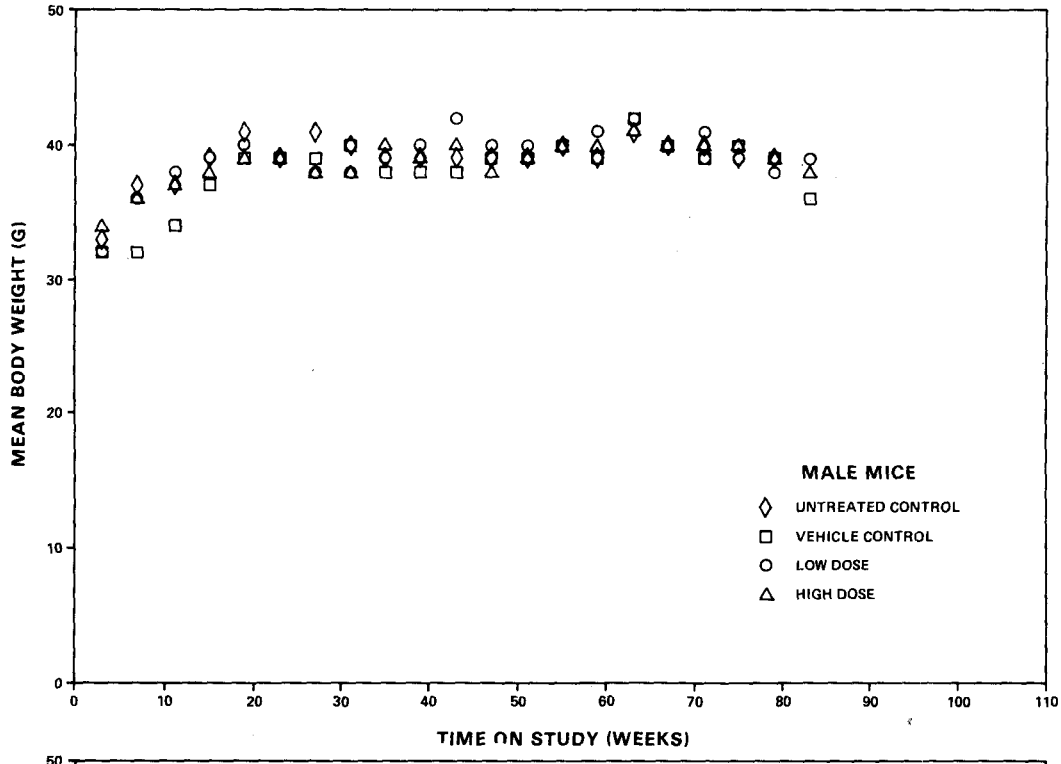
Mean body weight gains of dosed and control animals were similar throughout the study (Figure 1). During the first 13 weeks, the incidence of redness at the application site in both high-dose and low-dose female mice was less than 5%. After 14 weeks, the incidence of redness at the application site was 5% to 10% for low-dose males and 10% to 20% for the high-dose males. Severe skin irritation observed in the dosed mice in the subchronic study was not observed in the chronic study. No growths were observed in the skin of the low-dose mice, although wart-like growths were observed at the application site on six high-dose males and four high-dose females during the second year of the study. These lesions were examined histopathologically, but only one neoplasm was found. Scabs and scar tissue, observed in animals of the untreated-control, vehicle-control, and dosed groups, were probably due to itching and scratching in response to being clipped.

B. Survival

Estimates of the probabilities of survival for male and female mice administered Selsun® by dermal application, together with those of the vehicle and untreated controls, are shown by the Kaplan and Meier curves in Figure 2. The result of the Tarone test for dose-related trend in mortality is not significant in either sex.

In male mice, 41/50 (82%) of the high-dose group, 43/50 (86%) of the low-dose group, and 40/50 (80%) of the vehicle-control group were still alive at 52 weeks on study. In females, 41/50 (82%) of each study group were still alive at 52 weeks on study.

Survival declined rapidly after 52 weeks and the study was terminated at 88 weeks.



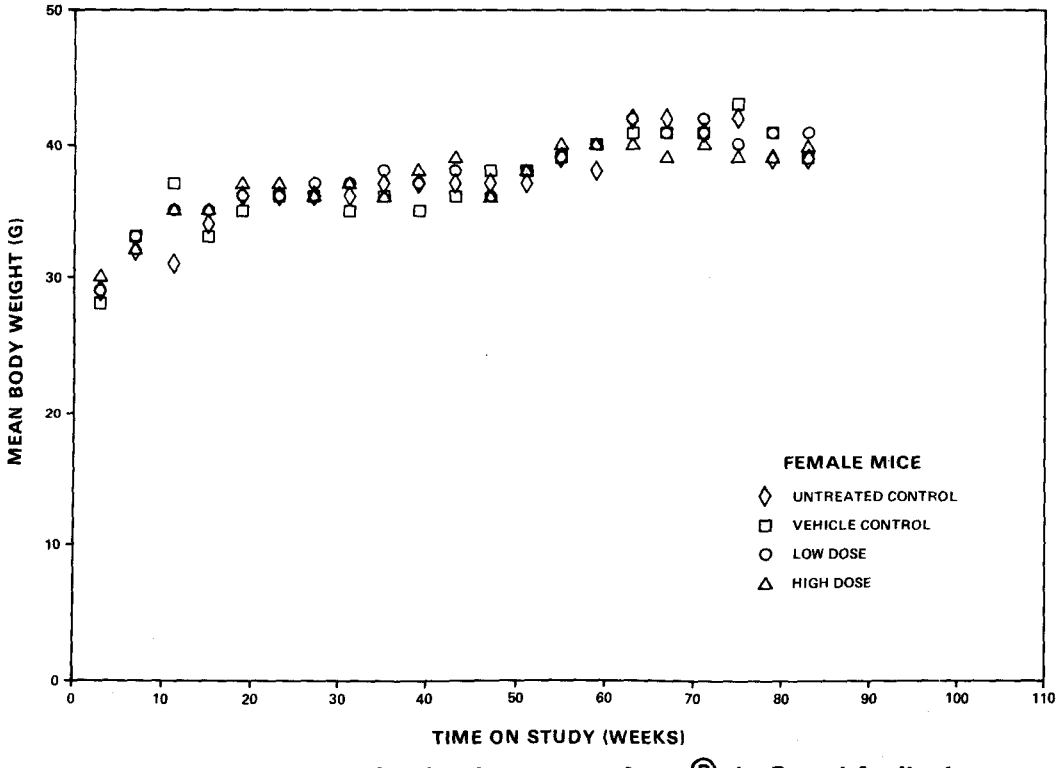


Figure 1. Growth Curves For Mice Administered Selsun® by Dermal Application

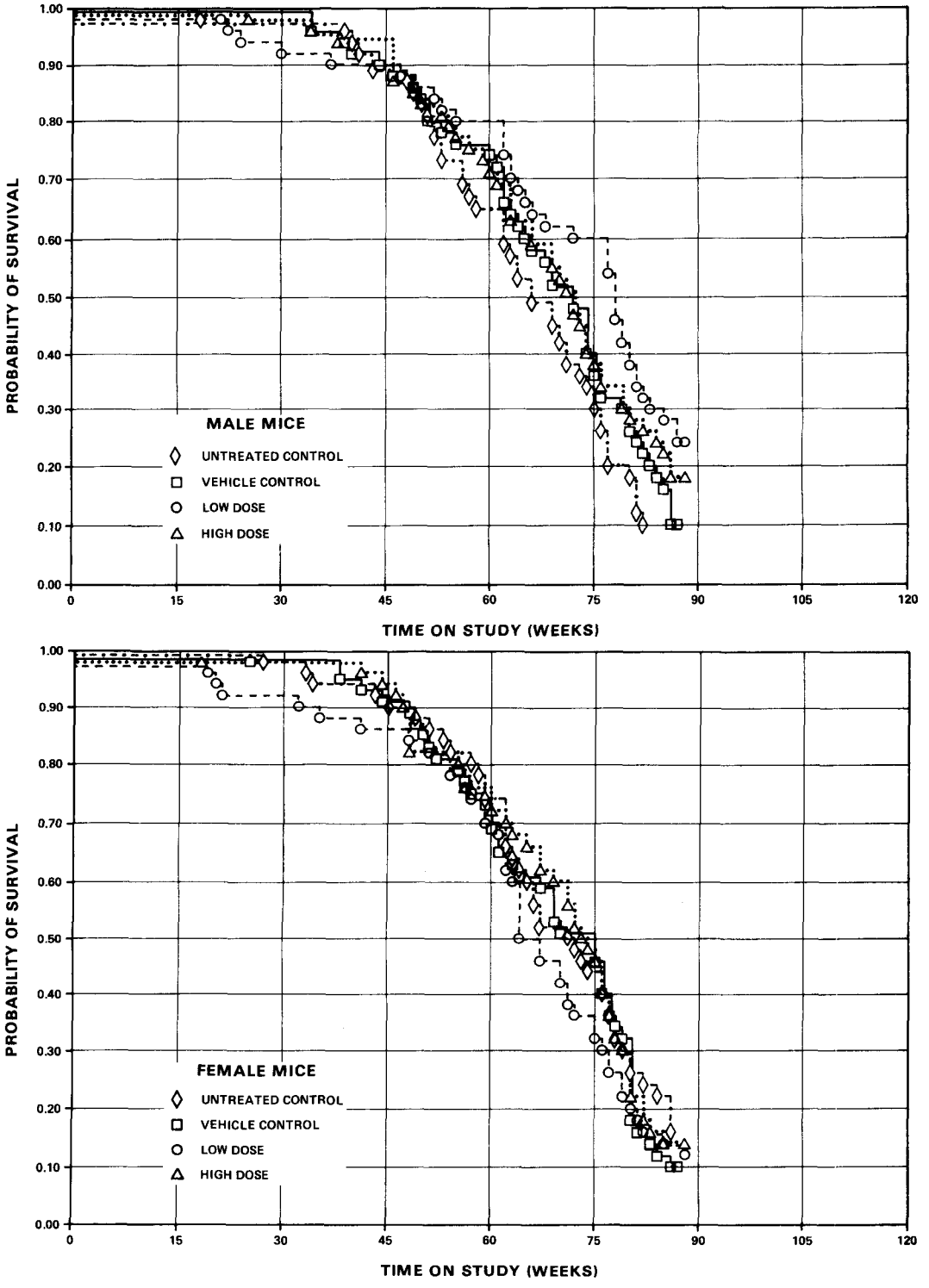


Figure 2. Survival Curves For Mice Administered Selsun® by Dermal Application

C. Pathology

Histopathologic findings on neoplasms in mice are summarized in Appendix A, Tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix B, Tables B1 and B2.

A variety of neoplasms were seen in control and dosed mice. All tumors noted were those commonly seen in mice of this strain, and they occurred in comparable numbers in both control and dosed mice. Only the incidence of lung tumors in male mice may have been related to dermal application of Selsun®. The incidences of alveolar/bronchiolar adenomas or carcinomas in male mice were 9/48 (19%) in the high-dose group, 7/50 (14%) in the low-dose group, 1/49 (2%) in the vehicle-control group, and 3/50 (6%) in the untreated-control group. The lung tumors were usually singular rather than multiple.

At the dermal application site, only one tumor (a papilloma) was found in a high-dose female mouse.

Nonneoplastic skin lesions at the site of compound application were induced in both sexes. The most common findings were acanthosis and hyperkeratosis, which were observed both in low-dose male mice (30% and 28%, respectively) and in high-dose male mice (42% and 38%, respectively). The incidences of these lesions were 8% and 10% in corresponding, untreated male controls and 4% and 0% in vehicle controls. Acanthosis and hyperkeratosis were found also in low-dose female mice (42% and 34%, respectively) and in high-dose females (56% and 46%, respectively). These two lesions each occurred in 2% of corresponding untreated female controls and in 2% of the vehicle controls. Acanthosis was characterized by an increase in the thickness of the prickle-cell layer with retention of normal cellular polarity and epidermal architecture. Thickness varied from a slight degree of 3 to 5 cells to more extensive thickening of 10 to 12 cells or more. Occasionally, slight elongation of the rete pegs occurred. Some animals also had parakeratosis and acute and chronic inflammatory infiltrates of the epidermis and upper dermis. The mortality in control and dosed mice appeared to be due to amyloidosis, which affected several organs, especially the liver, kidney, and spleen.

Histopathologic examination provided no convincing evidence of the carcinogenicity of Selsun® in ICR Swiss mice under the conditions of this bioassay.

D. Statistical Analyses of Results

Tables 4 and 5 contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and at an incidence of at least 5% in one or more groups. The incidence of lesions in the untreated group are shown in Appendixes A and B.

In male mice, the incidence of alveolar/bronchiolar adenomas or carcinomas in the untreated-controls was 3/50 (6%) compared with 1/49 (2%) in the vehicle-controls. The incidence in the untreated-controls is not statistically different from the vehicle-control. Nevertheless, the incidence in the former group is sufficiently higher than the vehicle controls so that the Fisher exact test indicates that no statistically significant differences exist between the untreated-controls and either dosed group (7/50, 14% in the low-dose group and 9/48, 19% in the high-dose group). However, a significant dose-related trend is noted (P=0.041). When the incidence in the matched vehicle-control is used in comparisons with the dosed groups, a significant positive linear trend in incidence is seen (P=0.008), and the Fisher exact test between the high-dose group and these vehicle-controls is statistically significant (P=0.007).

In a dermal study of selenium sulfide which was conducted concurrently with Selsun® at this laboratory, the incidence of lung tumors in the vehicle-controls for the male mice was 3/48 (6%). Therefore, there are two vehicle-control groups at this laboratory, each with an incidence of lung tumor of 6% or less. The vehicle controls from the dermal study of selenium sulfide were pooled with the vehicle controls in this study of Selsun® and subjected to statistical tests with the dosed groups reported here. The results indicate a linear trend (P=0.003) and a significantly higher incidence (P=0.006) in the high-dose group (9/48, 19%) than in the pooled control group (4/97, 4%). The comparison of either matched or pooled vehicle-control groups with the high-dose group indicates the possibility of an association of the administration of Selsun® with the occurrence of lung

tumors, since the incidence in the vehicle control is low. The incidence in the dosed groups of female mice was comparable with that in the control group. The results of the statistical tests on the tumor incidences in female mice are not significant.

Table 4. Analyses of the Incidence of Primary Tumors in Male Mice Administered Selsun® by Dermal Application (a)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	1/49 (2)	7/50 (14)	9/48 (19)
P Values (c,d)	P=0.008	P=0.032	P=0.007
Relative Risk (e)		6.860	9.188
Lower Limit		0.932	1.353
Upper Limit		302.197	392.598
Weeks to First Observed Tumor	87	68	49
Liver: Hepatocellular Carcinoma (b)	3/49 (6)	0/49 (0)	3/49 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.000	1.000
Lower Limit		0.000	0.140
Upper Limit		1.662	7.126
Weeks to First Observed Tumor	62	--	55
Liver: Hepatocellular Carcinoma or Adenoma (b)	5/49 (10)	1/49 (2)	4/49 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.200	o.800
Lower Limit			0.004
0.168			
Upper Limit			1.698
3.494			
Weeks to First Observed Tumor	62	79	55

(a) Dosed groups received doses of 25% or 50% Selsun® in distilled water, three times per week.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the vehicle-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95% confidence interval of the relative risk between each dosed group and the control group.

Table 5. Analyses of the Incidence of Primary Tumors in Female Mice Administered Selsun® by Dermal Application (a)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma (b)	7/49 (14)	10/49 (20)	7/49 (14)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.429	1.000
Lower Limit		0.536	0.324
Upper Limit		4.064	3.091
Weeks to First Observed Tumor	48	54	56
Hematopoietic System: Lymphoma (b)	4/50 (8)	2/50 (4)	2/50 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.500	0.500
Lower Limit		0.047	0.047
Upper Limit		3.318	3.318
Weeks to First Observed Tumor	51	67	18

(a) Dosed groups received doses of 25% or 50% Selsun® in distilled water three times per week.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the vehicle-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95% confidence interval of the relative risk between each dosed group and the control group.

IV. DISCUSSION

In some inbred strains of mice, decreased survival after 1 year has been related to increased occurrence of amyloidosis (Dunn, 1967). The survival of control and treated animals in the present study may be attributed to the high incidence of amyloidosis observed in both treated and control animals. Similarity of mean body weights of the low- and high-dose groups and of the untreated controls and the vehicle controls, as well as the lack of other life threatening or dose-related lesions, suggest that the test animals may have been able to tolerate exposure to greater amounts of the test substance. However, results of subchronic studies suggest that higher doses or more frequent application might not have been tolerated due to irritation at the application site.

In male mice, alveolar/bronchiolar carcinomas or adenomas occurred with a statistically significant dose-related trend (P=0.008). The result of the Fisher exact test comparing the incidence in the high-dose group with that in the vehicle controls is also significant; however, when comparisons were made with the untreated controls, the incidence in the high-dose group was not significant. Two of the alveolar/bronchiolar tumors occurring in male mice were carcinomas; the remainder were adenomas. The one alveolar/ bronchiolar carcinoma occurring in a high-dose male mouse was first observed at week 60; the one alveolar/bronchiolar carcinoma occurring in a vehicle-control male mouse was first observed at week 87. Alveolar/bronchiolar adenomas, first observed at week 53 in the untreated-control male mice, at week 68 in the low-dose male mice, and at week 49 in the high-dose male mice, were not considered the source of the early deaths because they were singular rather than multiple tumors. Hyperplasia of the alveolar epithelium was found in one vehicle-control male mouse, but not in any of the dosed male mice. In female mice, no tumors occurred in statistically significant numbers. Since alveolar/bronchiolar adenomas have been reported as indigenous tumors among aging Swiss mice (Witschi and Lock, 1979), the incidences of these tumors observed among male mice could not be clearly related to dermally applied Selsun®.

It should be noted that two additional tests have been conducted with selenium sulfide preparations. Selenium sulfide, administered by gavage, was found to be carcinogenic for male and female F344 rats and female B6C3F1 mice, inducing hepatocellular carcinomas in rats and female mice and alveolar/bronchiolar carcinomas or adenomas in female mice (NCI, 1980). However, in a concurrent bioassay (which was also terminated early), selenium sulfide applied dermally to ICR Swiss mice did not induce significant carcinogenic effects (NCI, 1980a). Toxic skin effects precluded applying dermal doses of Selsun® comparable with the gavage doses associated with tumor induction. Incidences of the lung and liver tumors observed in the bioassays involving selenium sulfide or Selsun® are summarized in Tables 6 and 7.

Reports in the literature concerning the extent of percutaneous absorption of selenium sulfide in humans are inconclusive. Ransone et al. (1961) reported in an uncontrolled case study that, when selenium sulfide in a shampoo formulation was applied to a scalp having open lesions, an elevated level of urinary selenium was measured. Increased urinary excretion of selenium attributed to percutaneous absorption of selenium sulfide was also reported by Sternberg et al. (1964); in this study, a cream containing 1% selenium sulfide was applied to the backs of human subjects. Two other studies indicated that selenium is not excreted after repeated application of shampoo containing selenium sulfide (Slinger and Hubbard, 1951; and Cummins and Kimura, 1971). Although skin lesions were observed in mice at the site of Selsun® application and may be attributed to the test chemical, measurements for levels of urinary selenium were not performed in the study reported here.

Table 6. Incidences of Alveolar/Bronchiolar Carcinomas or Adenomas in Concurrent Bioassays of Selenium Sulfide and Selsun® in Mice

Sex and Strain	Substance	Initial Dose/Week (mg SeS, High Dose/ Low Dose)	Route of Adminis-ration	Tumor Incidence (Percent) Untreated Control	Vehicle Control	Low Dose	High Dose	Duration of Bioassay (Weeks)	Statistical Significance of Results
Male B6C3F1	SeS	14/2.8	Gavage	18	8	20	26	104	Positive (veh. control)
Female B6C3F1	SeS	14/2.8	Gavage	4	0	6	24	104	Positive (for high dose)
Male ICR Swiss	SeS	3/1.5	Dermal Application	6(a)	6	18	8	87	Negative
Female ICR Swiss	SeS	3/1.5	Dermal Application	18(a)	4	8	16	87	Dose-related trend (veh. control)
Male ICR Swiss	Selsun®	1.8/0.9	Dermal Application	6(a)	2	14	17	88	Positive (veh. control)
Female ICR Swiss	Selsun®	1.8/0.9	Dermal Application	18(a)	14	20	14	88	Negative

(a) Untreated ICR Swiss mice controls were shared by the Selsun® and the selenium sulfide dermal studies.

Table 7. Incidences of Hepatocellular Carcinomas in Concurrent Bioassays of Selenium Sulfide and Selsun® in the Rat and Mouse

Sex, Strain, Species	Substance	Initial Dose/Week (mg SeS High Dose/ Low Dose)	Route of Administration	Tumor Incidence (percent) Untreated Control	Vehicle Control	Low Dose	High Dose	Duration of Bioassay (Weeks)	Statistical Significance of Results
Male F344 Rat	SeS	7.5/1.5	Gavage	2	0	0	29	104	Positive
Female F344 Rat	SeS	7.5/1.5	Gavage	0	0	0	42	104	Positive
Male B6C3F1 Mouse	SeS	14/2.8	Gavage	35	30	22	46	104	Negative
Female B6C3F1 Mouse	SeS	14/2.8	Gavage	4	0	2	45	104	Positive
Male ICR Swiss Mouse	SeS	3.0/1.5	Dermal Application	8(a)	2	4	2	87	Negative
Female ICR Swiss Mouse	SeS	3.0/1.5	Dermal Application	0(a)	0	2	4	87	Negative
Male ICR Swiss Mouse	Selsun®	1.8/0.9	Dermal Application	8(a)	10	2	8	88	Negative
Female ICR Swiss Mouse	Selsun®	1.8/0.9	Dermal Application	0(a)	0	2	0	88	Negative

(a) Untreated ICR Swiss mice controls were shared by the Selsun® and the selenium sulfide dermal studies.

V. CONCLUSIONS

Under the conditions of this bioassay, dermal application of Selsun® was not carcinogenic for ICR Swiss mice. The study was limited, however, by the relatively short lifespan of this strain of mouse.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS
IN MICE ADMINISTERED SELSUN®
BY DERMAL APPLICATION

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED SELSUN® BY DERMAL APPLICATION

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS NECROPSIED	50	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50	50
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(50)	(49)	(50)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (6%)		7 (14%)	8 (17%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)		1 (2%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	2 (4%)	
*SKIN	(50)	(50)	(50)	(50)
MAST-CELL TUMOR			2 (4%)	
CIRCULATORY SYSTEM				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
HEMANGIOSARCOMA		1 (2%)		
#LIVER	(50)	(49)	(49)	(49)
HEMANGIOSARCOMA	1 (2%)			
DIGESTIVE SYSTEM				
#LIVER	(50)	(49)	(49)	(49)
HEPATOCELLULAR ADENOMA	3 (6%)	2 (4%)	1 (2%)	1 (2%)
HEPATOCELLULAR CARCINOMA	1 (2%)	3 (6%)		3 (6%)
URINARY SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM				
#THYROID	(30)	(30)	(36)	(39)
FOLLICULAR-CELL ADENOMA			1 (3%)	
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
TOE				
FIBROSARCOMA		1		
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	50	50	50	50
NATURAL DEATH@	31	29	24	28
MORIBUND SACRIFICE	13	16	14	12
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED	1			1
TERMINAL SACRIFICE	5	5	12	9
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	9	12	11
TOTAL PRIMARY TUMORS	8	9	13	13
TOTAL ANIMALS WITH BENIGN TUMORS	6	2	9	8
TOTAL BENIGN TUMORS	6	2	9	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	7	2	4
TOTAL MALIGNANT TUMORS	2	7	2	4
TOTAL ANIMALS WITH SECONDARY TUMORS#				
TOTAL SECONDARY TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			2	
TOTAL UNCERTAIN TUMORS			2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED SELSUN® BY DERMAL APPLICATION

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS NECROPSIED	50	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50	49
INTEGUMENTARY SYSTEM				
*SKIN	(50)	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA				1 (2%)
*SUBCUT TISSUE	(50)	(50)	(50)	(50)
NEUROFIBROSARCOMA			1 (2%)	
RESPIRATORY SYSTEM				
#LUNG	(49)	(49)	(49)	(49)
ADENOCARCINOMA, NOS, METASTATIC				1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	8 (16%)	7 (14%)	10 (20%)	7 (14%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)			
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	2 (4%)	2 (4%)	2 (4%)	2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)		
#SPLEEN	(49)	(47)	(45)	(48)
MALIGNANT LYMPHOMA, NOS	1 (2%)			
#KIDNEY	(50)	(49)	(50)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)		
#THYMUS	(6)	(1)	(3)	(8)
MALIGNANT LYMPHOMA, NOS	1 (17%)			
CIRCULATORY SYSTEM				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
HEMANGIOSARCOMA	1 (2%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#SPLEEN	(49)	(47)	(45)	(48)
HEMANGIOMA			1 (2%)	
DIGESTIVE SYSTEM				
#LIVER	(50)	(50)	(50)	(49)
HEPATOCELLULAR CARCINOMA				1 (2%)
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(39)	(36)	(39)	(41)
ADENOMA, NOS			1 (3%)	
#ADRENAL	(44)	(43)	(46)	(48)
PHEOCHROMOCYTOMA			1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(50)	(50)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	1 (2%)	1 (2%)
#UTERUS	(46)	(47)	(49)	(46)
LEIOMYOSARCOMA	1 (2%)	1 (2%)		1 (2%)
#OVARY	(39)	(41)	(45)	(44)
PAPILLARY CYSTADENOMA, NOS			1 (2%)	
GRANULOSA-CELL TUMOR		1 (2%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	50	50	50	50
NATURAL DEATHa	29	23	32	30
MORIBUND SACRIFICE	13	21	12	13
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED		1		
TERMINAL SACRIFICE	8	5	6	7
ANIMAL MISSING				

a INCLUDES AUTOLYZED ANIMALS

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	13	17	14
TOTAL PRIMARY TUMORS	15	14	18	14
TOTAL ANIMALS WITH BENIGN TUMORS	8	7	14	9
TOTAL BENIGN TUMORS	8	7	14	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	6	4	5
TOTAL MALIGNANT TUMORS	7	6	4	5
TOTAL ANIMALS WITH SECONDARY TUMORS#				1
TOTAL SECONDARY TUMORS				1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1		
TOTAL UNCERTAIN TUMORS		1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED SELSUN® BY DERMAL APPLICATION

TABLE B1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED SELSUN® BY DERMAL APPLICATION

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS NECROPSIED	50	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50	50
INTEGUMENTARY SYSTEM				
*SKIN	(50)	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)			
ULCER, NOS	1 (2%)			2 (4%)
ULCER, FOCAL				1 (2%)
INFLAMMATION, ACUTE	2 (4%)		1 (2%)	2 (4%)
ULCER, ACUTE	2 (4%)	1 (2%)		
INFLAMMATION, ACUTE FOCAL	2 (4%)			2 (4%)
INFLAMMATION, CHRONIC				1 (2%)
HYPERKERATOSIS	5 (10%)		14 (28%)	19 (38%)
ACANTHOSIS	4 (8%)	2 (4%)	15 (30%)	21 (42%)
RESPIRATORY SYSTEM				
#LUNG/BRONCHIOLE	(50)	(49)	(50)	(48)
INFLAMMATION, ACUTE FOCAL		1 (2%)		
#LUNG	(50)	(49)	(50)	(48)
PNEUMONIA, ASPIRATION			1 (2%)	
PNEUMONIA, CHRONIC MURINE		3 (6%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)		
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
HYPERPLASIA, PLASMA CELL	1 (2%)			
*SKIN	(50)	(50)	(50)	(50)
PARAKERATOSIS	1 (2%)		3 (6%)	5 (10%)
LIPOMATOSIS	1 (2%)			
#SPLEEN	(49)	(47)	(47)	(48)
AMYLOIDOSIS			1 (2%)	2 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIESIS		1 (2%)		
ERYTHROPOIESIS	6 (12%)	1 (2%)		
#MANDIBULAR L. NODE	(27)	(24)	(44)	(34)
HYPERPLASIA, PLASMA CELL	7 (26%)			1 (3%)
HYPERPLASIA, LYMPHOID	1 (4%)			
#PANCREATIC L.NODE	(27)	(24)	(44)	(34)
HYPERPLASIA, PLASMA CELL	1 (4%)			
#MESENTERIC L. NODE	(27)	(24)	(44)	(34)
CONGESTION, NOS		2 (8%)	3 (7%)	5 (15%)
EDEMA, NOS				1 (3%)
HYPERPLASIA, PLASMA CELL		1 (4%)		
HYPERPLASIA, LYMPHOID			1 (2%)	2 (6%)
CIRCULATORY SYSTEM				
#MESENTERIC L. NODE	(27)	(24)	(44)	(34)
THROMBUS, ORGANIZED			1 (2%)	
#HEART	(50)	(49)	(48)	(49)
ENDOCARDITIS, BACTERIAL			1 (2%)	1 (2%)
FIBROSIS, DIFFUSE	5 (10%)	11 (22%)	6 (13%)	12 (24%)
PERIARTERITIS			1 (2%)	
#HEART/ATRIUM	(50)	(49)	(48)	(49)
THROMBUS, MURAL	6 (12%)	9 (18%)	11 (23%)	6 (12%)
#MYOCARDIUM	(50)	(49)	(48)	(49)
INFLAMMATION, SUPPURATIVE		1 (2%)		
INFLAMMATION, ACUTE	1 (2%)			
INFLAMMATION, ACUTE FOCAL	1 (2%)		1 (2%)	
#ENDOCARDIUM	(50)	(49)	(48)	(49)
INFLAMMATION, CHRONIC FOCAL		1 (2%)		
#SALIVARY GLAND	(47)	(45)	(49)	(46)
THROMBOSIS, NOS			1 (2%)	
PERIVASCULITIS			1 (2%)	
#LIVER	(50)	(49)	(49)	(49)
THROMBOSIS, NOS				1 (2%)
#KIDNEY/GLOMERULUS	(50)	(49)	(50)	(49)
EMBOLUS, SEPTIC				1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM				
#LIVER	(50)	(49)	(49)	(49)
CYST, NOS			2 (4%)	
HEMATOMA, ORGANIZED				1 (2%)
INFLAMMATION, ACUTE		1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)	1 (2%)		
NECROSIS, FOCAL				1 (2%)
ANGIECTASIS				1 (2%)
#BILE DUCT	(50)	(49)	(49)	(49)
CYST, NOS			2 (4%)	
#PANCREAS	(45)	(47)	(47)	(49)
CYSTIC DUCTS		1 (2%)		1 (2%)
INFLAMMATION, INTERSTITIAL		1 (2%)		1 (2%)
INFLAMMATION, ACUTE		1 (2%)		
INFLAMMATION, CHRONIC		1 (2%)	1 (2%)	1 (2%)
NECROSIS, NOS			1 (2%)	
#ESOPHAGUS	(47)	(43)	(45)	(46)
HYPERKERATOSIS		1 (2%)		
#PERIESOPHAGEAL TISSU	(47)	(43)	(45)	(46)
INFLAMMATION, CHRONIC				1 (2%)
#GASTRIC MUCOSA	(49)	(45)	(48)	(46)
INFLAMMATION, ACUTE				1 (2%)
CALCIFICATION, NOS	1 (2%)			
#STOMACH WALL	(49)	(45)	(48)	(46)
INFLAMMATION, CHRONIC			1 (2%)	
#SMALL INTESTINE	(45)	(44)	(34)	(36)
AMYLOIDOSIS	1 (2%)			
#DUODENUM	(45)	(44)	(34)	(36)
AMYLOIDOSIS		1 (2%)		
#JEJUNUM	(45)	(44)	(34)	(36)
AMYLOIDOSIS	1 (2%)			
#ILEUM	(45)	(44)	(34)	(36)
AMYLOIDOSIS		1 (2%)	4 (12%)	4 (11%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#COLON	(42)	(44)	(36)	(39)
NEMATODIASIS		3 (7%)	2 (6%)	1 (3%)
URINARY SYSTEM				
#KIDNEY	(50)	(49)	(50)	(49)
HYDRONEPHROSIS	1 (2%)	3 (6%)		1 (2%)
PYELONEPHRITIS, NOS	1 (2%)			
INFLAMMATION, SUPPURATIVE	2 (4%)			1 (2%)
PYELONEPHRITIS SUPPURATIVE		3 (6%)	1 (2%)	2 (4%)
PYELONEPHRITIS, ACUTE	1 (2%)	2 (4%)		
INFLAMMATION, CHRONIC	1 (2%)	1 (2%)		
PYELONEPHRITIS, CHRONIC			1 (2%)	
INFLAMMATION, CHRONIC DIFFUSE	1 (2%)			
GLOMERULOSCLEROSIS, NOS	2 (4%)			
NECROSIS, MEDULLARY		1 (2%)		
AMYLOIDOSIS		4 (8%)		1 (2%)
#KIDNEY/CORTEX	(50)	(49)	(50)	(49)
CYST, NOS		1 (2%)		
#KIDNEY/PELVIS	(50)	(49)	(50)	(49)
INFLAMMATION, SUPPURATIVE		1 (2%)		
#URINARY BLADDER	(35)	(26)	(34)	(32)
ULCER, ACUTE				1 (3%)
INFLAMMATION, ACUTE NECROTIZING		1 (4%)		
INFLAMMATION, CHRONIC	1 (3%)	1 (4%)	1 (3%)	2 (6%)
#U.BLADDER/SUBMUCOSA	(35)	(26)	(34)	(32)
NECROSIS, FOCAL	1 (3%)			
ENDOCRINE SYSTEM				
#THYROID	(30)	(30)	(36)	(39)
CYSTIC FOLLICLES		4 (13%)		
REPRODUCTIVE SYSTEM				
*PENIS	(50)	(50)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#PROSTATE	(43)	(35)	(45)	(35)
INFLAMMATION, SUPPURATIVE	1 (2%)	4 (11%)	1 (2%)	4 (11%)
INFLAMMATION, ACUTE	1 (2%)	1 (3%)		
INFLAMMATION, CHRONIC			1 (2%)	
*SEMINAL VESICLE	(50)	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE				2 (4%)
#TESTIS/TUBULE	(49)	(48)	(50)	(50)
DEGENERATION, NOS		1 (2%)	1 (2%)	
CALCIFICATION, FOCAL		1 (2%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*MEDIASTINUM	(50)	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)			
*PERITONEUM	(50)	(50)	(50)	(50)
INFLAMMATION, ACUTE		1 (2%)		
*MESENTERY	(50)	(50)	(50)	(50)
ABSCESS, NOS	1 (2%)			
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
AMYLOIDOSIS	43 (86%)	36 (72%)	39 (78%)	36 (72%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
OMENTUM				
NECROSIS, FAT		1		
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	1			1
AUTO/NECROPSY/HISTO PERF	1			
AUTO/NECROPSY/NO HISTO		1		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED SELSUN® BY DERMAL APPLICATION

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS NECROPSIED	50	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50	49
INTEGUMENTARY SYSTEM				
*SKIN	(50)	(50)	(50)	(50)
INFLAMMATION, NOS				1 (2%)
INFLAMMATION, FOCAL			1 (2%)	4 (8%)
INFLAMMATION, ACUTE			3 (6%)	4 (8%)
INFLAMMATION, ACUTE FOCAL			1 (2%)	3 (6%)
INFLAMMATION, CHRONIC	1 (2%)	1 (2%)		1 (2%)
HYPERKERATOSIS	1 (2%)	1 (2%)	17 (34%)	23 (46%)
ACANTHOSIS		1 (2%)	21 (42%)	26 (52%)
RESPIRATORY SYSTEM				
#LUNG/BRONCHUS	(49)	(49)	(49)	(49)
INFLAMMATION, CHRONIC	1 (2%)			
#LUNG/BRONCHIOLE	(49)	(49)	(49)	(49)
INFLAMMATION, FOCAL		1 (2%)		
INFLAMMATION, CHRONIC		1 (2%)		
INFLAMMATION OBLITERATIVE				1 (2%)
#LUNG	(49)	(49)	(49)	(49)
BRONCHOPNEUMONIA, ACUTE	1 (2%)			
ABSCESS, NOS				2 (4%)
HEMATOPOIETIC SYSTEM				
*SKIN	(50)	(50)	(50)	(50)
PARAKERATOSIS			7 (14%)	2 (4%)
#BONE MARROW	(41)	(48)	(39)	(36)
GRANULOPOIESIS			1 (3%)	
#SPLEEN	(49)	(47)	(45)	(48)
AMYLOIDOSIS	1 (2%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIESIS	1 (2%)	1 (2%)	1 (2%)	1 (2%)
ERYTHROPOIESIS	8 (16%)	5 (11%)	5 (11%)	3 (6%)
#LYMPH NODE	(35)	(34)	(32)	(38)
HYPERPLASIA, PLASMA CELL	3 (9%)			
#MANDIBULAR L. NODE	(35)	(34)	(32)	(38)
HYPERPLASIA, PLASMA CELL	3 (9%)	1 (3%)		
#MEDIASTINAL L.NODE	(35)	(34)	(32)	(38)
INFLAMMATION, ACUTE		1 (3%)		
HYPERPLASIA, PLASMA CELL				1 (3%)
HYPERPLASIA, LYMPHOID		1 (3%)		
#PANCREATIC L.NODE	(35)	(34)	(32)	(38)
HYPERPLASIA, PLASMA CELL	1 (3%)			
#MESENTERIC L. NODE	(35)	(34)	(32)	(38)
CONGESTION, NOS		2 (6%)		2 (5%)
HEMORRHAGE	1 (3%)			
HYPERPLASIA, PLASMA CELL	1 (3%)			
HYPERPLASIA, LYMPHOID		1 (3%)		
#ADRENAL	(44)	(43)	(46)	(48)
HEMATOPOIESIS		1 (2%)		
CIRCULATORY SYSTEM				
#MEDIASTINAL L.NODE	(35)	(34)	(32)	(38)
THROMBUS, CANALIZED		1 (3%)		
#HEART	(49)	(50)	(50)	(49)
ENDOCARDITIS, BACTERIAL			3 (6%)	2 (4%)
FIBROSIS, DIFFUSE	7 (14%)	4 (8%)	5 (10%)	3 (6%)
#HEART/ATRIUM	(49)	(50)	(50)	(49)
THROMBUS, MURAL	3 (6%)	3 (6%)	2 (4%)	5 (10%)
#MYOCARDIUM	(49)	(50)	(50)	(49)
INFLAMMATION, ACUTE				1 (2%)
INFLAMMATION, ACUTE FOCAL		1 (2%)		
INFLAMMATION, ACUTE NECROTIZING		1 (2%)		
*PULMONARY ARTERY	(50)	(50)	(50)	(50)
THROMBOSIS, NOS			2 (4%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
CALCIFICATION, NOS		1 (2%)		
*UTERINE ARTERY	(50)	(50)	(50)	(50)
INFLAMMATION, ACUTE	1 (2%)			
NECROSIS, FIBRINOID			2 (4%)	
*PULMONARY VEIN	(50)	(50)	(50)	(50)
THROMBOSIS, NOS				3 (6%)
#UTERUS	(46)	(47)	(49)	(46)
THROMBOSIS, NOS		1 (2%)		
#OVARY	(39)	(41)	(45)	(44)
THROMBOSIS, NOS			1 (2%)	
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(47)	(43)	(46)	(44)
AMYLOIDOSIS				1 (2%)
#LIVER	(50)	(50)	(50)	(49)
CYST, NOS			2 (4%)	
MULTILOCULAR CYST	1 (2%)			
NECROSIS, NOS	1 (2%)	1 (2%)	1 (2%)	
NECROSIS, FOCAL		1 (2%)		1 (2%)
BASOPHILIC CYTO CHANGE				1 (2%)
*GALLBLADDER	(50)	(50)	(50)	(50)
INFLAMMATION, ACUTE	1 (2%)			
#BILE DUCT	(50)	(50)	(50)	(49)
CYST, NOS	1 (2%)		1 (2%)	
#PANCREAS	(46)	(47)	(43)	(47)
DILATATION/DUCTS			1 (2%)	
INFLAMMATION, ACUTE				1 (2%)
INFLAMMATION, CHRONIC			1 (2%)	
NECROSIS, FOCAL				1 (2%)
#PERIPANCREATIC TISSU	(46)	(47)	(43)	(47)
NECROSIS, FAT				1 (2%)
#ESOPHAGUS	(44)	(45)	(43)	(47)
INFLAMMATION, ACUTE	1 (2%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#PERIESOPHAGEAL TISSU	(44)	(45)	(43)	(47)
INFLAMMATION, ACUTE			1 (2%)	1 (2%)
#STOMACH	(50)	(46)	(43)	(49)
INFLAMMATION, CHRONIC			1 (2%)	
METAPLASIA, SQUAMOUS			1 (2%)	
#GASTRIC MUCOSA	(50)	(46)	(43)	(49)
CALCIFICATION, NOS				1 (2%)
#JEJUNUM	(39)	(39)	(36)	(41)
AMYLOIDOSIS	1 (3%)			
#ILEUM	(39)	(39)	(36)	(41)
AMYLOIDOSIS	2 (5%)	1 (3%)	3 (8%)	5 (12%)
#COLON	(37)	(38)	(34)	(43)
INFLAMMATION, ACUTE FOCAL		1 (3%)		
NEMATODIASIS		2 (5%)	3 (9%)	2 (5%)
URINARY SYSTEM				
#KIDNEY	(50)	(49)	(50)	(49)
PYELONEPHRITIS SUPPURATIVE			1 (2%)	
GLOMERULONEPHRITIS, ACUTE	1 (2%)			
PYELONEPHRITIS, ACUTE	2 (4%)			
INFLAMMATION, CHRONIC		1 (2%)	1 (2%)	1 (2%)
PYELONEPHRITIS, CHRONIC	1 (2%)	3 (6%)		
INFLAMMATION, CHRONIC DIFFUSE	1 (2%)			
SCLEROSIS				1 (2%)
GLOMERULOSCLEROSIS, NOS	1 (2%)			1 (2%)
AMYLOIDOSIS		1 (2%)	1 (2%)	1 (2%)
INCLUSION, NUCLEAR		1 (2%)		
#KIDNEY/CORTEX	(50)	(49)	(50)	(49)
CYST, NOS		1 (2%)		
SCAR			1 (2%)	1 (2%)
#KIDNEY/TUBULE	(50)	(49)	(50)	(49)
CALCIFICATION, NOS	1 (2%)			
ENDOCRINE SYSTEM				
#THYROID	(39)	(39)	(32)	(36)
CYSTIC FOLLICLES		2 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM				
#UTERUS	(46)	(47)	(49)	(46)
HYDROMETRA		1 (2%)		
CYST, NOS	1 (2%)			
HEMORRHAGE		1 (2%)	1 (2%)	
ANGIECTASIS				2 (4%)
#UTERUS/ENDOMETRIUM	(46)	(47)	(49)	(46)
INFLAMMATION, SUPPURATIVE	1 (2%)		2 (4%)	
HYPERPLASIA, CYSTIC	25 (54%)	35 (74%)	33 (67%)	36 (78%)
#OVARY/OVIDUCT	(46)	(47)	(49)	(46)
INFLAMMATION, SUPPURATIVE			1 (2%)	
#OVARY	(39)	(41)	(45)	(44)
CYST, NOS	3 (8%)	5 (12%)	4 (9%)	11 (25%)
HEMORRHAGE			1 (2%)	
HEMORRHAGIC CYST	1 (3%)		3 (7%)	1 (2%)
AMYLOIDOSIS			1 (2%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(50)	(50)	(50)	(50)
NECROSIS, FAT			1 (2%)	
*PERICARDIUM	(50)	(50)	(50)	(50)
INFLAMMATION, FIBRINOUS				1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC		1 (2%)		1 (2%)
INFLAMMATION, CHRONIC FOCAL				1 (2%)
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
AMYLOIDOSIS	39 (78%)	41 (82%)	40 (80%)	37 (74%)
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED		1	1	
AUTO/NECROPSY/HISTO PERF	3	1		
AUTO/NECROPSY/NO HISTO				1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX C

ANALYSIS OF THE CARBON DISULFIDE EXTRACT OF SELSUN®

MIDWEST RESEARCH INSTITUTE

APPENDIX C

Analysis of the Carbon Disulfide Extract of Selsun®
Midwest Research Institute

X-Ray Diffraction

Instrument: Debye-Scherrer camera fitted with filtered copper radiation, 50 kv, and 30 mamp.

Procedure: About 3 ml of a test solution of the Selsun® used in the bioassay was extracted with 50 ml of carbon disulfide. The extract was allowed to evaporate in a beaker covered with aluminum foil. The resulting orange-colored needles were used for the X-ray analysis. Intensities were recorded as approximations expressed in terms varying from "very weak" to "very strong." Only two literature references could be found that gave d-spacings for selenium monosulfide (Smith, 1960; Virodov, 1964), and none could be found for the disulfide. However, the approximations of intensities at different d values, as observed for the test material used in the bioassay, were consistent with the numerical values of intensities given in the literature for selenium monosulfide. Literature values and those observed in the current analysis are presented in Table C-1.

Table C1. X-Ray Diffraction Values

Literature Values(a)		Values Found(b)	
d	intensity	d	intensity
6.67	10	6.78-6.18	band
6.28	20		
5.13	10	5.11	very weak
4.42	50	4.37	medium
		4.16	very weak
3.77	100	3.75	very strong
3.70	50		
3.54	50	3.51	medium
3.34	10	3.35	weak +
3.22	60	3.21	medium
3.14	40	3.11	medium -
3.06	30	3.04	weak +
2.97	10	2.95	weak
2.78	10	2.76	weak
2.63	20	2.63	weak +
2.58	10	2.56	weak
2.52	40	2.51	medium
2.44	10	2.43	very weak
2.24	20	2.24	weak +
		2.14-2.09	band
2.01	20	2.01	weak +
1.97	10	1.97	weak
1.92	10		
1.89	20	1.89	weak
1.83	10	1.83	weak -
1.78	30	1.79	weak +
1.74	10	1.74	very weak
1.71	20	1.71	weak
1.66	20	1.66	weak
1.63	20	1.63	weak
1.57	20	1.57	weak
1.53	10	1.53	weak
1.48	10	1.48	very weak
1.46	10	1.46	very weak

(a) Reported in Smith (1960) and Virodov (1964).
(b) The approximations of intensities at different d values, as observed for the test material used in the bioassay, were consistent with the numerical values of intensities given in the literature for selenium monosulfide.

APPENDIX D

ANALYSIS OF SELSUN® SOLUTION

APPENDIX D

METHOD OF ANALYSIS OF SELSUN SOLUTION

The entire sample of Selsun® was extracted with 25 ml of carbon disulfide three times. The extracts were combined and a 30-ml aliquot was taken to dryness using a flash evaporator. A 5-ml concentrated nitric acid solution was added to the residue and the acid was heated until no more brown gases evolved. At this point the solution was clear. The digest was transferred to a volumetric flask with distilled water and the volume was adjusted to the mark. An analytical standard was prepared using a known amount of selenium sulfide. These known weights of selenium sulfide were dissolved in carbon disulfide and were taken through the above procedure. The above samples, including the control, were analyzed for selenium using atomic absorption. Duplicate assays were not performed.

Review of the Bioassay of Selsun® * for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

February 15, 1980

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of Selsun® for carcinogenicity.

The primary reviewer for the report on the bioassay of Selsun®, agreed with the conclusion that the compound was not carcinogenic, under the conditions of test. The reviewer briefly described the experimental design and conditions of test. The relatively short lifespan of the mice may have been an experimental shortcoming, although the strain was selected because of its supposed sensitivity. The reviewer suggested that this point be more strongly made in the report. Also, some comment should be made as to whether Selsun® shortened the animals' natural lifespan. The reviewer opined that the dosages applied were justified, based on the toxicity at higher concentrations.

The secondary reviewer thought that the maximum tolerated doses were not achieved and, therefore, the study was inadequate from this standpoint. However, he agreed that the report accurately reflected what occurred.

The primary reviewer moved that the report on the bioassay of Selsun® be accepted as written. The motion was seconded and approved.

Members present were:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
David B. Clayson, Eppley Institute for Research in Cancer
Joseph Highland, Environmental Defense Fund
William Lijinsky, Federick Cancer Research Center
Henry C. Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Louise Strong, University of Texas Health Sciences Center

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

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