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BIOASSAY OF SELENIUM SULFIDE FOR POSSIBLE CARCINOGENICITY (Dermal Study)

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FOREWORD

This report presents the results of the bioassay of selenium sulfide 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 conditions. 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 selenium sulfide 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 of mice in the subchronic study were performed by Dr. D. A. Banas (4) and reviewed by Dr. R. W. Voelker (4), and the histopathologic examinations in 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 (8). 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 (9).

Chemicals used in this bioassay were analyzed at Midwest Research Institute (10), 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 (1,11).

This report was prepared at Tracor Jitco (1) 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. 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. Harry A. Milman, Dr. Thomas W. Orme, Dr. A. R. Patel (12), Dr. Marcelina B. Powers, Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

Selenium is an essential nutrient, and various selenium compounds have industrial and medical uses.

The possible carcinogenicity of selenium sulfide (a component in shampoos) was investigated by applying a suspension of this substance to the skin of ICR Swiss mice. Groups of 50 mice of each sex were treated by applying 0.5 mg or 1.0 mg selenium sulfide three times a week for 86 weeks to a clipped 2- x 3-cm dorsal surface. The selenium sulfide was suspended in a 0.05 ml saline solution containing 0.5% carboxymethylcellulose.

Mean body weights of all dosed and control groups were comparable throughout the study. Amyloidosis, previously reported as a cause of death in Swiss mice, was a factor in the deaths of most treated and control mice after 1 year, and the study was terminated after 88 weeks when the majority of animals in all dosed and control groups had died.

Under the conditions of this bioassay, dermal application of selenium sulfide did not produce a carcinogenic effect in ICR Swiss mice, but the study was limited by the relatively short lifespan of this strain of mouse.

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I. INTRODUCTION

SeS

SELENIUM SULFIDE

Selenium sulfide (CAS 7446-34-6; NCI C50033) is used in the treatment of seborrheic dermatitis, seborrheic sicca (dandruff), and tinea versicolor (Rook et al., 1972; Swinyard, 1975; AMA Dept. of Drugs, 1977). Selenium sulfide is present in some shampoos sold over the counter at concentrations of 1% and in some prescription shampoos at a concentration of 2.5% (Physicians' Desk Reference, 1977). The shampoos are applied once or twice a week, left in contact with the skin for 2 to 3 minutes, rinsed, and then applied a second time for a similar time period. Selenium sulfide shampoos are also used on dogs as cleansing agents and for the removal of skin debris associated with eczema or superficial dermatoses (Federal Register, 1978). Residues of selenium sulfide remain on the scalp after rinsing (AMA Dept. of Drugs, 1977).

^{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). Sodium selenate and selenite have been used in animal feeds to prevent selenium deficiency diseases in livestock and poultry (Federal Register, 1974).

Selenium and its compounds are used industrially in the manufacture of glass; in electronic rectifiers; in photoelectric cells; as a constituent in 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).

Two hundred kilograms of selenium sulfide are produced annually for use as an antidandruff agent (IARC, 1975), and 160,000 kg of waste from the medicinal industry, containing 320 kg selenium sulfide, are generated annually (EPA, 1976). Production of other selenium compounds in the United States is estimated at 1 million kilograms per year (Stone, 1973).

The oral LD_{50} of selenium sulfide in male Sprague-Dawley rats is 138 mg/kg body weight, whereas 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. The insolubility of selenium sulfide in water has been suggested as the reason that the acute oral toxicity of selenium sulfide is lower than that of sodium selenite and selenate (Cummins and Kimura, 1971). Peak particle size in the Henschler and Kirschner study was 5-15 μ compared with 5-10 μ in the Cummins and Kimura study.

Some shampoo formulations containing selenium sulfide incorporated with wetting agents, sequestrants, a fungicide, and other ingredients (Physicians' Desk Reference, 1977) have been reported to 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) respectively. 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).

An antimitotic mechanism of action is suggested by data showing that selenium sulfide decreases the rate of incorporation of radioactive-labeled thymidine into the DNA of dermal epithelial cells of human scalps (Plewig and Kligman, 1969).

Mutagenicity tests have been performed with sodium selenite (+4 oxidation state) and sodium selenate (+6 oxidation state). Sodium selenite was not mutagenic in Salmonella typhimurium test systems, but sodium selenate was (Lofroth and Ames, 1978). At doses varying from $8x10^{-5}$ to $3x10^{-3}$ M, sodium selenite induced DNA fragmentation, DNA-repair synthesis, chromosome aberrations, and mitotic inhibition in cultured human fibroblasts (Lo et al., 1978). Sodium selenite produced more chromosomal breaks in cultured human leukocytes than did sodium selenate, and in recombinant assays with two strains of Bacillus subtilis selenite induced more DNA damage than did selenate (Nakamuro et al., 1976).

Sodium selenite has been widely used in media to culture Salmonella. Data on the possible teratogenic effects on fetuses of pregnant laboratory workers handling sodium selenite as an ingredient in culture media for Salmonella are presented by Robertson (1970).

Selenium is an essential nutritional trace element for several species. In rats, the threshold for selenium deficiency disease is 10 ng/kg (National Academy of Sciences, 1976). In man, three enzyme catalyzed oxidation-reduction reactions have been shown to require selenium. When organisms

receive higher concentrations of selenium than is normally required, the excess selenium replaces sulfur in many cellular constituents and thus interferes with cellular metabolism (Stadtman, 1974).

A dermal route of administration was selected for testing selenium sulfide so that the test results could be compared with those from other dermal tests of a commercial shampoo formulation containing this ingredient. These other tests were conducted under identical protocols and were reported separately (NCI, 1980a). Also reported separately is a bioassay of selenium sulfide administered by gavage (NCI, 1980).

II. MATERIALS AND METHODS

A. Chemical

Selenium sulfide is a bright orange powder that is insoluble in water. The selenium sulfide used in the bioassay was obtained as a single batch (Lot No. 47E204) from City Chemical Corporation (New York, N. Y.). Analyses performed at Midwest Research Institute included elemental analysis, melting point, and X-ray diffraction (Appendix C). The results of elemental analyses are consistent with a mixture of selenium mono and disulfides, or a mixture of selenium monosulfide, selenium, and sulfur. However, the melting point of the test sample, 115° to 117°C, was nearer to the 118° to 119°C reported for the monosulfide (Weast, 1974-1975) than to the 100°C reported for the disulfide. The X-ray diffraction patterns were consistent with those reported for selenium monosulfide (Smith, 1960; Virodov, 1964). The test material is referred to in this report by the common name, selenium sulfide.

B. Dosage Preparation and Administration

The test material was stored in a glass container at 4°C for the duration of the bioassay. Selenium sulfide was suspended in 0.5% sodium carboxymethylcellulose (Sigma Chemicals, St. Louis, Mo.) in saline and mixed in a tissue grinder. Stock suspensions, prepared weekly and stored at 4°C, were stirred continuously with a magnetic stirring bar before and during the animal dosing procedures to ensure that the chemical remained in suspension. The particle size distribution was not determined.

Test suspensions selected at two or three monthly intervals were analyzed by atomic absorption for correctness of dose level as described in Appendix D. The mean concentration of nine samples having a theoretical value of 20 mg/ml was 18.9±2.9. The coefficient of variation was 15.3%, and the range was 14.8 to 23.0 mg/ml.

The stability of the test compound in the vehicle (0.5% carboxymethylcellulose in saline) was studied by X-ray diffraction. The procedures used

to determine the effect of the vehicle on the selenium sulfide in the mixture are described in Appendix E. Various suspensions of the chemical were prepared as described and then extracted and analyzed. X-ray diffraction patterns from all samples had similar d spacings and the same major line, but the relative intensities of the lines differed from sample to sample. The major band in all samples corresponded with that of selenium sulfide (Se_4S_4; empirical formula SeS). The variations in the line intensities obtained indicate that the samples may have contained varying amounts of selenium and sulfur molecular species in addition to selenium sulfide.

Test animals and vehicle controls were clipped weekly to expose a 2- x 3-cm dorsal surface. Test suspensions were applied to the skin via an automatic pipette (Cornwall, Becton Dickinson) and spread evenly over the surface with a glass rod.

C. Animals

Male and female ICR Swiss mice were obtained from Charles River Breeding Laboratories (Wilmington, Mass.) at 5 weeks of age. Upon receipt, the mice were acclimated for 2 weeks before being assigned to dosed or control groups.

D. Animal Maintenance

Mice were housed in a room with the temperature maintained at 22°C to 24°C and the relative humidity at 45% to 55%. The air handling system was a single pass-through system that provided 7 to 10 changes of room air per hour. Exhaust 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). The cages were suspended in 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. 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 using Acclaim® detergent (Economics Laboratory, St. Paul, Minn.).

In addition to the dermal study of selenium sulfide, a similar study on Selsun was conducted concurrently in the same room. All controls for these tests were housed in this room. Untreated controls, but not vehicle controls, were shared between the two tests.

E. Subchronic Studies

In 90-day subchronic tests performed to establish the doses of selenium sulfide to be used in the chronic study, groups of 10 male and female mice were treated 5 times per week with the vehicle or with 1, 5, 10, 25, or 50 mg of selenium sulfide suspended in 0.1 ml of 0.5% sodium carboxymethylcellulose in saline. Untreated controls and treated mice were weighed weekly and observed daily for mortality, skin irritation, and other toxic signs. After 13 weeks, all survivors were killed by cervical dislocation. Necropsies were performed on all animals, and representative tissues, including the site of application to the skin, were examined. Doses administered, survival of animals in each dosed group at the end of the study, and changes in mean body weights of dosed groups relative to those of controls as of week 13, are shown in Table 1.

Most of the mice given daily applications of 1, 5, or 10 mg developed local redness and irritation at the test site after 39, 14, and 14 applications, respectively, but these conditions were not severe enough to require discontinuation of the treatment. All these animals survived to the end of the test. Mice given 25 or 50 mg displayed arched spines, cyanosis, initial weight loss, tremors, and rough hair coats within the first week. They were also less active than controls. Deaths occurred in three males and five females given 25 mg and in all females and eight males given 50 mg. Because of this mortality and severe skin damage at the site of application, testing was discontinued in the 25- and 50-mg groups after 17 applications.

Skin sections were histologically normal in the mice that received 17 applications of 25 or 50 mg and that survived to the end of the test period. Slight acanthosis and hyperkeratosis were observed histologically in a skin section from one male that died following treatment with 25 mg. The alterations of the skin observed in histologic preparations that were attributed

Table 1. Doses, Survival, and Mean Body Weights of Mice Following Dermal Exposure to Selenium Sulfide for 90 Days

Dose(a) (mg)	Survival(b)	Mean Body Weight (grams) Initial	Final	Gain	Weight Change Relative to Controls (%)
Males					
0 (c)	10/10	28.9	35.4	6.5	
1	10/10	28.2	35.7	7.5	+15
5	10/10	28.8	38.1	9.3	+43
10	10/10	28.4	37.7	9.3	+45
25 (d)	7/10	29.9	37.7	7.8	+20
50 (d)	2/10	28.5	37.5	9.0	+38
Females					
0 (c)	10/10	24.1	30.8	6.7	
1	10/10	23.9	31.0	7.1	+6
5	10/10	24.6	32.8	8.2	+22
10	10/10	25.0	33.1	8.1	+21
25 (d)	5/10	23.5	32.5	9.0	+34
50 (d)	0/10	23.1	-	-	-

(a) Compound was administered in 0.1 ml saline containing 0.5% sodium carboxymethylcellulose, five times per week.
(b) Number surviving/number in group.
(c) Vehicle controls received 0.1 ml saline containing 0.5% sodium carboxymethylcellulose, five times per week.
(d) Treatment discontinued after 17 applications.

to the application of 5 or 10 mg selenium sulfide consisted of minimal to moderate acanthosis in 80% to 90% of both male and female mice and occasional scattered foci of inflammatory cells. Skin sections were normal from the group receiving 1 mg.

Focal coagulation necrosis of the liver was observed in one animal given 5 mg, in one given 10 mg, in two of the eight survivors given 25 mg, and in one of the two survivors given 50 mg. Focal calcification of the liver occurred in one female given 5 mg. Incidental lesions in the livers included microgranulomas and minimal nonsuppurative pericholangitis. Except for the animals given the 1-mg dose, all treatment groups had an increased incidence and severity of interstitial nephritis when compared with controls.

Dose levels of 0.5 and 1 mg selenium sulfide were selected for the chronic dermal study.

F. Chronic Study

The number of animals per group, doses administered, and duration of the chronic study are shown in Table 2. This study was terminated at 86 weeks because of poor survival in the dosed and control groups.

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 by intraperitoneal injections of 0.3 to 0.5 ml Diabutal® containing 60 mg/kg sodium pentobarbital (Diamond Laboratories, Inc., Des Moines, Iowa) and necropsied.

Gross and microscopic examinations were performed on major tissues and organs and on all gross lesions from killed animals. 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, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, pancreas, stomach, small

Table 2. Design of the Selenium Sulfide Chronic Dermal Study in Mice

Sex and Test Group	Initial No. of Animals	Selenium Sulfide Dose (mg/day)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Males				
Untreated Control(a)	50	0	0	83
Vehicle Control (b)	50	0	86	2
Low Dose (c)	50	0.5	86	2
High Dose (c)	50	1	86	2
Females				
Untreated Control(a)	50	0	0	87
Vehicle Control (b)	50	0	86	2
Low Dose (c)	50	0.5	86	2
High Dose (c)	50	1	86	2

(a) Untreated controls were clipped weekly.
(b) Vehicle controls were clipped weekly and dosed three times per week with 0.05 ml of saline containing 0.5% carboxymethylcellulose.
(c) Doses were administered in 0.05 ml of saline containing 0.5% carboxymethylcellulose, three times per week.

intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, and brain.

Necropsies were also 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 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 (e.g., skin or mammary tumors) before histologic sampling 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. When results for two dosed groups are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 is made. The Bonferroni inequality (Miller, 1966) was used. When two comparisons are made, this method requires that the P value for any comparison be less than or equal to 0.05/2 (0.025).

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. When a linear trend is assumed, 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 weights of dosed and control animals were similar throughout the study (Figure 1). Redness and irritation were present at the treatment site in 50% of the male and 10% of the female mice in the low-dose group and in 50% of the male and 50% of the female mice in the high-dose group. A few dorsal growths were observed clinically in the high-dose mice, and none were seen in the low-dose mice. The lesions were examined histopathologically, but no diagnoses of neoplasms were made. Scaling and the formation of scar tissue that occurred on the dorsal skin in low- and high-dose mice were also evident in untreated and vehicle controls and were believed to be caused by scratching. A dark deposit was visible on the skin of the dosed animals.

B. Survival

Estimates of the probabilities of survival for male and female mice administered selenium sulfide dermally at the doses of this bioassay, together with those of the vehicle and untreated controls, are shown by the Kaplan and Meier curves in Figure 2. The untreated-control group is not included in the statistical analysis because the test condition of the vehicle controls resembles more closely that of the dosed groups. The result of the Tarone test for dose-related trend in mortality is not significant in either sex.

In male mice, 43/50 (86%) of the high-dose group, 41/50 (82%) of the low-dose group, and 44/50 (88%) of the vehicle-control group were still alive at 52 weeks on study. In females, 42/50 (84%) of the high-dose group, 41/50 (82%) of the low-dose group, and 40/50 (80%) of the vehicle-control group were still alive at 52 weeks on study. Survival declined rapidly after 52 weeks. The study was terminated at 86-88 weeks because so few animals remained.

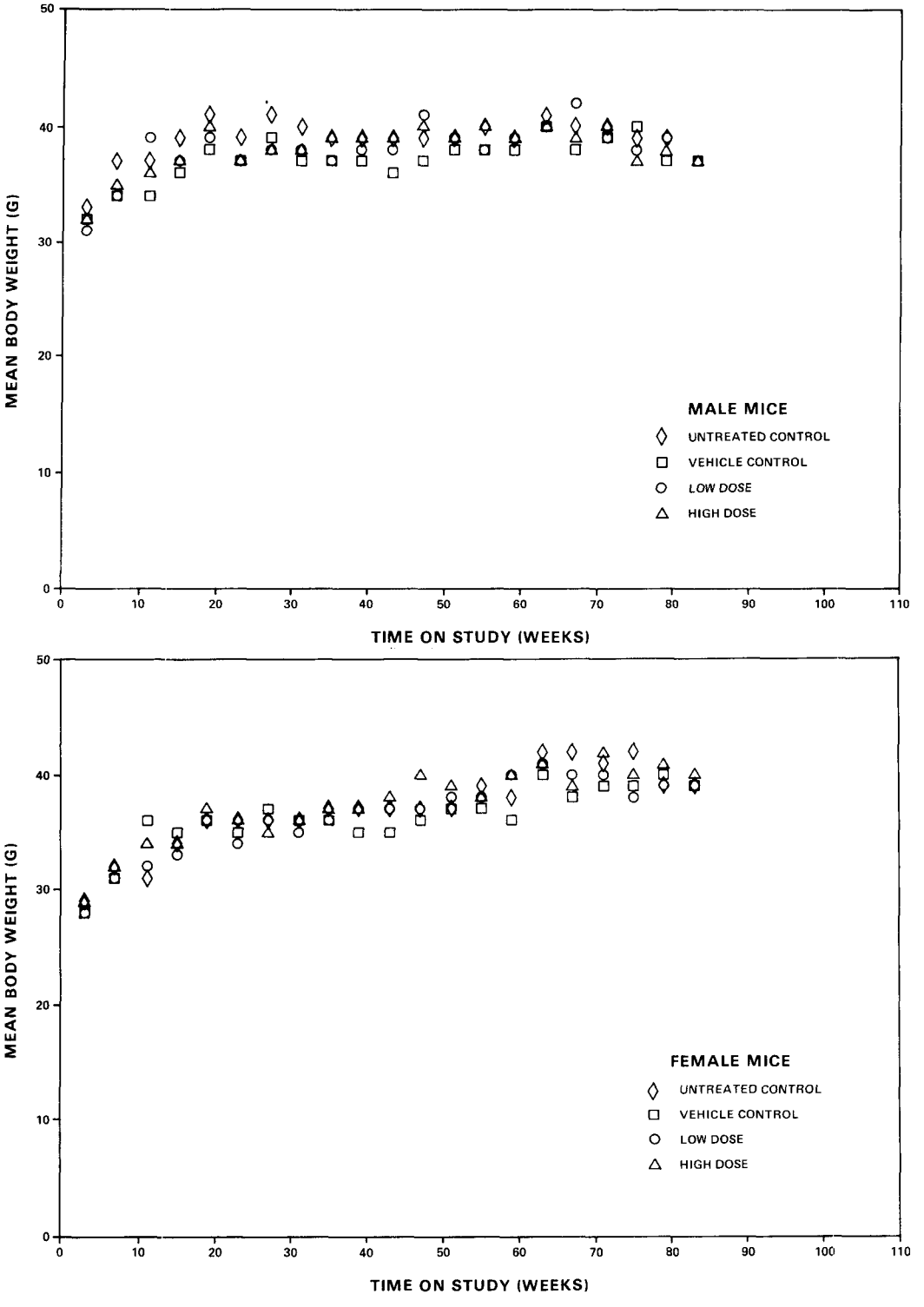

Figure 1. Growth Curves for Mice Administered Selenium Sulfide by Dermal Application

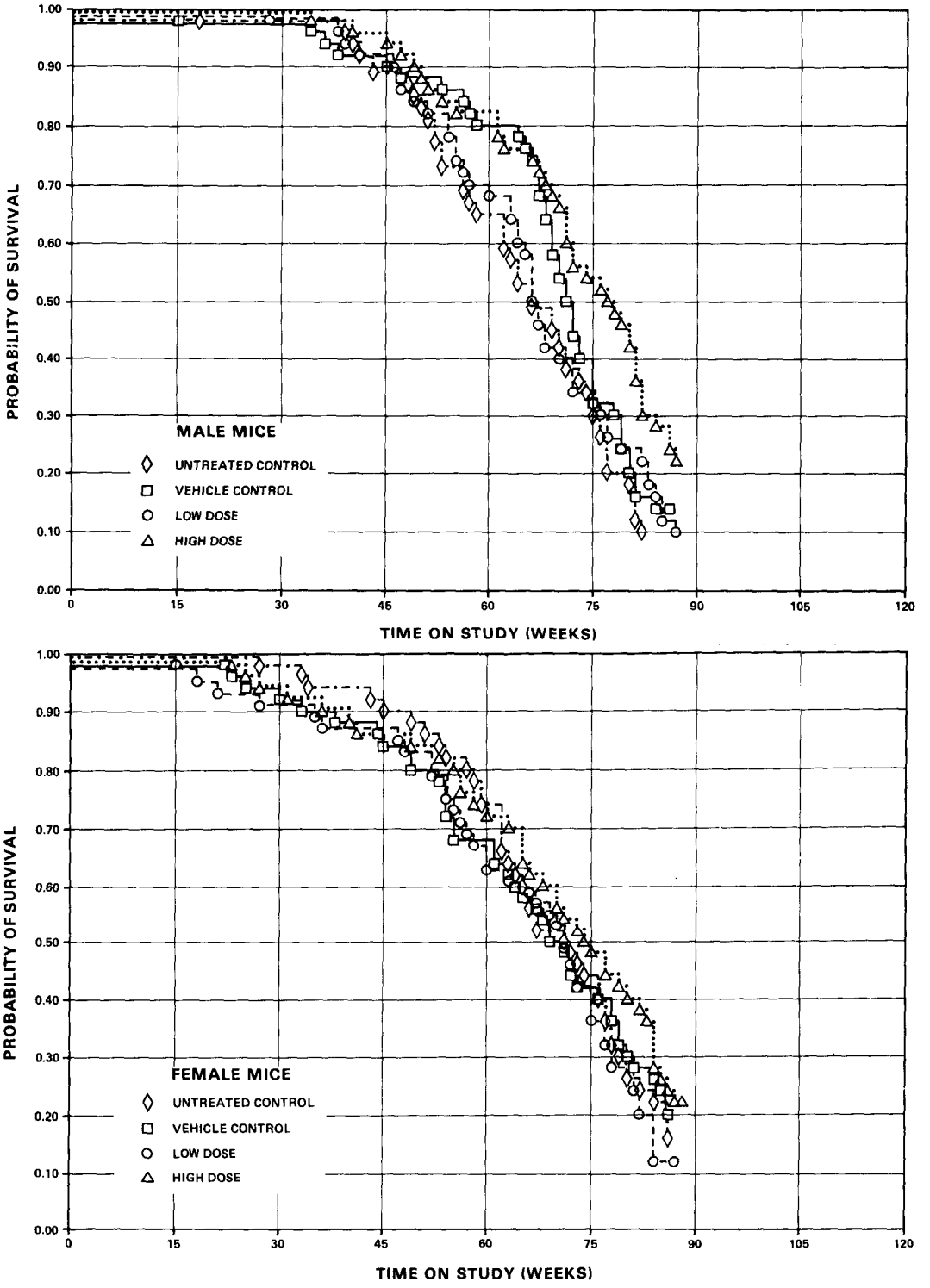

Figure 2. Survival Curves for Mice Administered Selenium Sulfide by Dermal Application

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. The tumors, which were those commonly seen in mice of this strain, occurred in comparable numbers in both control and dosed mice.

A variety of nonneoplastic lesions were seen in similar incidences in control and dosed male and female mice, except for those of the skin. At the site of application, acanthosis was seen in 20% to 30% of the mice in all dosed groups. The incidence in control groups was 0% to 8%. Some mice also had hyperkeratosis and acute and chronic inflammatory skin lesions. The high mortality in control and dosed mice appeared to be a result of generalized amyloidosis, especially involving the liver, kidney, and spleen.

Histopathologic examination provided no evidence that selenium sulfide was carcinogenic in ICR Swiss mice when applied to the skin under the conditions of this study.

D. Statistical Analyses of Results

Tables 3 and 4 contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and with an incidence of at least 5% in one or more groups. The untreated-control group is not included in the tables of statistical analysis because the test condition of the vehicle-control group resembles more closely that of the dosed groups. With one exception, incidences of untreated controls with tumors at specific sites did not differ from those in the vehicle controls. The incidence of alveolar/bronchiolar adenomas and carcinomas in female mice was 9/49 (18%) in the untreated controls compared with 2/50 (4%) in the vehicle-control group.

In male mice, the results are not significant for the Cochran-Armitage test for dose-related trend in incidences of tumors and for the Fisher exact test comparing the incidences of tumors in the control group with those in each dosed group.

According to the Cochran-Armitage test, the incidence of female mice with either alveolar/bronchiolar carcinomas or adenomas is significant (P=0.028). Tumors were detected as early as the 25th week of the study in high-dose female mice. The Fisher exact test, comparing the incidence in the high-dose group with that in the vehicle-control group, shows a P value of 0.043. This value is above the 0.025 level of significance for an overall significance level of P=0.05.

The result of the Cochran-Armitage test for the incidence of female mice with either hemangiomas or hemangiosarcomas is significant (P=0.026), but the results of the Fisher exact test are not. The untreated-control group exhibited an incidence of 1/50 (2%).

In each of the 95% confidence intervals for relative risk shown in the tables, one is included; this inclusion indicates the absence of significant positive results. It should also be noted that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of tumor induction by selenium sulfide, which could not be detected under the conditions of this test.

Table 3. Analyses of the Incidence of Primary Tumors in Male Mice Administered Selenium Sulfide by Dermal Application (a)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	0/48 (0)	3/49 (6)	0/48 (0)
P Values (c)	N.S.	N.S.	--
Departure from Linear Trend (d)	P=0.014	P=0.014	
Relative Risk (e)		Infinite	--
Lower Limit		0.590	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	46	--
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	3/48 (6)	9/49 (18)	4/48 (8)
P Values (c)	N.S.	N.S.	N.S.
Departure from Linear Trend (d)	P=0.044		
Relative Risk (e)		2.939	1.333
Lower Limit		0.789	0.238
Upper Limit		15.979	8.665
Weeks to First Observed Tumor	69	46	72

(a) Dosed groups received doses of 0.5 or 1.0 mg, 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) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.
(e) The 95% confidence interval of the relative risk between each dosed group and the control group.

Table 4. Analyses of the Incidence of Primary Tumors in Female Mice Administered Selenium Sulfide by Dermal Application (a)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	2/50 (4)	4/49 (8)	8/49 (16)
P Values (c)	P=0.028	N.S.	P=0.043
Relative Risk (d)		2.041	4.082
Lower Limit		0.308	0.868
Upper Limit		21.737	37.876
Weeks to First Observed Tumor	86	66	25
Hematopoietic System: Lymphoma or Leukemia (b)	5/50 (10)	5/50 (10)	3/50 (6)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (d)		1.000	0.600
Lower Limit		0.245	0.098
Upper Limit		4.082	2.910
Weeks to First Observed Tumor	30	36	84
All Sites: Hemangioma or Hemangiosarcoma (b)	0/50 (0)	1/50 (2)	4/50 (8)
P Values (c)	P=0.026	N.S.	N.S.
Relative Risk (d)		Infinite	Infinite
Lower Limit		0.054	0.927
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	87	63

Table 4. Analyses of the Incidence of Primary Tumors in Female Mice Administered Selenium Sulfide by Dermal Application (a)

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Pituitary: Carcinoma, NOS or Adenoma, NOS (b)	2/40 (5)	0/36 (0)	0/36 (0)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (d)		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		3.719	3.719
Weeks to First Observed Tumor	86	--	--

(a) Dosed groups received doses of 0.5 or 1.0 mg, 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) The 95% confidence interval of the relative risk between each dosed group and the control group.

IV. DISCUSSION

The study was terminated at 86-88 weeks because of the low survival of the control and the treated ICR Swiss white mice. This low survival is attributed to the high incidence of amyloidosis. Amyloidosis as a cause of early death in ICR mice has been reported by Homburger, et al. (1975). The similarity of mean body weights in the low- and high-dose groups, the untreated controls, and the vehicle controls, as well as the lack of other life-threatening or dose-related lesions, suggest that the treated animals may have been able to tolerate exposure to greater amounts of the test substance; however, higher doses induced severe skin irritation in the subchronic studies.

In a dermal bioassay conducted concurrently in the same room, 50 ICR Swiss mice of either sex received doses of diluted Selsun® containing a total of 1.8 or 0.9 mg selenium sulfide per week (NCI, 1980a). In the present study, the mice received a total of 3.0 or 1.5 mg selenium sulfide per week. Low survival (comparable with the present study) was also observed in all groups of ICR Swiss mice in the Selsun® study.

In the subchronic 90-day dermal toxicity study in ICR Swiss mice, slight but detectable effects were found in both liver and kidney tissues of all dosed groups, except those administered 1 mg. There was also an increase in the incidence and severity of interstitial nephritis in all treatment groups compared with controls. Although these results suggest that some selenium sulfide was percutaneouly absorbed, similar effects were not observed at the end of the chronic study.

There was no statistically significant evidence of carcinogenicity in male mice. According to the Cochran-Armitage test and the Fisher exact test, tumor incidences were not significant.

In female mice, alveolar/bronchiolar carcinomas or adenomas occurred with a dose-related trend (P=0.028). The result of the Fisher exact test comparing incidence in the high-dose group with that in the vehicle-control group was a P value of 0.043. This value is greater than the 0.025 level required for overall significance by the multiple comparison criterion. The

incidence of alveolar/bronchiolar carcinomas or adenomas in the female untreated-control group was higher than the incidence in either the vehicle-control or the high-dose group.

Hemangiomas or hemangiosarcomas occurred in female mice with a dose-related trend that is significant (P=0.026). The results of the Fisher exact test, however, are not significant.

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, the level of urinary selenium was elevated. 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; Cummins and Kimura, 1971).

Selenium sulfide was administered by gavage in concurrent tests and was found to be carcinogenic for male and female F344 rats and female B6C3F1 mice. Hepatocellular carcinomas were induced in male and female rats and female mice and alveolar/bronchiolar carcinomas and adenomas were induced in female mice (NCI, 1980).

V. CONCLUSION

Under the conditions of this bioassay, selenium applied to the skin did not induce a carcinogenic effect in ICR Swiss mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADMINISTERED SELENIUM SULFIDE BY DERMAL APPLICATION

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED SELENIUM SULFIDE 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	50
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(50)	(48)	(49)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (6%)	3 (6%)	6 (12%)	4 (8%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			3 (6%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)	
#MANDIBULAR L. NODE	(27)	(30)	(34)	(35)
MALIGNANT LYMPHOMA, NOS				1 (3%)
#LIVER	(50)	(48)	(48)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)		
#THYMUS	(2)	(4)	(3)	(3)
MALIGNANT LYMPHOMA, NOS			1 (33%)	
CIRCULATORY SYSTEM				
#SPLEEN	(49)	(47)	(46)	(48)
HEMANGIOSARCOMA		1 (2%)		1 (2%)
#LIVER	(50)	(48)	(48)	(50)
HEMANGIOSARCOMA	1 (2%)			

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
DIGESTIVE SYSTEM				
#LIVER	(50)	(48)	(48)	(50)
HEPATOCELLULAR ADENOMA	3 (6%)		1 (2%)	
HEPATOCELLULAR CARCINOMA	1 (2%)	1 (2%)	1 (2%)	1 (2%)
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
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
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	50	50	50	50
NATURAL DEATH@	31	23	27	25
MORIBUND SACRIFICE	13	20	18	14
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED	1			
TERMINAL SACRIFICE	5	7	5	11
ANIMAL MISSING				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	6	13	6
TOTAL PRIMARY TUMORS	8	6	13	7
TOTAL ANIMALS WITH BENIGN TUMORS	6	3	7	4
TOTAL BENIGN TUMORS	6	3	7	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	3	6	3
TOTAL MALIGNANT TUMORS	2	3	6	3
TOTAL ANIMALS WITH SECONDARY TUMORS#				
TOTAL SECONDARY TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

@ INCLUDES AUTOLYZED ANIMALS

* 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 SELENIUM SULFIDE 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	50
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(49)	(50)	(49)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	8 (16%)	2 (4%)	3 (6%)	7 (14%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	2 (4%)	3 (6%)	2 (4%)	3 (6%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)		
GRANULOCYTIC LEUKEMIA			1 (2%)	
#SPLEEN	(49)	(48)	(48)	(49)
MALIGNANT LYMPHOMA, NOS	1 (2%)			
#MESENTERIC L. NODE	(35)	(34)	(36)	(37)
MALIGNANT LYMPHOMA, NOS		1 (3%)		
#LIVER	(50)	(49)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)	
#THYMUS	(6)	(5)	(4)	(2)
MALIGNANT LYMPHOMA, NOS	1 (17%)			
CIRCULATORY SYSTEM				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
HEMANGIOSARCOMA	1 (2%)			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
#UTERUS	(46)	(48)	(47)	(49)
HEMANGIOMA				3 (6%)
HEMANGIOSARCOMA			1 (2%)	
DIGESTIVE SYSTEM				
#LIVER	(50)	(49)	(50)	(50)
HEPATOCELLULAR ADENOMA			1 (2%)	1 (2%)
HEPATOCELLULAR CARCINOMA				1 (2%)
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(39)	(40)	(36)	(36)
CARCINOMA,NOS		1 (3%)		
ADENOMA, NOS		1 (3%)		
#THYROID	(39)	(41)	(36)	(35)
FOLLICULAR-CELL ADENOMA		1 (2%)		
REPRODUCTIVE SYSTEM				
#UTERUS	(46)	(48)	(47)	(49)
LEIOMYOSARCOMA	1 (2%)			
ENDOMETRIAL STROMAL POLYP			1 (2%)	
#OVARY	(39)	(43)	(43)	(46)
LUTEOMA		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				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
SARCOMA, NOS		1 (2%)		
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	50	50	50	50
NATURAL DEATH@	29	26	25	25
MORIBUND SACRIFICE	13	14	18	14
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED			1	
TERMINAL SACRIFICE	8	10	6	11
ANIMAL MISSING				

@ 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	11	12	16
TOTAL PRIMARY TUMORS	15	12	12	17
TOTAL ANIMALS WITH BENIGN TUMORS	8	5	5	11
TOTAL BENIGN TUMORS	8	5	5	11
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	7	7	6
TOTAL MALIGNANT TUMORS	7	7	7	6
TOTAL ANIMALS WITH SECONDARY TUMORS#				
TOTAL SECONDARY TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
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 SELENIUM SULFIDE BY DERMAL APPLICATION

TABLE B1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED SELENIUM SULFIDE 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	50
INTEGUMENTARY SYSTEM				
*SKIN	(50)	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)			
ULCER, NOS	1 (2%)			
INFLAMMATION, NECROTIZING		1 (2%)		
INFLAMMATION, ACUTE	2 (4%)			
ULCER, ACUTE	2 (4%)			2 (4%)
INFLAMMATION, ACUTE FOCAL	2 (4%)			
INFLAMMATION ACUTE PUSTULAR				1 (2%)
INFLAMMATION, CHRONIC			2 (4%)	1 (2%)
HYPERKERATOSIS	5 (10%)	4 (8%)	2 (4%)	6 (12%)
ACANTHOSIS	4 (8%)	3 (6%)	10 (20%)	10 (20%)
RESPIRATORY SYSTEM				
#LUNG	(50)	(48)	(49)	(48)
INFLAMMATION, NOS			1 (2%)	
BRONCHOPNEUMONIA, ACUTE			1 (2%)	
PNEUMONIA, CHRONIC MURINE		1 (2%)		
FIBROSIS, DIFFUSE			1 (2%)	
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%)			
LIPOMATOSIS	1 (2%)			
#SPLEEN	(49)	(47)	(46)	(48)
AMYLOIDOSIS			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
HEMATOPOIESIS				1 (2%)
ERYTHROPOIESIS	6 (12%)	8 (17%)	4 (9%)	1 (2%)
#LYMPH NODE	(27)	(30)	(34)	(35)
INFLAMMATION, ACUTE			1 (3%)	
#MANDIBULAR L. NODE	(27)	(30)	(34)	(35)
HYPERPLASIA, PLASMA CELL	7 (26%)	1 (3%)	1 (3%)	
HYPERPLASIA, LYMPHOID	1 (4%)			
#PANCREATIC L.NODE	(27)	(30)	(34)	(35)
HYPERPLASIA, PLASMA CELL	1 (4%)			
#MESENTERIC L. NODE	(27)	(30)	(34)	(35)
CONGESTION, NOS				5 (14%)
HEMORRHAGE			1 (3%)	
HYPERPLASIA, PLASMA CELL				1 (3%)
#LIVER	(50)	(48)	(48)	(50)
HEMATOPOIESIS		1 (2%)		
CIRCULATORY SYSTEM				
#MESENTERIC L. NODE	(27)	(30)	(34)	(35)
THROMBUS, FIBRIN		1 (3%)		
#HEART	(50)	(49)	(50)	(49)
ENDOCARDITIS, BACTERIAL			2 (4%)	
FIBROSIS, DIFFUSE	5 (10%)	11 (22%)	5 (10%)	6 (12%)
AMYLOIDOSIS				1 (2%)
#HEART/ATRIUM	(50)	(49)	(50)	(49)
THROMBUS, MURAL	6 (12%)	15 (31%)	5 (10%)	14 (29%)
#MYOCARDIUM	(50)	(49)	(50)	(49)
INFLAMMATION, ACUTE	1 (2%)		2 (4%)	
INFLAMMATION, ACUTE FOCAL	1 (2%)			1 (2%)
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(47)	(45)	(49)	(49)
AMYLOIDOSIS				1 (2%)
#LIVER	(50)	(48)	(48)	(50)
INFLAMMATION, CHRONIC	1 (2%)	1 (2%)	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
NECROSIS, FOCAL		1 (2%)		1 (2%)
ANGIECTASIS		1 (2%)		
#LIVER/CENTRILOBULAR	(50)	(48)	(48)	(50)
NECROSIS, NOS			1 (2%)	
#PANCREAS	(45)	(47)	(45)	(50)
INFLAMMATION, CHRONIC			1 (2%)	
#ESOPHAGUS	(47)	(45)	(40)	(47)
INFLAMMATION, CHRONIC				1 (2%)
#STOMACH	(49)	(46)	(50)	(44)
INFLAMMATION, CHRONIC				1 (2%)
#GASTRIC MUCOSA	(49)	(46)	(50)	(44)
CALCIFICATION, NOS	1 (2%)	2 (4%)		
#SMALL INTESTINE	(45)	(42)	(39)	(37)
AMYLOIDOSIS	1 (2%)			
#JEJUNUM	(45)	(42)	(39)	(37)
AMYLOIDOSIS	1 (2%)			
#ILEUM	(45)	(42)	(39)	(37)
INFLAMMATION, CHRONIC		1 (2%)		
AMYLOIDOSIS		4 (10%)	3 (8%)	8 (22%)
#COLON	(42)	(43)	(36)	(37)
NEMATODIASIS		1 (2%)	3 (8%)	4 (11%)
URINARY SYSTEM				
#KIDNEY	(50)	(49)	(50)	(50)
HYDRONEPHROSIS	1 (2%)	1 (2%)		
PYELONEPHRITIS, NOS	1 (2%)			
INFLAMMATION, SUPPURATIVE	2 (4%)		1 (2%)	
PYELONEPHRITIS SUPPURATIVE				3 (6%)
PYELONEPHRITIS, ACUTE	1 (2%)	3 (6%)		
INFLAMMATION, CHRONIC	1 (2%)	1 (2%)	1 (2%)	2 (4%)
PYELONEPHRITIS, CHRONIC		2 (4%)		
INFLAMMATION, CHRONIC DIFFUSE	1 (2%)			
GLOMERULOSCLEROSIS, NOS	2 (4%)		1 (2%)	
AMYLOIDOSIS		1 (2%)		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
#URINARY BLADDER	(35)	(29)	(31)	(35)
INFLAMMATION, ACUTE		1 (3%)		
INFLAMMATION, ACUTE SUPPURATIVE				2 (6%)
INFLAMMATION, CHRONIC	1 (3%)	1 (3%)		2 (6%)
#U.BLADDER/SUBMUCOSA	(35)	(29)	(31)	(35)
NECROSIS, FOCAL	1 (3%)			
ENDOCRINE SYSTEM				
#ADRENAL	(38)	(45)	(44)	(47)
AMYLOIDOSIS			1 (2%)	
#THYROID	(30)	(37)	(32)	(30)
FOLLICULAR CYST, NOS		1 (3%)		
REPRODUCTIVE SYSTEM				
*PENIS	(50)	(50)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)			
#PROSTATE	(43)	(38)	(43)	(37)
INFLAMMATION, SUPPURATIVE	1 (2%)	1 (3%)		1 (3%)
INFLAMMATION, ACUTE	1 (2%)			3 (8%)
#TESTIS/TUBULE	(49)	(49)	(49)	(48)
DEGENERATION, NOS			1 (2%)	
CALCIFICATION, NOS			1 (2%)	1 (2%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				

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
BODY CAVITIES				
*MEDIASTINUM	(50)	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)			
*PERITONEUM	(50)	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE		1 (2%)		
*MESENTERY	(50)	(50)	(50)	(50)
ABSCESS, NOS	1 (2%)		1 (2%)	
NECROSIS, FAT			1 (2%)	
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
AMYLOIDOSIS	43 (86%)	41 (82%)	40 (80%)	35 (70%)
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	1		1	1
AUTO/NECROPSY/HISTO PERF	1	1	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 SELENIUM SULFIDE 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	50
INTEGUMENTARY SYSTEM				
*SKIN	(50)	(50)	(50)	(50)
INFLAMMATION, NOS		1 (2%)		
ULCER, NOS			1 (2%)	
INFLAMMATION, ACUTE			1 (2%)	
ULCER, ACUTE		1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		2 (4%)	4 (8%)
HYPERKERATOSIS	1 (2%)	1 (2%)	8 (16%)	10 (20%)
ACANTHOSIS		2 (4%)	9 (18%)	16 (32%)
RESPIRATORY SYSTEM				
#LUNG/BRONCHUS	(49)	(50)	(49)	(49)
INFLAMMATION, CHRONIC	1 (2%)			
#LUNG	(49)	(50)	(49)	(49)
BRONCHOPNEUMONIA, ACUTE	1 (2%)			
PNEUMONIA, CHRONIC MURINE			1 (2%)	
HYPERPLASIA, ADENOMATOUS		1 (2%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM				2 (4%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
HEMATOPOIESIS		1 (2%)		
ERYTHROPOIESIS		1 (2%)		
#BONE MARROW	(41)	(38)	(43)	(46)
GRANULOPOIESIS				1 (2%)
#SPLEEN	(49)	(48)	(48)	(49)
AMYLOIDOSIS	1 (2%)		1 (2%)	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%)	
ERYTHROPOIESIS	8 (16%)	9 (19%)	10 (21%)	8 (16%)
#LYMPH NODE	(35)	(34)	(36)	(37)
HYPERPLASIA, PLASMA CELL	3 (9%)			
#MANDIBULAR L. NODE	(35)	(34)	(36)	(37)
INFLAMMATION, ACUTE/CHRONIC		1 (3%)		
HYPERPLASIA, PLASMA CELL	3 (9%)	1 (3%)		1 (3%)
#PANCREATIC L.NODE	(35)	(34)	(36)	(37)
HYPERPLASIA, PLASMA CELL	1 (3%)			
#MESENTERIC L. NODE	(35)	(34)	(36)	(37)
CONGESTION, NOS			2 (6%)	1 (3%)
HEMORRHAGE	1 (3%)			
INFLAMMATION, ACUTE			1 (3%)	
HYPERPLASIA, PLASMA CELL	1 (3%)		1 (3%)	
HYPERPLASIA, LYMPHOID			1 (3%)	
#LIVER	(50)	(49)	(50)	(50)
GRANULOPOIESIS				1 (2%)
CIRCULATORY SYSTEM				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
PERIARTERITIS			1 (2%)	
#LUNG	(49)	(50)	(49)	(49)
THROMBOSIS, NOS				2 (4%)
#HEART	(49)	(48)	(48)	(49)
ENDOCARDITIS, BACTERIAL		1 (2%)	1 (2%)	5 (10%)
FIBROSIS, DIFFUSE	7 (14%)	8 (17%)	5 (10%)	5 (10%)
PERIVASCULITIS				1 (2%)
AMYLOIDOSIS			1 (2%)	
#HEART/ATRIUM	(49)	(48)	(48)	(49)
THROMBUS, MURAL	3 (6%)	4 (8%)	2 (4%)	4 (8%)
#MYOCARDIUM	(49)	(48)	(48)	(49)
INFLAMMATION, SUPPURATIVE				1 (2%)
INFLAMMATION, ACUTE			1 (2%)	1 (2%)
*AORTA	(50)	(50)	(50)	(50)
MEDIAL CALCIFICATION		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
*UTERINE ARTERY	(50)	(50)	(50)	(50)
INFLAMMATION, ACUTE	1 (2%)			
NECROSIS, FIBRINOID			1 (2%)	
#THYMUS	(6)	(5)	(4)	(2)
THROMBUS, ORGANIZED				1 (50%)
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(47)	(43)	(43)	(46)
AMYLOIDOSIS		1 (2%)		
#LIVER	(50)	(49)	(50)	(50)
CYST, NOS				1 (2%)
MULTILOCULAR CYST	1 (2%)			1 (2%)
INFLAMMATION, CHRONIC			2 (4%)	1 (2%)
INFLAMMATION, GRANULOMATOUS			1 (2%)	
NECROSIS, NOS	1 (2%)			
NECROSIS, FOCAL		1 (2%)	1 (2%)	
AMYLOIDOSIS		1 (2%)		
GROUND-GLASS CYTO CHANGE		1 (2%)		
ANGIECTASIS		1 (2%)		1 (2%)
*GALLBLADDER	(50)	(50)	(50)	(50)
INFLAMMATION, ACUTE	1 (2%)			
#BILE DUCT	(50)	(49)	(50)	(50)
CYST, NOS	1 (2%)	1 (2%)		
#PANCREAS	(46)	(48)	(46)	(49)
INFLAMMATION, CHRONIC				1 (2%)
#PANCREATIC ACINUS	(46)	(48)	(46)	(49)
ATROPHY, NOS			1 (2%)	
#ESOPHAGUS	(44)	(45)	(45)	(42)
INFLAMMATION, ACUTE	1 (2%)			
#JEJUNUM	(39)	(35)	(40)	(39)
AMYLOIDOSIS	1 (3%)			1 (3%)
#ILEUM	(39)	(35)	(40)	(39)
AMYLOIDOSIS	2 (5%)	3 (9%)	3 (8%)	

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
#COLON	(37)	(41)	(44)	(39)
INFLAMMATION, ACUTE			1 (2%)	
NEMATODIASIS		1 (2%)	3 (7%)	1 (3%)
URINARY SYSTEM				
#KIDNEY	(50)	(49)	(49)	(50)
HYDRONEPHROSIS			2 (4%)	
INFLAMMATION, SUPPURATIVE			1 (2%)	
GLOMERULONEPHRITIS, ACUTE	1 (2%)			
PYELONEPHRITIS, ACUTE	2 (4%)			
INFLAMMATION, CHRONIC		1 (2%)		
PYELONEPHRITIS, CHRONIC	1 (2%)	6 (12%)		
INFLAMMATION, CHRONIC DIFFUSE	1 (2%)			
SCLEROSIS		2 (4%)		
GLOMERULOSCLEROSIS, NOS	1 (2%)			
NECROSIS, MEDULLARY		1 (2%)		
AMYLOIDOSIS		1 (2%)	2 (4%)	
#KIDNEY/CORTEX	(50)	(49)	(49)	(50)
SCAR				1 (2%)
#RENAL PAPILLA	(50)	(49)	(49)	(50)
CALCIFICATION, NOS				1 (2%)
#KIDNEY/TUBULE	(50)	(49)	(49)	(50)
CALCIFICATION, NOS	1 (2%)	1 (2%)		
ENDOCRINE SYSTEM				
#ADRENAL	(44)	(49)	(47)	(45)
AMYLOIDOSIS		1 (2%)		
#THYROID	(39)	(41)	(36)	(35)
CYSTIC FOLLICLES		2 (5%)		
INFLAMMATION, CHRONIC				1 (3%)
INFLAMMATION, CHRONIC FOCAL				1 (3%)
REPRODUCTIVE SYSTEM				
#UTERUS	(46)	(48)	(47)	(49)
HAMARTOMA				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
CYST, NOS	1 (2%)			
INFLAMMATION, SUPPURATIVE		1 (2%)		
INFLAMMATION, ACUTE		1 (2%)		
#UTERUS/ENDOMETRIUM	(46)	(48)	(47)	(49)
INFLAMMATION, SUPPURATIVE	1 (2%)			
INFLAMMATION, CHRONIC				1 (2%)
HYPERPLASIA, CYSTIC	25 (54%)	30 (63%)	34 (72%)	32 (65%)
#OVARY	(39)	(43)	(43)	(46)
CYST, NOS	3 (8%)	6 (14%)	7 (16%)	7 (15%)
HEMORRHAGIC CYST	1 (3%)	3 (7%)	4 (9%)	1 (2%)
INFLAMMATION, SUPPURATIVE			1 (2%)	
AMYLOIDOSIS			1 (2%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
AMYLOIDOSIS	39 (78%)	37 (74%)	32 (64%)	44 (88%)
OMENTUM				
NECROSIS, FAT		1		
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED		1	1	1

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
AUTO/NECROPSY/HISTO PERF	3	1		

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

APPENDIX C

ANALYSIS OF SELENIUM SULFIDE

APPENDIX C

Analysis of Selenium Sulfide

Midwest Research Institute

A. Elemental Analysis

Element	Se	S
Theory		
SeS	71.12	28.88
SeS_2	55.18	44.82
Observed 11/15/74	61.0±0.6	40.93
		41.08
Observed 7/19/79	59.97	40.7±0.2
	59.88	

Melting Point

Literature	
SeS	118°-119°C (Weast, 1974-1975)
SeS_2	less than 100°C (Weast, 1974-1975)
Observed	115°-117°C

C. X-Ray Diffraction

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

Procedure: The X-ray diffraction pattern of the selenium sulfide powder used in this study was determined. Since a suitable standard was not available, intensities were recorded as approximations expressed in terms varying from "very weak" to "very strong." X-ray diffraction values are reported in Table C1.

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 SELENIUM SULFIDE SUSPENSION

APPENDIX D

Analysis of Selenium Sulfide Suspension
Midwest Research Institute

The entire sample of selenium sulfide in 0.5% carboxymethylcellulose in saline 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. Five milliliters of 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 quantitatively to a volumetric flask and the volume was adjusted to the mark with distilled water. An analytical standard was prepared by adding a known amount of selenium sulfide to carboxymethylcellulose (0.5%), extracting it with carbon disulfide, and repeating the procedure outlined above. The samples, including the control, were analyzed using atomic absorption.

APPENDIX E

STABILITY OF SELENIUM SULFIDE SUSPENSIONS

APPENDIX E

Stability of Selenium Sulfide Suspensions

SPECIAL STABILITY STUDY

I. PURPOSE

To determine if either of the following mixtures in any way decomposed or altered the selenium sulfide used in the bioassay:

a. Carboxymethylcellulose in saline.

b. Carboxymethylcellulose in deionized water.

II. ANALYSIS

A. SAMPLE PREPARATION

1. Sample 1: A 100 ml-solution of 0.5% carboxymethylcellulose, sodium salt in normal saline (0.9% sodium chloride) was prepared. A 960-mg sample of selenium sulfide was weighed into a 50-ml volumetric flask and brought to volume with the above saline carboxymethylcellulose (CMS) solution and mixed for 30 minutes on a vortex mixer. It was then left open to the atmosphere in the light for the next 30 minutes, with occasional shaking. The mixture was then shaken in a 125-ml separatory funnel for 2 minutes with 50 ml of carbon disulfide and allowed to separate. The bottom layer (CS_2 layer) was drained into a 100-ml beaker.

2. Sample 2: A control sample of the same approximate weight was dissolved in 50 ml of carbon disulfide in a 100-ml beaker. Both the sample and control beakers (Samples 1 and 2) were placed in a glove box on a marble slab covered by watchglasses and allowed to evaporate slowly overnight.

3. Sample 3: The method of preparation was the same as that for Sample 1, except that deionized water was substituted for saline solution and a 750-mg sample weight was used.

4. Sample 4: The method of preparation was the same as that for Sample 2, but a 750-mg sample was used.

5. Sample 5: The selenium sulfide sample was untreated and had been stored refrigerated.

6. Sample 6: The selenium sulfide sample used had been exposed overnight at room temperature in a beaker.

B. DESCRIPTION OF SAMPLES

Samples 1 through 4 crystallized with multiple crystal forms. Sample 1 contained red "needles," red "platelets," and yellow crystals. The control (Sample 2) contained mostly red "platelets" with a few red "needles" and some yellow crystals.

Sample 3 contained reddish orange crystals of 1-3 mm in length, while Sample 4 contained crystals of the same color of about 1 mm in length. Both Samples 3 and 4 had the yellow crystals seen in 1 and 2.

Sample 6 was unchanged in appearance from Sample 5.

C. X-RAY DIFFRACTION

X-ray diffraction analyses were performed on the following:

a. Samples 1 and 2: Individual crystals from both, and a total mix of all crystal types from each sample.

b. Samples 3 and 4: Total mix of all crystal types from each sample.

c. Samples 5 and 6: Representative sample of homogeneous material from each.

III. RESULTS

X-RAY DIFFRACTION

The x-ray diffraction patterns for all the samples had similar d spacings and all had the same major line. However, the relative intensities of the lines differed from sample to sample. The d spacing of the sample total mixes (1 and 3) and the control total mixes (2 and 4) corresponded well with each other and with the previous untreated selenium sulfide sample (report dated 11/15/74). Samples 5 and 6 also corresponded well with each other and with the previous untreated selenium sulfide sample.

Review of the Bioassay of Selenium Sulfide* 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 Selenium Sulfide for carcinogenicity.

The primary reviewer for the report on the bioassay of selenium sulfide agreed with the conclusion that the compound was not carcinogenic, under the conditions of test. After a brief description of the experimental design and toxicity findings, the reviewer opined that selenium sulfide would not pose any significant human risk, based on results of the bioassay study.

The secondary reviewer noted that no attempt was made to determine how much of the selenium sulfide was absorbed. He said that the results of the subchronic study indicated that higher chronic dosages could have been administered and added that he was disturbed by the high early mortality of the animals. Based on these deficiencies, the reviewer questioned the validity of the study for assessing the potential risk of selenium sulfide for human beings.

The primary reviewer indicated that the study was not intended to determine if selenium sulfide had systemic effects, since a previous study done by gavage was meant for that purpose. The reviewer added that dermal toxicity had been demonstrated in this bioassay and that the administration of higher dosages could have resulted in excessive toxicity. In regard to the excessive mortality, the reviewer pointed out that the mouse strain used was selected because it was supposed to be particularly sensitive, although its lifespan was relatively shorter than other strains. The reviewer added that the validity of the study would depend upon how much of their natural lifespan the animals had lived. A Program staff member indicated that the survival of the animals was consistent with the longevity displayed by this strain in other studies. Another

staff member commented that, despite the study's limitations, it was sufficiently adequate that the results should be reported.

One Clearinghouse member said that selenium was a conundrum in that it is carcinogenic when given at high levels by gavage but it is an essential element at low levels. He added that there is some evidence that it may even act as an anti-carcinogen. Another member pointed out that sodium selenite or selanate is the form of selenium that is essential. He said it is a conundrum similar to cobalt, in which one form is an essential element and another a carcinogen. It was suggested that a paragraph be added to the bioassay report indicating the differences in the various forms of selenium. The primary reviewer moved that the report on the bioassay of selenium sulfide by dermal exposure be accepted as written. The motion was seconded and approved unanimously.

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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