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BIOASSAY OF

RESERPINE

FOR POSSIBLE CARCINOGENICITY

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BIOASSAY OF
RESERPINE
FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
National Cancer Institute/National Toxicology Program

FOREWORD

This report presents the results of the bioassay of reserpine 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 reserpine was conducted by Southern Research Institute, Birmingham, Alabama, initially under direct contract to NCI (1) and later under a subcontract to Tracor Jitco, Inc., Rockville, Maryland, prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design and doses were determined by Drs. J. D. Prejean (2) and O. G. Fitzhugh (3,4). The principal investigator was Dr. Prejean (2). Mr. J. Belzer (2) and Mr. I. Brown (2) were responsible for the care of the laboratory animals and the administration of the test chemical. Data management and retrieval were performed by Ms. C. A. Prejean (2). Histopathologic examinations were performed by Drs. R. B. Thompson (2) and J. C. Peckham (2). The diagnoses included in this report represent the interpretations of Drs. Thompson and Peckham, whose findings were reviewed by Drs. Morton H. Levitt (5), John Sagartz (3), and Sherman Stinson (1) with Quality Review by Dr. Jerry Hardisty (6).

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (7). Statistical analyses were performed by Dr. J. R. Joiner (3), using methods selected for the bioassay program by Dr. J. J. Gart (8). Chemicals used in this bioassay were analyzed at Midwest Research Institute (9) under the direction of Dr. E. Murrill. Dosage analysis was performed by Ms. L. Burford (2) and Ms. R. James (2). The results of these analyses were reviewed by Dr. C. W. Jameson (3,10).

This report was prepared at Tracor Jitco (3) under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. C. R. Angel, Acting Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Dr. J. F. Robens (11), toxicologist; Drs. R. L. Schueler and J. W. Sagartz, pathologists; Ms. L. A. Owen and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The following scientists at NCI/NTP were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto Jr. (12), Dr. J. Fielding Douglas, Dr. Richard A. Griesemer, Dr. Charles K. Grieshaber, Dr. Thomas E. Hamm, Dr. William V. Hartwell, Dr. Morton H. Levitt, Dr. Harry Mahar, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. A. R. Patel (13), Dr. Marcelina B. Powers, Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

A bioassay for possible carcinogenicity of reserpine, an antihypertensive drug for human use, was conducted by administering the test chemical in feed to F344 rats and B6C3F1 mice.

Groups of 50 rats and 50 mice of each sex were administered reserpine at two doses, 5 ppm or 10 ppm, for 103 weeks and then observed for an additional 2 weeks. Matched controls consisted of groups of 50 untreated rats and 50 untreated mice of each sex. All surviving animals were killed and necropsied at the end of 104 or 105 weeks.

The significant effects that could be related to administration of reserpine at the doses used were decreased body weight and increased tumor formation in dosed male rats and in mice of both sexes. Dosed male rats had an increased incidence of adrenal medullary pheochromocytomas. Among dosed mice, some males developed undifferentiated carcinomas of the seminal vesicles, which rarely occur in control mice, and females had an increased incidence of mammary cancer.

It was concluded that, under the conditions of the bioassay, reserpine was carcinogenic in male rats and in mice of both sexes, producing three different kinds of cancers. Reserpine was not carcinogenic for female rats, but they may not have received a high enough dose for maximum test sensitivity.

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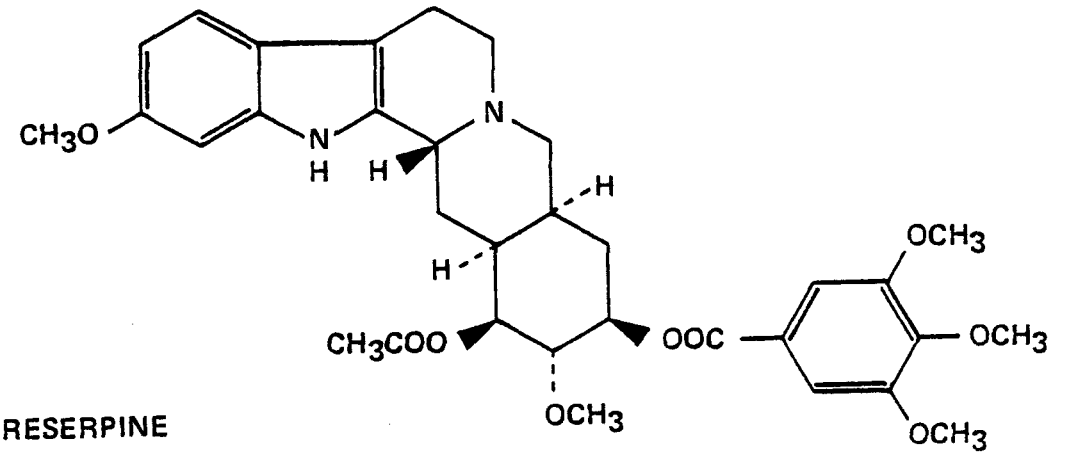
I. INTRODUCTION



RESERPINE

Reserpine (CAS 50-55-5; NCI C50157) is one of several biologically active alkaloids derived from Rauwolfia serpentina, a plant native to several southeast Asian countries (IARC, 1976). Reserpine is a prescription medication used in lowering blood pressure and is recommended primarily for patients with milder forms of hypertension (AMA Department of Drugs, 1977). Reserpine also has limited use as a peripheral vasodilator for Raynaud's syndrome and as a tranquilizer in various anxiety states. The therapeutic dose of reserpine used to treat hypertension ranges from 0.1 to 1.0 mg daily, and daily doses as high as 5 mg reserpine have been used to control psychoses (Byck, 1975). The widespread psychiatric uses of the drug in the 1950's have been largely abandoned because the drug produces mental depression (Byck, 1975; AMA Department of Drugs, 1977). The use of reserpine in animal feed (0.0001%) is permitted for preventing aortic rupture in turkeys (Code of Federal Regulations, 1977).

U. S. production data for the pharmaceutical product are not available because the chemical is obtained from natural sources and is not synthesized commercially (IARC, 1975). However, estimates obtained from surveys of pharmacies showed that, in 1977, U. S. physicians wrote almost 10,000 new prescriptions for reserpine or a combination of Rauwolfia alkaloids that included reserpine (National Prescription Audit, 1978). Women over 65 years of age comprised the single largest group of patients treated with this drug (National Drug and Therapeutic Index, 1977-1978).

The Rauwolfia alkaloids deplete norepinephrine from tissues (Byck, 1975). This hormone normally binds to receptors on post-ganglionic nerve endings of the sympathetic nervous system, where it serves as a transmitter substance. Following its synthesis, norepinephrine is stored in cytoplasmic granules to prevent its destruction by intracellular monoamine oxidase (Koelle, 1975). Extremely low concentrations of reserpine block the ATP-catalyzed uptake of norepinephrine at these storage sites, resulting in a sharp reduction in the physiological levels of norepinephrine (Koelle, 1975). Depletion occurs in the brain, the heart, and in the adrenal medulla as well as in adrenergic nerve endings (Gosselin et al., 1976; Innes and Nickerson, 1975). The cardiovascular effects, i.e., the antihypertensive effect, and many other pharmacological effects of reserpine are attributed to this blocking action (Nickerson and Collier, 1975; AMA Department of Drugs, 1977). Reserpine also has other actions, including peripheral vasodilatation in normal and sympathectomized human extremities when administered intraarterially, direct depression of myocardial function, and a variety of endocrine actions in experimental animals, e.g., altering secretion of hypothalamic regulatory hormones and increasing prolactin secretion (Nickerson and Collier, 1975).

Reserpine-C^{14} given intravenously to rats was cleared from the blood rapidly and reached a peak in tissues in 1 hour. After 24 hours, the label was found only in fat tissue and the liver (Sheppard et al., 1955). In mice, 70% of a radiolabeled material was recovered in urine 24 hours after oral feeding of reserpine C^{14} (Numerof et al., 1954).

Reserpine is rapidly absorbed from the gastrointestinal tract in man (Maass et al., 1969; Byck, 1975). The rate of clearance from plasma is biphasic, with the first phase having a half-life of 4.5 hours and the second 271 hours. Although between 63% and 75% of the drug is excreted in 4 days, elimination is still apparent at 11 days (Maass et al., 1969). Pharmacological effects persist after elimination is complete (Byck, 1975).

In a study to test the effects of alkaloids on chromosomes, cultures of human peripheral leucocytes were exposed to aqueous suspensions of reserpine. At concentrations ranging from 2.5 to 25.0 μg/ml, no chromosomal aberrations were observed, but a definite increase in the number of mitotic figures occurred at all concentrations (Bishun et al., 1975).

The LD_{50} of reserpine administered intravenously to rats of unidentified strain was reported to be 15 mg/kg body weight (Usdin and Efron, 1972). In mice (unidentified strain), the oral LD_{50} was 500 mg/kg and the intraperitoneal LD_{50} was 70 mg/kg (Usdin and Efron, 1972).

Reserpine was selected by the Carcinogenesis Bioassay Program because reports from the Boston Collaborative Drug Surveillance Program (1974) as well as other epidemiological studies indicated that women receiving long-term therapy with reserpine had an increased incidence of breast cancer. Although these results have not been corroborated (IARC, 1976), they indicated the necessity for a bioassay for possible carcinogenicity of this drug.

II. MATERIALS AND METHODS

A. Chemical

The reserpine used for the study was obtained in a single batch (Lot No. 6230-LOA-2) from S. B. Penick and Company, Lyndhurst, New Jersey. The purity of this batch, according to the manufacturer, met United States Pharmacopeia (USP) specifications. The identity and purity of the batch was confirmed and the USP specifications verified by analysis at Midwest Research Institute. The analyses included elemental analysis, melting point, optical rotation, nonaqueous titration with perchloric acid, thin-layer and high pressure liquid chromatography, and ultraviolet, infrared, and nuclear magnetic resonance spectrometry (Appendix E). The melting point was 260° to 267°C with decomposition. Elemental analyses (C, H, N) were correct for $C_{33}H_{40}N_2O_9$, the molecular formula of reserpine. A purity of 97.8%±1.0% was determined by comparing a sample of the test compound with a USP reserpine reference standard using visible spectrometric analysis. Nonaqueous titration of secondary amine groups with perchloric acid indicated a purity of 101.0%±0.7(δ)%. Thin-layer chromatography showed a major spot that was identical to that of a USP reserpine reference standard along with two trace impurities. High-pressure liquid chromatography indicated a 0.5% impurity that was not identified. Ultraviolet, visible, infrared and nuclear magnetic resonance spectra were consistent with the structure.

The chemical was stored in the original container at room temperature. Values similar to those at the initiation of the bioassay were obtained upon reanalysis of this batch at the completion of the bioassay.

B. Dietary Preparation

Dosed feed mixtures were prepared every 2 weeks by mixing a known amount of the reserpine with a small amount of Wayne® Lab-Blox Meal (Allied Mills,

Chicago, Ill.) and then adding this premix to the required amount of animal meal and mixing in an 8-quart twin-shell blender for 10 to 15 minutes.

The batch was divided and the half used for feeding the first week was kept at 22°C. The other half was stored at 5°C and used for feeding the second week. During use, the second portion was stored at 22°C.

Theoretical concentrations of reserpine in formulated diets were checked analytically at intervals during the chronic study to assess the accuracy of the diet preparation and the homogeneity of the mixture. Results are summarized in Appendix F. At each dietary concentration (5 ppm and 10 ppm), the mean of the analytical concentration for the samples checked was within 12% of the theoretical concentration, and the coefficient of variation did not exceed 22.9%.

The stability of reserpine in feed was satisfactory, as indicated by its presence in aliquots for analysis of chemical/vehicle mixtures stored at 5°C for various lengths of time up to 11 months and in chemical/vehicle mixtures stored up to a week (at 22°C, the conditions of the bioassay). For example, mean values were 10.4 ppm ($\pm$0.2δ) for the high dose (10 ppm) stored at 5°C and 11.7 ppm ($\pm$0.1δ) for the dose stored at 22°C for approximately 1 week (Appendix G).

C. Animals

Male and female F344 rats and B6C3F1 mice were obtained as 4-week old weanlings, all within 3 days of the same age, from the NCI Frederick Cancer Research Center (Frederick, Md.). The animals were acclimated within the test facility for 1 week and then assigned to cages on a weight basis. The initial weights of male rats used in the chronic study ranged from 73 to 106 g; female rats, 62 to 89 g; male mice, 15 to 21 g; and female mice, 13 to 17 g. Individual animals were identified by ear punch.

D. Animal Maintenance

The animals were housed five per cage in stainless steel solid bottom cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.): rats in 16" x 16" x 6" cages, and mice in 9" x 6" x 4" cages. The bedding used was heat-treated hardwood chips (Beta-Chips, Northeastern Products Corp., Warrensburg, N.Y.). Rat and mouse cages were covered with disposable filter caps (Negus Animal Container Co., Madison, Wis.). Wayne® Lab-Blox meal (Allied Mills, Inc., Chicago, Ill.) was used during the period the reserpine was administered and pellets were used thereafter. The feed was provided ad libitum and replenished once per week. Tap water (with no further additives) was supplied ad libitum.

Cages, cage covers, and fresh bedding were replaced twice a week. Feed hoppers and water bottles with sipper tubes and stoppers were provided once a week. All equipment was washed in industrial tunnel-type cage washers using Elect detergent (sodium tripolyphosphate, sodium silicate, sodium carbonate, and modified polyethyloxylated alcohol; percentages not available) at 82°C. Disposable cage filter bonnets were changed once a month.

Animal rooms were maintained at 20° to 24°C, and relative humidity was 40% to 60%. Incoming air and exhaust air were passed through fiberglass roughing filters at a rate that allowed 15 changes per hour. Air pressure in the animal rooms was positive to the hallway and negative to the rest of the building, with no movement of air between rooms. Fluorescent lighting was used 9 hours per day.

Rats fed reserpine were housed in the same room with rats fed FANFT (2-formylamino-4-(5-nitro-2-furyl)thiazole) (CAS 24554-26-5) between day 94 and day 289 of the reserpine study. On day 229 of the reserpine study, FANFT diets were replaced with control diets and the animals that had been treated with FANFT were observed for an additional 60 days. Mice were housed with mice being treated with the following chemicals:

(CAS 23214-92-8)	adriamycin
(CAS 645-05-6)	hexamethylmelamine
(CAS 7008-42-6)	acronycine
(CAS 3546-10-9)	phenesterin

At week 80, the mice fed reserpine and their controls were moved to a separate room.

E. Subchronic Studies

Two subchronic feeding studies were conducted to determine the concentrations to be used in the chronic studies (referred to in this report as "low" and "high" doses).

Preliminary to the chronic study, a 14-day feeding study was performed with groups of five male and five female rats and mice which were fed diets containing one of five doses. Table 1 shows the doses fed, survival of animals, and mean body weight of each dosed group at the end of the 14-day study. At the end of the 14 days, the animals were killed and necropsied. Histopathologic changes consisted of suppressed spermatogenesis in male rats at 600 or 200 ppm, spleen and thymus atrophy in most male or female rats at 600 or 200 ppm and in most male or female mice at 20 ppm or greater, and bone marrow atrophy in most male and female rats at 60 ppm or greater. At 20 ppm, 2/5 male and 0/5 female mice survived, with bone marrow atrophy in 2/5 females. No lesions, except thymus atrophy in one female mouse, occurred in the rats or mice fed 6 ppm, the concentration selected as the high dose for the 13-week subchronic study.

In the 13-week subchronic study, groups of 10 male and 10 female rats and mice were fed diets containing reserpine at one of five doses, and groups of 10 control animals of each sex were fed basal diet. Each animal was observed twice a day and was weighed once a week. Table 2 shows the doses fed, the survival of animals in each dosed group at the end of the study, and the mean body weight of each dosed group at week 13, expressed as a percentage of the mean body weight of the corresponding controls. At the end of the 13 weeks, the animals were killed and necropsied.

No deaths occurred in any of the control or dosed groups of rats or mice. No decreases in mean body weight were observed in the rats; but measurable depression occurred in the mean body weights of dosed groups of mice. No gross or microscopic lesions that could be related to feeding of reserpine were observed in either species.

The low and high doses for the chronic studies were set at 5 ppm and 10 ppm for both rats and mice.

Table 1. Dose Levels, Survival, and Mean Body Weights of Rats and Mice Fed Reserpine for 14 Days

Dose (ppm)	Male		Female	
	Survival(a)	Mean Body Weight at Day 14 (grams)	Survival(a)	Mean Body Weight at Day 14 (grams)
Rats				
6	5/5	186	5/5	129
20	5/5	153	5/5	116
60	5/5	115	5/5	79
200	4/5	60	0/5	--
600	0/5	--	0/5	--
Mice				
6	5/5	17	5/5	15
20	2/5	13	0/5	--
60	0/5	--	0/5	--
200	0/5	--	0/5	--
600	0/5	--	0/5	--

(a) Number surviving/number in group.

Table 2. Dose Levels, Survival, and Mean Body Weights of F344 Rats and B6C3F1 Mice Fed Reserpine for 13 Weeks

Dose (ppm)	Male		Female	
	Survival(a)	Mean Weight at Week 13 as % of Control	Survival(a)	Mean Weight at Week 13 as % of Control
Rats				
0	10/10	100	10/10	100
0.4	10/10	107	10/10	105
0.8	10/10	106	10/10	107
1.5	10/10	100	10/10	101
3.0	10/10	100	10/10	101
6.0	10/10	103	10/10	106
Mice				
0	10/10	100	10/10	100
0.4	10/10	91	10/10	93
0.8	10/10	94	10/10	89
1.5	10/10	85	10/10	93
3.0	10/10	88	10/10	93
6.0	10/10	85	10/10	85

(a) Number surviving/number in group.

F. Chronic Studies

The number of animals per group, doses administered, and durations of the chronic feeding studies are shown in Table 3.

G. Clinical Examinations and Pathology

All animals were observed twice daily, and observations of sick, tumor-bearing, and moribund animals were recorded. Clinical examination and palpation for masses were performed each month, and the animals were weighed at least once per month. Moribund animals and animals that survived to the end of the bioassay were killed using carbon dioxide and necropsied.

Gross and microscopic examinations were performed on major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The 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 (abdominal), lungs and bronchi, trachea, bone, bone marrow (femur) and thigh muscle, spleen, lymph nodes, thymus, heart, salivary glands, liver, pancreas, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, mammary gland, uterus, ovary, brain, epididymus, eye, and all tissue masses. Peripheral blood smears also were made for all animals, whenever possible.

Necropsies were performed on all animals found dead, unless precluded in whole or in part by autolysis or cannibalization. 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.

Table 3. Experimental Design for Chronic Reserpine Feeding Studies in F344 Rats and B6C3F1 Mice

Sex and Test Group	Initial No. of Animals(a)	Reserpine in Diet (b) (ppm)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male				
Matched-Control	50	0		104
Low-Dose	50	5	103	2
High-Dose	50	10	103	1-2
Female				
Matched-Control	50	0		104
Low-Dose	50	5	103	2
High-Dose	50	10	103	2

(a) All animals were 5 weeks of age when placed on study.
(b) Test and control diets were provided ad libitum 7 days per week.

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 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. 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 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.025.

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 percent confidence interval for the relative risk of each dosed group compared to 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 - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of the dosed male rats were lower than those of the corresponding controls through week 80 of the bioassay but were unaffected thereafter. Mean body weights of the female rats were unaffected by the test chemical (Figure 1). Drooping eyelids, noted as soon as the second day, occurred in each animal in the dosed groups, but not in the controls.

B. Survival (Rats)

Estimates of the probabilities of survival for male and female rats fed reserpine in diets at the doses of this bioassay, and for the matched controls, are shown by the Kaplan and Meier curves in Figure 2. The result of the Tarone test is not significant in either sex.

In males, 44/50 (88%) of the high-dose, 40/50 (80%) of the low-dose, and 38/50 (76%) of the control groups survived for 90 weeks, and in females, 39/50 (78%) of the high-dose, 40/50 (80%) of the low-dose, and 44/50 (88%) of the control groups survived for 90 weeks.

Sufficient numbers of rats of each sex were at risk for the development of late-appearing tumors.

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are summarized in Appendix A, Tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix C, Tables C1 and C2.

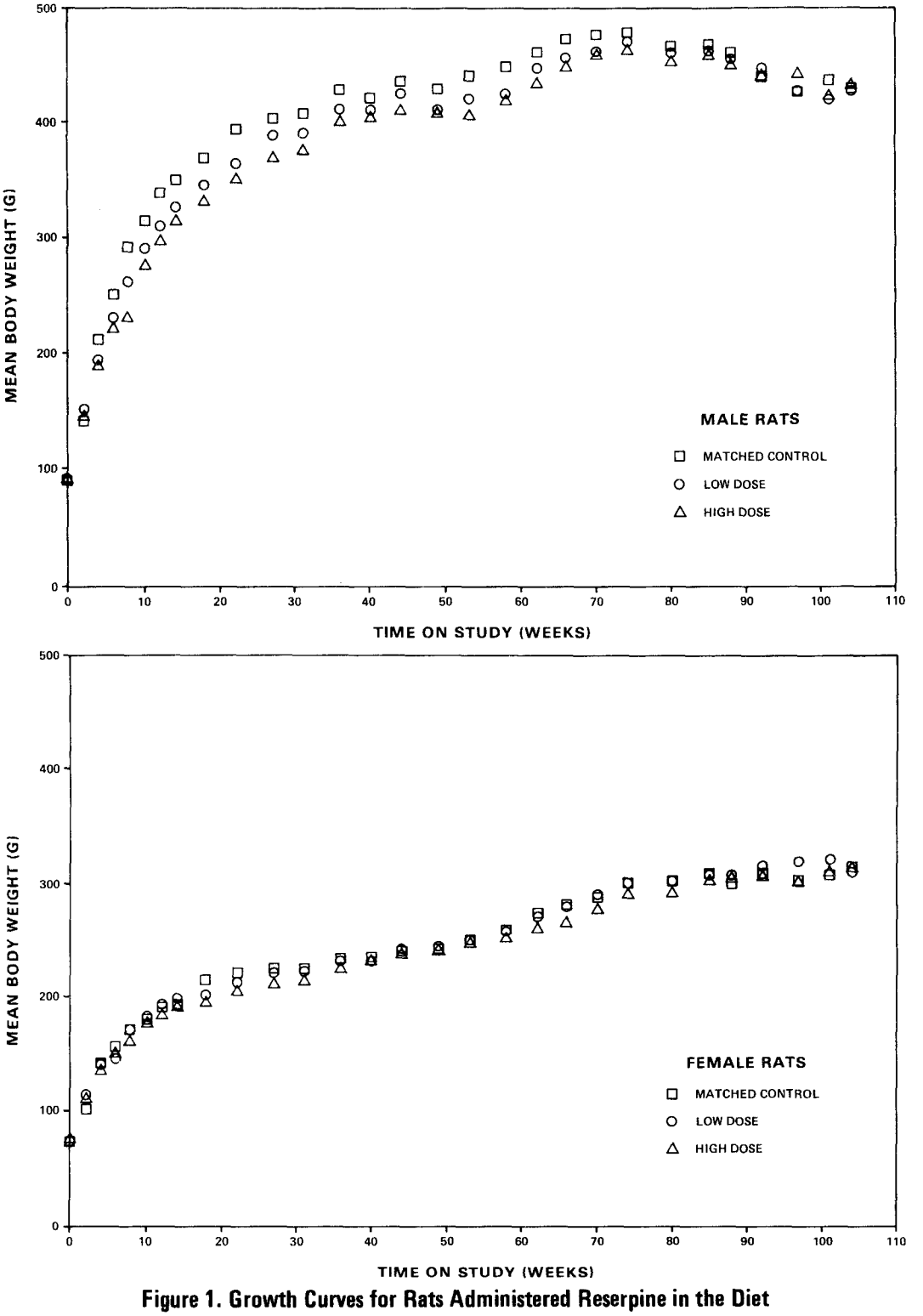


Figure 1. Growth Curves for Rats Administered Reserpine in the Diet

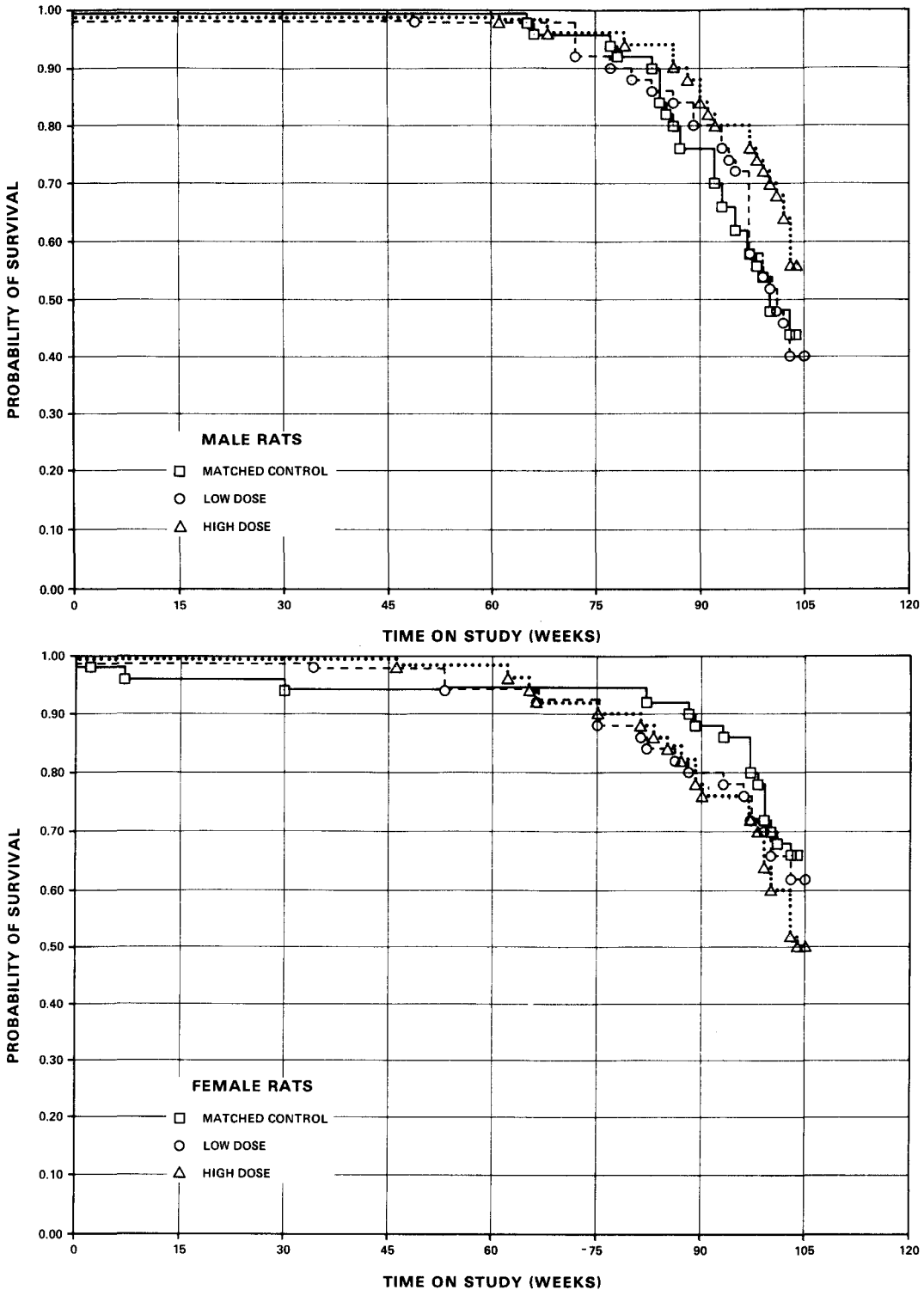


Figure 2. Survival Curves for Rats Administered Reserpine in the Diet

A variety of neoplasms occurred in both the matched-control and dosed groups. Some types of neoplasms occurred only, or with a greater frequency, in rats of dosed groups as compared with controls. These lesions, except for those of the adrenal, appeared independent of any administration of the test chemical.

Table 4 indicates that an increased incidence of adrenal medullary tumors in the dosed male rats appeared to be related to administration of the test chemical.

Pheochromocytomas diagnosed as malignant were usually characterized by extensive growth with only a compressed rim of cortex remaining. In some instances, the proliferating medullary cells extended to the capsule. The cell nuclei were usually large and hyperchromatic with a reticulated chromatin pattern and a large prominent nucleolus. Cells were arranged in clumps or packets and were separated by thin connective tissue septa. Binucleate cells were seen in some tumors.

Benign pheochromocytomas were usually arranged in well-demarcated islands of proliferating medullary cells. These cells appeared to be well differentiated. Cells varied in appearance; some cells had small round basophilic nuclei and scant cytoplasm while others had a more open nucleus and abundant vacuolated cytoplasm. Focal hyperplasias of the adrenal medulla were small foci of pheochromocytes with nuclei resembling those of normal medullary cells and slightly basophilic cytoplasm.

A number of degenerative, proliferative, and inflammatory changes encountered in animals of the dosed and control groups are commonly seen in aged F344 rats.

The histopathologic examination provided evidence that an increased incidence of adrenal pheochromocytomas occurred in male F344 rats fed reserpine under conditions of this bioassay.

D. Statistical Analyses of Results (Rats)

Tables 5 and 6 contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

Table 4. Incidence of Adrenal Medullary Tumors in Male Rats

	Matched Control	Low Dose	High Dose
Number of Rats Examined Microscopically	48	49	48
Morphology[a]			
Focal Hyperplasia	3(6%)	4(8%)	3(6%)
Pheochromocytoma	2(4%)	14(29%)	15(31%)
Pheochromocytoma, Malignant	1(2%)	4(8%)	9(19%)

(a) Most advanced lesion diagnosed for each rat with a lesion.

In male rats, the result of the Cochran-Armitage test for the incidence of pheochromocytomas of the adrenal is significant (P less than 0.001). The Fisher exact test shows that the incidence in each dosed group is significantly higher than that in the controls (P less than 0.001). The results of the statistical tests on the incidence of adrenal tumors in female rats are not significant. The results of statistical analysis indicates that the incidence of adrenal tumors in male rats is associated with the administration of reserpine.

In female rats, results of the Fisher exact test used to compare the incidences of chromophobe carcinomas of the pituitary in the low-dose and control groups (P=0.031) were above the 0.025 level required for significance by the Bonferroni inequality criterion for multiple comparison. The historical incidence of this tumor in untreated female rats is 401/2,194 (18%), which is much lower than the 21/46 (46%) seen in the untreated controls in this study. The results of the statistical tests are also not significant when the incidences of chromophobe carcinomas and adenomas are combined for analysis.

Significant results in the negative direction are observed in the incidence of tumors of the hematopoietic system and in the combined incidence of chromophobe carcinomas and adenomas of the pituitary in male rats as well as in the incidences of C-cell carcinomas of the thyroid and of endometrial stromal polyps of the uterus in female rats. The results of the statistical analysis suggest that the incidence of adrenal medullary tumors in male rats is associated with the administration of reserpine.

Table 5. Analyses of the Incidence of Primary Tumors in Male Rats Administered Reserpine in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Fibroma of the Subcutaneous Tissue (b)	0/49 (0)	1/50 (2)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		Infinite	Infinite
Lower Limit		0.053	0.590
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	83	98
Hematopoietic System: Lymphoma or Leukemia (b)	21/49 (43)	18/50 (36)	13/50 (26)
P Values (c,d)	P=0.049 (N)	N.S.	N.S.
Relative Risk (e)		0.840	0.607
Lower Limit		0.488	0.319
Upper Limit		1.438	1.117
Weeks to First Observed Tumor	66	72	86
Pituitary: Chromophobe Carcinoma (b)	4/49 (8)	3/49 (6)	0/47 (0)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.750	0.000
Lower Limit		0.115	0.000
Upper Limit		4.201	1.123
Weeks to First Observed Tumor	95	77	--
Pituitary: Chromophobe Carcinoma or Adenoma (b)	17/49 (35)	13/49 (27)	6/47 (13)
P Values (c,d)	P=0.009 (N)	N.S.	P=0.011 (N)
Relative Risk (e)		0.765	0.368
Lower Limit		0.386	0.131
Upper Limit		1.481	0.883
Weeks to First Observed Tumor	77	49	104

Table 5. Analyses of the Incidence of Primary Tumors in Male Rats Administered Reserpine in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant (b)	3/48 (6)	18/49 (37)	24/48 (50)
P Values (c,d)	P less than 0.001	P less than 0.001	P less than 0.001
Relative Risk (e)		5.878	8.000
Lower Limit		1.875	2.684
Upper Limit		29.103	38.147
Weeks to First Observed Tumor	104	93	86
Thyroid: Follicular-cell Adenoma or Carcinoma (b)	1/49 (2)	3/49 (6)	0/50 (0)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		3.000	0.000
Lower Limit		0.251	0.000
Upper Limit		154.197	18.285
Weeks to First Observed Tumor	83	103	--
Thyroid: C-cell Adenoma or Carcinoma (b)	3/49 (6)	2/49 (4)	2/50 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.667	0.653
Lower Limit		0.058	0.057
Upper Limit		5.565	5.457
Weeks to First Observed Tumor	97	97	104
Pancreatic Islets: Islet-cell Carcinoma (b)	2/49 (4)	0/47 (0)	4/49 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.000	2.000
Lower Limit		0.000	0.302
Upper Limit		3.519	21.298
Weeks to First Observed Tumor	103	--	100

Table 5. Analyses of the Incidence of Primary Tumors in Male Rats Administered Reserpine in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pancreatic Islets: Islet-cell Carcinoma or Adenoma (b)	4/49 (8)	2/47 (4)	5/49 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.521	1.250
Lower Limit		0.049	0.286
Upper Limit		3.450	5.947
Weeks to First Observed Tumor	103	94	100
Testis: Interstitial-cell Tumor (b)	43/49 (88)	37/48 (77)	38/50 (76)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.878	0.866
Lower Limit		0.739	0.730
Upper Limit		1.080	1.067
Weeks to First Observed Tumor	78	80	61

(a) Dosed groups received 5 or 10 ppm.
(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 matched-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 6. Analyses of the Incidence of Primary Tumors in Female Rats Administered Reserpine in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia (b)	15/50 (30)	10/49 (20)	14/50 (28)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.680	0.933
Lower Limit		0.304	0.469
Upper Limit		1.453	1.845
Weeks to First Observed Tumor	7	75	62
Pituitary: Chromophobe Carcinoma (b)	0/46 (0)	5/48 (10)	0/45 (0)
P Values (c,d)	N.S.	P=0.031	--
Departure from Linear Trend (f)	P=0.002		
Relative Risk (e)		Infinite	--
Lower Limit		1.212	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	100	--
Pituitary: Chromophobe Adenoma or Carcinoma (b)	21/46 (46)	27/48 (56)	28/45 (62)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.232	1.363
Lower Limit		0.797	0.894
Upper Limit		1.914	2.066
Weeks to First Observed Tumor	89	53	62
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant (b)	1/49 (2)	3/48 (6)	4/49 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		3.063	4.000
Lower Limit		0.257	0.415
Upper Limit		157.336	192.766
Weeks to First Observed Tumor	104	75	99

Table 6. Analyses of the Incidence of Primary Tumors in Female Rats Administered Reserpine in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma (b)	4/48 (8)	2/48 (4)	0/48 (0)
P Values (c,d)	P=0.037 (N)	N.S.	N.S.
Relative Risk (e)		0.500	0.000
Lower Limit		0.047	0.000
Upper Limit		3.311	1.077
Weeks to First Observed Tumor	104	103	--
Thyroid: C-cell Carcinoma or Adenoma (b)	6/48 (13)	5/48 (10)	2/48 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.833	0.333
Lower Limit		0.215	0.034
Upper Limit		3.055	1.754
Weeks to First Observed Tumor	104	88	100
Mammary Gland: Fibroadenoma (b)	14/50 (28)	18/49 (37)	14/50 (28)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.312	1.000
Lower Limit		0.698	0.496
Upper Limit		2.509	2.018
Weeks to First Observed Tumor	97	75	97
Uterus: Endometrial Stromal Polyp (b)	10/50 (20)	5/49 (10)	2/49 (4)
P Values (c,d)	P=0.010 (N)	N.S.	P=0.015 (N)
Relative Risk (e)		0.510	0.204
Lower Limit		0.147	0.023
Upper Limit		1.510	0.894
Weeks to First Observed Tumor	30	66	100

Table 6. Analyses of the Incidence of Primary Tumors in Female Rats Administered Reserpine in the Diet (a)

(continued)

(a) Dosed groups received 5 or 10 ppm.
(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 matched-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.
(f) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of all dosed groups of male and female mice were lower than those of corresponding controls and were dose related throughout the bioassay (Figure 3). Partial closure or ptosis of the eyelids occurred in each animal among the dosed male and female mice but not in controls and was first observed as early as 2 days from the start of the study.

B. Survival (Mice)

Estimates of the probabilities of survival for male and female mice administered reserpine in the diet at the doses of this bioassay, together with those of the matched controls, are shown by the Kaplan and Meier curves in Figure 4. The result of the Tarone test is not significant in either sex.

In males, 41/50 (82%) of the high-dose, 35/50 (70%) of the low-dose, and 38/50 (76%) of the matched-control groups lived to the end of the study, and in females, 38/50 (76%) of the high-dose, 35/50 (70%) of the low-dose, and 44/50 (88%) of the matched-control groups lived to the end of the study.

C. Pathology (Mice)

Histopathologic findings on neoplasms in mice are summarized in Appendix B, Tables B1 and B2; findings on nonneoplastic lesions are summarized in Appendix D, Tables D1 and D2.

A variety of neoplasms occurred in both the matched-control and dosed groups. With the exception of proliferative and neoplastic lesions of the seminal vesicles in males and mammary glands in females, the neoplasms appeared with approximately equal frequency in dosed and control groups of mice. The incidence of inflammatory, proliferative, and neoplastic lesions

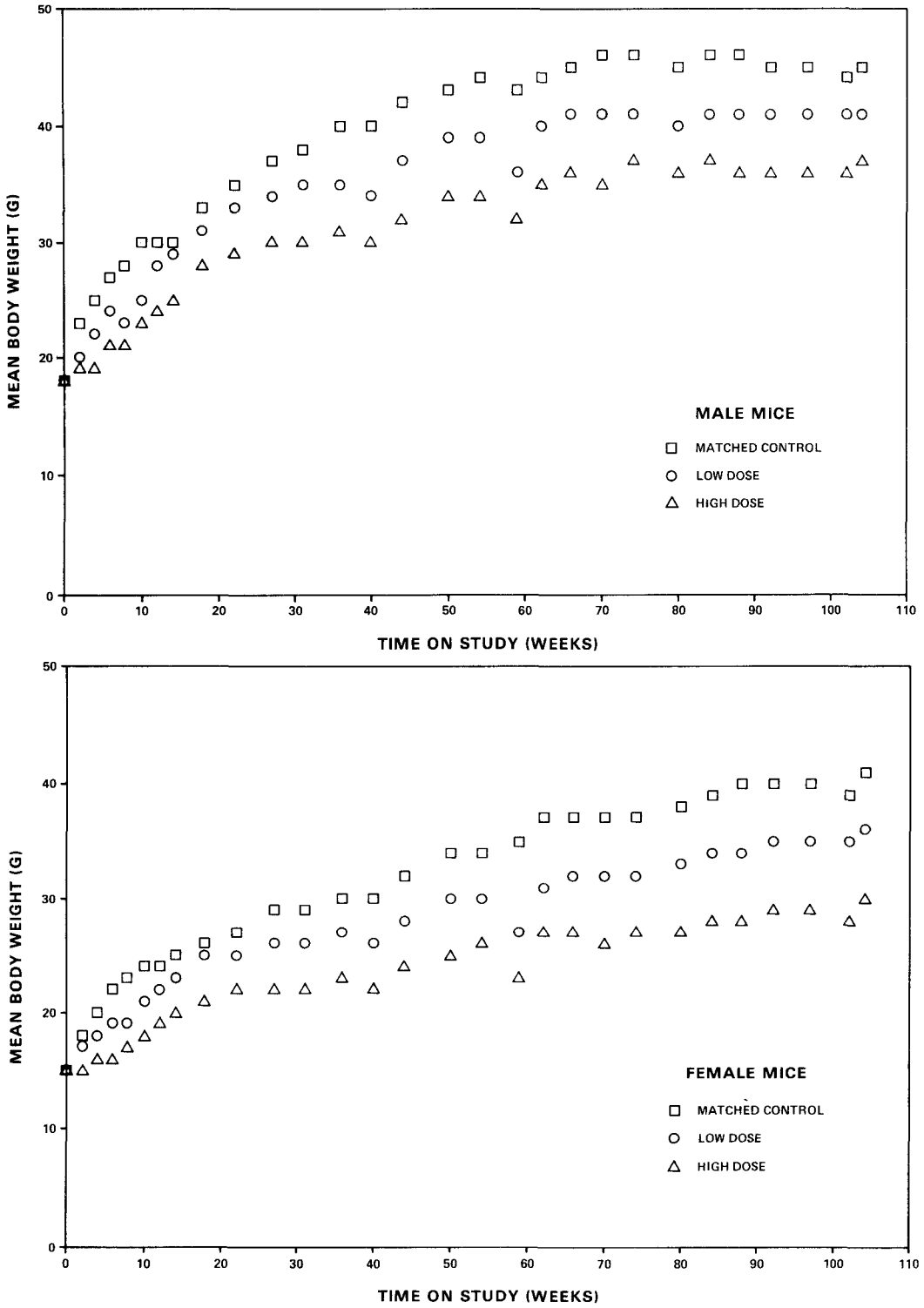


Figure 3. Growth Curves for Mice Administered Reserpine in the Diet

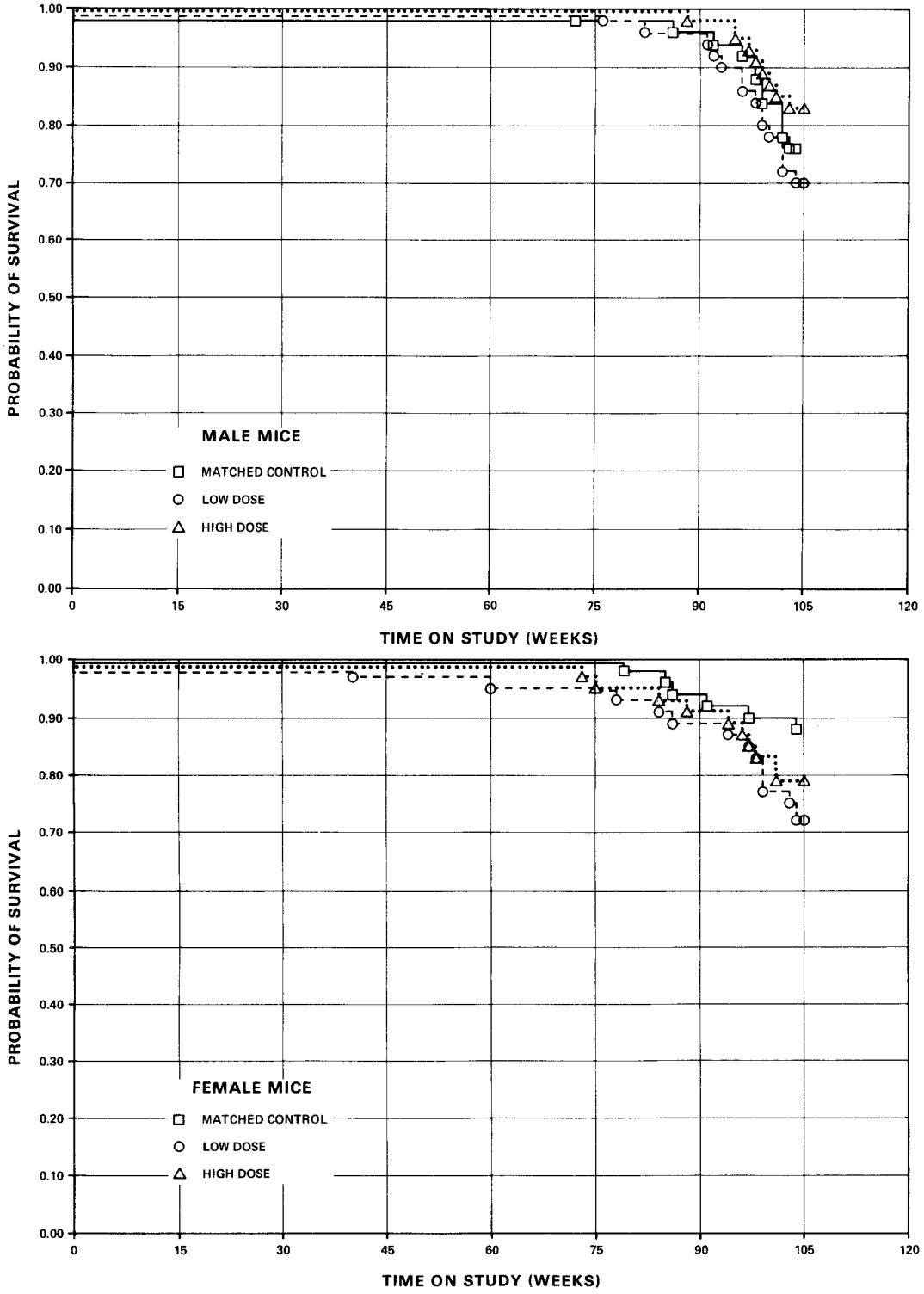


Figure 4. Survival Curves for Mice Administered Reserpine in the Diet

of the seminal vesicles in males and mammary glands in females are shown in Table 7.

The unusual neoplastic lesions in the seminal vesicles were reported as undifferentiated carcinomas. These tumors were characterized by considerable cellular pleomorphism and appeared to involve the glandular epithelium and muscular wall of the seminal vesicles, although their origin was uncertain. There were some areas of extensive spindle-cell proliferation with multiple discrete areas of anaplastic epithelial-like cells. The spindle cells had oval or elongated nuclei with variable amounts of streaming cytoplasm and appeared to be derived from the anaplastic cells. The anaplastic cells were pleomorphic with round, oval, or angular nuclei. The nuclei were often vesicular with a prominent nucleolus. Cytoplasm was usually abundant, but the borders were indistinct. In the areas of two tumors the cells were arranged in trabecular pattern.

Inflammatory lesions involving the seminal vesicles consisted of lymphocytic infiltration and fibrosis of the smooth muscle wall. A few dosed mice had focal papillary hyperplasia of the seminal vesicle epithelium. Focal areas of hyperplasia had variable degrees of atypia such as nuclear enlargement, prominent nucleoli, and vacuolated pale cytoplasm. Cells often appeared columnar rather than cuboidal. Neoplastic and inflammatory lesions of the seminal vesicles were confined to the dosed male mice; no lesions were observed in the control male mice.

An increased incidence of mammary gland tumors was observed in the dosed female mice as compared with the matched controls. Malignant mixed tumors have also been classified as adenocarcinoma Type C and fibroadenomas and were interpreted as being low-grade malignant. Mammary tumors in this study appeared to be compound related since none of the lesions occurred in the control females.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were also encountered in animals of the dosed and control groups. For the most part, the nonneoplastic lesions are commonly seen in aged mice and were not associated with increased mortalities or decreased life spans, except for those previously noted.

Table 7. Incidence of Inflammatory, Proliferative, and Neoplastic Lesions of the Seminal Vesicles in Male and Mammary Glands in Female Mice

Topography: Morphology	Matched Control	Low Dose	High Dose
SEMINAL VESICLES:			
(No. of Animals Examined)	50	50	49
Inflammation, Chronic	0(0%)	10(20%)	8(16%)
Fibrosis	0(0%)	13(26%)	5(10%)
Hyperplasia, Epithelial	0(0%)	4(8%)	4(8%)
Undifferentiated Carcinoma	0(0%)	1(2%)	5(10%)
MAMMARY GLANDS			
(No. of Animals Examined)	50	49	48
Adenocarcinoma, NOS	0(0%)	2(4%)	1(2%)
Adenosquamous Carcinoma	0(0%)	1(2%)	1(2%)
Mixed Tumor, Malignant	0(0%)	4(8%)	5(10%)
Cystic Ducts	0(0%)	4(8%)	6(13%)

The histopathologic examination provided evidence that, under the conditions of this bioassay, reserpine increased the incidences of undifferentiated carcinomas and inflammatory lesions of the seminal vesicles in males and of mammary tumors in female B6C3F1 mice.

D. Statistical Analysis of Results (Mice)

Tables 8 and 9 contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In male mice, the result of the Cochran-Armitage test on the incidence of undifferentiated carcinomas of the seminal vesicle is significant (P=0.010). The Fisher exact comparison of the incidences of the high-dose and control groups shows a P value of 0.027, which is above the 0.025 level required for significance when the Bonferroni inequality is used for multiple comparison. However, the historical records of this laboratory show no such tumor in a total of 586 B6C3F1 male mice. Using 1/586 as the parameter and assuming a binomial distribution, the probability of obtaining 5 or more tumors out of a sample size of 49 is less than 0.001.

The result of the Cochran-Armitage test on the incidence of female mice with all types of malignant tumors of the mammary gland is significant (P=0.011). Also, the result of the Fisher exact test shows that the incidence in each dosed group is significantly higher than that in the control group, P=0.006 in low-dose group and P=0.005 in high-dose group.

Significant results in the negative direction are observed in the combined incidence of hepatocellular carcinomas and adenomas of the liver in male mice.

The results of the statistical evaluation indicate that the incidences of undifferentiated carcinomas of the seminal vesicle in male mice and of tumors of the mammary gland in female mice are associated with administration of the test chemical.

Table 8. Analyses of the Incidence of Primary Tumors in Male Mice Administered Reserpine in the Diet (a)

Topography:Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	5/50 (10)	3/50 (6)	1/46 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.600	0.217
Lower Limit		0.098	0.005
Upper Limit		2.910	1.841
Weeks to First Observed Tumor	96	98	105
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	9/50 (18)	9/50 (18)	6/46 (13)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.000	0.725
Lower Limit		0.384	0.229
Upper Limit		2.603	2.093
Weeks to First Observed Tumor	72	93	14
Hematopoietic System: Lymphoma or Leukemia (b)	8/50 (16)	6/50 (12)	8/49 (16)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.750	1.020
Lower Limit		0.231	0.363
Upper Limit		2.281	2.869
Weeks to First Observed Tumor	86	76	88
Liver: Hepatocellular Carcinoma (b)	6/50 (12)	8/50 (16)	1/48 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.333	0.174
Lower Limit		0.438	0.004
Upper Limit		4.331	1.355
Weeks to First Observed Tumor	92	96	95
Liver: Hepatocellular Carcinoma or Adenoma (b)	12/50 (24)	14/50 (28)	4/48 (8)
P Values (c,d)	P=0.038 (N)	N.S.	P=0.033 (N)
Relative Risk (Matched Control)(e)		1.167	0.347
Lower Limit		0.559	0.087
Upper Limit		2.475	1.054
Weeks to First Observed Tumor	92	96	95
Seminal Vesicle: Undifferentiated Carcinoma (b)	0/50 (0)	1/50 (2)	5/49 (10)
P Values (c,d)	P=0.010	N.S.	P=0.027
Relative Risk (Matched Control)(e)		Infinite	Infinite
Lower Limit		0.054	1.287
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor		99	98
Harderian Gland: Adenona,NOS (b)	2/50 (4)	2/50 (4)	4/49 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (Matched Control)(e)		1.000	2.041
Lower Limit		0.075	0.308
Upper Limit		13.326	21.737
Weeks to First Observed Tumor	102	91	97

(a) Dosed groups received doses of 5 or 10 ppm in the diet.
(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 untreated-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 percent confidence interval of the relative risk between each dosed group and the control group.

Table 9. Analyses of the Incidence of Primary Tumors in Female Mice Administered Reserpine in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	4/50 (8)	4/49 (8)	4/48 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.020	1.042
Lower Limit		0.201	0.205
Upper Limit		5.183	5.286
Weeks to First Observed Tumor	104	105	94
Hematopoietic System: Lymphoma or Leukemia (b)	11/50 (22)	7/49 (14)	10/48 (21)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.649	0.947
Lower Limit		0.232	0.398
Upper Limit		1.676	2.223
Weeks to First Observed Tumor	85	84	73
Mammary Gland: Malignant Tumors, All Types (b)	0/50 (0)	7/49 (14)	7/48 (15)
P Values (c,d)	P=0.011	P=0.006	P=0.005
Relative Risk (e)		Infinite	Infinite
Lower Limit		1.981	2.023
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	60	84

(a) Dosed groups received 5 or 10 ppm.
(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 matched-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.

V. DISCUSSION

Mean body weights, slightly lower among dosed male rats than those of the corresponding controls through week 80, were essentially unaffected among females receiving the test chemical. Mean body weights of dosed groups of male and female mice were lower than those of corresponding controls and were dose-related throughout the study. Survival in the dosed and control groups of rats and mice was 76% or greater at week 90. The female rats may have been able to tolerate higher doses.

In male F344 rats, the incidence of pheochromocytomas of the adrenal medulla was dose-related (P less than 0.001), and the incidence in each dose group was significantly higher (P less than 0.021) when compared directly with the control group. Pheochromocytomas have been reported among 6.7% of 119 aging male F344 rats examined by Sass et al. (1975) and in the adrenal medulla of 8.8% of 158 two-year old male F344 rats by Goodman et al. (1979).

In the study reported here, pheochromocytomas in male F344 rats were detected in 6% (3/48) of the matched control, 37% (18/49) of the low-dose, and 50% (24/48) of the high-dose group. To verify these findings and assure accuracy of the diagnoses, specimens including tissues containing the lesions were examined by each member of an independent panel of experts who had no knowledge of the treatment groups (Capen, Holland and Nielsen, 1979). Within minor variations in tumor incidences and related descriptive phraseology, the findings of the panel (Appendix H) corroborated the conclusion of tumor induction based on findings reported by the pathologists in residence at the performing laboratory and the quality assurance panel that reviewed the initial diagnosis. In addition, according to Hollander and Snell (1976), it may be difficult to distinguish between hyperplasia and small pheochromocytomas. Thus, the majority of proliferative medullary lesions were neoplasms, as we have diagnosed, rather than hyperplasias. These findings relate the incidence of adrenal medullary pheochromocytomas in male F344 rats to the administration of reserpine. The induction of pheochromocytomas, which usually secrete epinephrine and norepinephrine (Chalfie and Perlman, 1976), possibly may be due to the existence of a compensating mechanism of the adrenal gland involving its depletion of bioamine stores by reserpine.

In female F344 rats, the occurrence of chromophobe carcinomas of the pituitary cannot be clearly related to the administration of reserpine because the only statistically significant incidence, which occurred in the low-dose group (P=0.031), was greater than the level required by the Bonferroni criterion for multiple comparison (P=0.025).

In previous studies, dietary administration of reserpine to rats has been associated with a shortening of the time to tumor appearance. Wistar rats fed a semi-liquid diet containing 100 ppm of reserpine for 18 months developed lymphosarcomas and hepatomas at an incidence of 16% among 135 rats (43 males and 92 females), compared with the absence of such tumors in a group of 20 males and 30 females administered control diet in solid form. First tumors occurred among females at 8 to 8.5 months and among males 2 months later. In another phase of this study, a combination of lymphosarcomas and hepatomas occurred as early as the 12th month in 13% of a group of 50 male and 80 female Wistar rats fed semi-liquid control diets (Tuchmann-Duplessis and Mercier-Parot, 1962). In a more recent study in which Wistar rats were fed diets containing 30 or 60 ppm reserpine for 75 weeks, no tumors or other histopathologic changes in the dosed groups were related to the test chemical (Tatematsu et al., 1978). Sprague-Dawley rats fed 8 ppm reserpine in the feed simultaneously with dimethylaminoazobenzene at 600 ppm developed a marginally higher incidence of tumors than did animals fed dimethylaminoazobenzene alone (Hurst et al., 1958). All studies previously reported for rats were considered as inadequate to assess the possible carcinogenicity of reserpine (IARC, 1976).

In B6C3F1 male mice, the cell origin of undifferentiated carcinomas of the seminal vesicles is not clear. Similarly, undifferentiated tumors have been reported in the seminal vesicles of Mastomys which were considered by some reviewers to be sarcomas rather than undifferentiated carcinomas. Nevertheless, all reviewers agreed that these Mastomys tumors were malignant neoplasms that probably arose in the seminal vesicles (Hollander and Higginson, 1971). The occurrence of the tumors in B6C3F1 mice was dose related (control 0/50, low-dose 1/50, high-dose 5/49; P=0.010). These tumors occurred with a significant incidence in the high-dose group (P=0.027), but they were not reported in the historical records of 586 male control mice at this laboratory. This level of significance is above that (P=0.025)

required for significance by the Bonferroni criterion for multiple comparison, but if a binomial distribution is assumed and the parameter of 1/586 is used, the probability of obtaining 5 or more of these tumors in a sample size of 49 is less than 0.001. In addition, the occurrence of nonneoplastic lesions of the seminal vesicles of dosed animals, including inflammation, fibrosis, and epithelial hyperplasia, supports the conclusion that the undifferentiated carcinomas of the seminal vesicle were associated with the administration of reserpine.

In B6C3F1 female mice, the occurrence of malignant tumors of the mammary gland was associated with administration of the test chemical. The incidences in the dosed groups were significantly higher (high-dose, P=0.005; low-dose, P=0.006) than those in the control group, and the results of the test for a dose-related trend were significant (P=0.011). Malignant tumors of the mammary gland may be related to the known action of reserpine in stimulating mammary growth and lactation in experimental animals (Gaunt et al., 1963).

In previous studies, dietary administration of reserpine to female mice predisposed to mammary cancer (45% - 55% spontaneous incidence) has been associated with shortening the time to tumor appearance as well as with a possible increase in the incidence of mammary tumors. Female C3H/He mice fed an average dietary dose of 0.24 micrograms of reserpine per day for 18 months developed mammary tumors at an incidence of 15/24 (62.5%), compared with an incidence of 12/22 (54.5%) among controls. The earliest tumors in the dosed animals appeared by day 216, with most occurring between the 8th and 11th months; the earliest tumors in the controls appeared by day 320, with all occurring between the 11th and 17th months (Lacassagne and Duplan, 1959). In comparable tests using a resistant strain of female mice (XVII nc), no tumors developed in either dosed or control groups. These studies were considered as inadequate to assess the possible carcinogenicity of reserpine in mice.

Several epidemiological studies have been performed on the incidence of tumors among humans exposed to reserpine. Available evidence does not link the use of reserpine with tumor incidence in males. Occurrence of breast cancer among women has been associated with the use of reserpine in several surveys, but the indicated risk from exposure is inconsistent. The lack of

adequate testing in earlier experimental animal studies precluded any conclusion regarding the existence of a risk (IARC, 1976). Our present study in rats and mice strongly indicates a potential risk to humans.

Under the conditions of this bioassay, reserpine was carcinogenic in male F344 rats, inducing pheochromocytomas of the adrenal, and in B6C3F1 mice, inducing malignant tumors of the mammary gland in females and undifferentiated carcinomas of the seminal vesicles in males. Reserpine was not carcinogenic for female F344 rats.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED RESERPINE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED RESERPINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(50)
SQUAMOUS CELL PAPILLOMA			1 (2%)
SQUAMOUS CELL CARCINOMA	1 (2%)		
SEBACEOUS ADENOMA			2 (4%)
KERATOACANTHOMA	1 (2%)		1 (2%)
*SUBCUT TISSUE	(49)	(50)	(50)
SARCOMA, NOS	1 (2%)		
FIBROMA		1 (2%)	3 (6%)
FIBROSARCOMA			2 (4%)
LIPOMA		1 (2%)	
OSTEOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(49)	(49)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)		
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
C-CELL CARCINOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(50)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	3 (6%)	1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
UNDIFFERENTIATED LEUKEMIA	18 (37%)	16 (32%)	11 (22%)
LYMPHOCYTIC LEUKEMIA			1 (2%)
#THYMUS	(48)	(49)	(48)
THYMOMA			1 (2%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
*TONGUE	(49)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	2 (4%)	1 (2%)	1 (2%)
SQUAMOUS CELL CARCINOMA			1 (2%)
#LIVER	(49)	(49)	(50)
HEPATOCELLULAR ADENOMA		1 (2%)	1 (2%)
#PANCREAS	(49)	(47)	(49)
ACINAR-CELL ADENOMA	1 (2%)		1 (2%)
#STOMACH	(49)	(49)	(50)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
#SMALL INTESTINE	(49)	(44)	(49)
ADENOCARCINOMA, NOS	2 (4%)		
MUCINOUS ADENOCARCINOMA			1 (2%)
#JEJUNUM	(49)	(44)	(49)
FIBROSARCOMA			1 (2%)
URINARY SYSTEM			
#URINARY BLADDER	(46)	(45)	(47)
TRANSITIONAL-CELL PAPILLOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(49)	(49)	(47)
CHROMOPHOBE ADENOMA	13 (27%)	10 (20%)	6 (13%)
CHROMOPHOBE CARCINOMA	4 (8%)	3 (6%)	
ACIDOPHIL ADENOMA	1 (2%)		
#ADRENAL	(48)	(49)	(48)
CORTICAL CARCINOMA	1 (2%)		1 (2%)
PHEOCHROMOCYTOMA	3 (6%)	14 (29%)	15 (31%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (2%)	4 (8%)	9 (19%)
#ADRENAL MEDULLA	(48)	(49)	(48)
NEUROBLASTOMA		1 (2%)	
#THYROID	(49)	(49)	(50)
FOLLICULAR-CELL ADENOMA	1 (2%)	2 (4%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
FOLLICULAR-CELL CARCINOMA		1 (2%)	
C-CELL ADENOMA	2 (4%)	1 (2%)	1 (2%)
C-CELL CARCINOMA	1 (2%)	1 (2%)	1 (2%)
#PANCREATIC ISLETS	(49)	(47)	(49)
ISLET-CELL ADENOMA	2 (4%)	2 (4%)	1 (2%)
ISLET-CELL CARCINOMA	2 (4%)		4 (8%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(50)	(50)
ADENOCARCINOMA, NOS	1 (2%)		
FIBROADENOMA	1 (2%)	1 (2%)	
*PREPUTIAL GLAND	(49)	(50)	(50)
ADENOMA, NOS	1 (2%)		1 (2%)
*SEMINAL VESICLE	(49)	(50)	(50)
ADENOMA, NOS	1 (2%)		
#TESTIS	(49)	(48)	(50)
INTERSTITIAL-CELL TUMOR	43 (88%)	37 (77%)	38 (76%)
NERVOUS SYSTEM			
#BRAIN	(49)	(49)	(49)
SARCOMA, NOS, UNC PRIM OR META	1 (2%)		
SPECIAL SENSE ORGANS			
*EAR CANAL	(49)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(49)	(50)	(50)
LIPOMA		1 (2%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*TUNICA VAGINALIS	(49)	(50)	(50)
MESOTHELIOMA, NOS	1 (2%)		1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(49)	(50)	(50)
SARCOMA, NOS		1 (2%)	
SARCOMA, NOS, METASTATIC	1 (2%)		
MESOTHELIOMA, NOS			2 (4%)
MESOTHELIOMA, MALIGNANT		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	4	7	8
MORIBUND SACRIFICE	24	23	14
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	22	20	28
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	49	48	48
TOTAL PRIMARY TUMORS	109	105	109
TOTAL ANIMALS WITH BENIGN TUMORS	47	44	44
TOTAL BENIGN TUMORS	73	74	72
TOTAL ANIMALS WITH MALIGNANT TUMORS	29	28	29
TOTAL MALIGNANT TUMORS	34	31	34
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	
TOTAL SECONDARY TUMORS	1	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1		3
TOTAL UNCERTAIN TUMORS	1		3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC	1		
TOTAL UNCERTAIN TUMORS	1		

* 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 RATS ADMINISTERED RESERPINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(49)	(50)
BASAL-CELL CARCINOMA			1 (2%)
SARCOMA, NOS	1 (2%)		
FIBROMA	1 (2%)		2 (4%)
FIBROSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(48)	(48)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
SARCOMA, NOS, METASTATIC	2 (4%)	1 (2%)	
OSTEOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(50)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (2%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
UNDIFFERENTIATED LEUKEMIA	14 (28%)	10 (20%)	12 (24%)
LYMPHOCYTIC LEUKEMIA			1 (2%)
#PANCREATIC L.NODE	(50)	(48)	(50)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*TONGUE	(50)	(49)	(50)
SQUAMOUS CELL PAPILLOMA		1 (2%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(50)	(49)	(50)
HEPATOCELLULAR ADENOMA	1 (2%)		
#JEJUNUM	(47)	(48)	(48)
LEIOMYOSARCOMA	1 (2%)		
*RECTAL MUCOUS MEMBRA	(50)	(49)	(50)
SARCOMA, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY/PELVIS	(50)	(49)	(47)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(48)	(45)
ADENOMA, NOS		1 (2%)	
CHROMOPHOBE ADENOMA	21 (46%)	22 (46%)	28 (62%)
CHROMOPHOBE CARCINOMA		5 (10%)	
#ADRENAL	(49)	(48)	(49)
CORTICAL ADENOMA	1 (2%)	1 (2%)	1 (2%)
PHEOCHROMOCYTOMA	1 (2%)	3 (6%)	3 (6%)
PHEOCHROMOCYTOMA, MALIGNANT			1 (2%)
#THYROID	(48)	(48)	(48)
FOLLICULAR-CELL ADENOMA			1 (2%)
FOLLICULAR-CELL CARCINOMA			1 (2%)
C-CELL ADENOMA	2 (4%)	3 (6%)	2 (4%)
C-CELL CARCINOMA	4 (8%)	2 (4%)	
#PANCREATIC ISLETS	(50)	(48)	(49)
ISLET-CELL ADENOMA		1 (2%)	
ISLET-CELL CARCINOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(49)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
CYSTADENOMA, NOS			1 (2%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CYSTADENOCARCINOMA, NOS			1 (2%)
PAPILLARY CYSTADENOMA, NOS			1 (2%)
ACINAR-CELL ADENOMA	1 (2%)		
FIBROADENOMA	14 (28%)	18 (37%)	14 (28%)
*PREPUTIAL GLAND	(50)	(49)	(50)
CARCINOMA,NOS			1 (2%)
ADENOMA, NOS		2 (4%)	
#UTERUS	(50)	(49)	(49)
CARCINOMA,NOS		1 (2%)	
SARCOMA, NOS			1 (2%)
ENDOMETRIAL STROMAL POLYP	10 (20%)	5 (10%)	2 (4%)
ENDOMETRIAL STROMAL SARCOMA		1 (2%)	
#UTERUS/ENDOMETRIUM	(50)	(49)	(49)
ADENOCARCINOMA, NOS	1 (2%)		
#UTERUS/MYOMETRIUM	(50)	(49)	(49)
LEIOMYOMA	1 (2%)		
NERVOUS SYSTEM			
#BRAIN	(50)	(48)	(49)
EPENDYMOMA			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*FEMUR	(50)	(49)	(50)
OSTEOSARCOMA		1 (2%)	
*TIBIA	(50)	(49)	(50)
OSTEOSARCOMA		1 (2%)	
BODY CAVITIES			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(49)	(50)
SARCOMA, NOS	1 (2%)	2 (4%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	5	4	4
MORIBUND SACRIFICE	12	15	21
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	33	31	25
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	42	45	45
TOTAL PRIMARY TUMORS	77	86	76
TOTAL ANIMALS WITH BENIGN TUMORS	37	39	35
TOTAL BENIGN TUMORS	53	57	55
TOTAL ANIMALS WITH MALIGNANT TUMORS	23	22	20
TOTAL MALIGNANT TUMORS	24	29	21
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	2	
TOTAL SECONDARY TUMORS	2	2	
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 NEOPLASMS IN
MICE ADMINISTERED RESERPINE IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED RESERPINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(49)
SQUAMOUS CELL PAPILLOMA		1 (2%)	1 (2%)
SQUAMOUS CELL CARCINOMA	1 (2%)	1 (2%)	
SARCOMA, NOS	1 (2%)		
FIBROMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(46)
ALVEOLAR/BRONCHIOLAR ADENOMA	4 (8%)	7 (14%)	5 (11%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	5 (10%)	3 (6%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)	2 (4%)	3 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	3 (6%)	1 (2%)	
MALIGNANT LYMPHOMA, MIXED TYPE	1 (2%)		1 (2%)
LYMPHOCYTIC LEUKEMIA	2 (4%)		
#MANDIBULAR L. NODE	(50)	(50)	(48)
MAST-CELL TUMOR		1 (2%)	
#MESENTERIC L. NODE	(50)	(50)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			2 (4%)
#LIVER	(50)	(50)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#PEYERS PATCH	(50)	(50)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	2 (4%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(49)
HEMANGIOSARCOMA	1 (2%)		
#SPLEEN	(50)	(50)	(47)
HEMANGIOMA	1 (2%)		
HEMANGIOSARCOMA		1 (2%)	1 (2%)
#LIVER	(50)	(50)	(48)
HEMANGIOMA	1 (2%)		
*MESENTERY	(50)	(50)	(49)
HEMANGIOSARCOMA		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(48)
HEPATOCELLULAR ADENOMA	7 (14%)	7 (14%)	3 (6%)
HEPATOCELLULAR CARCINOMA	6 (12%)	8 (16%)	1 (2%)
#STOMACH	(50)	(50)	(48)
SIGNET RING CARCINOMA	1 (2%)		
#DUODENUM	(50)	(50)	(48)
ADENOCARCINOMA, NOS	1 (2%)		
URINARY SYSTEM			
#URINARY BLADDER	(50)	(50)	(48)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	
ENDOCRINE SYSTEM			
#ADRENAL	(50)	(50)	(48)
CORTICAL ADENOMA	1 (2%)	1 (2%)	
PHEOCHROMOCYTOMA, MALIGNANT			1 (2%)
#THYROID	(48)	(49)	(45)
FOLLICULAR-CELL ADENOMA		1 (2%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(50)	(50)	(49)
UNDIFFERENTIATED CARCINOMA		1 (2%)	5 (10%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(49)
ADENOMA, NOS	2 (4%)	2 (4%)	4 (8%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(50)	(50)	(49)
LIPOMA		2 (4%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(49)
SARCOMA, NOS		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	2	6	4
MORIBUND SACRIFICE	10	9	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	38	35	41
ANIMAL MISSING			1

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	30	34	25
TOTAL PRIMARY TUMORS	40	45	31
TOTAL ANIMALS WITH BENIGN TUMORS	14	18	13
TOTAL BENIGN TUMORS	16	21	14
TOTAL ANIMALS WITH MALIGNANT TUMORS	22	18	14
TOTAL MALIGNANT TUMORS	24	23	17
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED RESERPINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			2
ANIMALS NECROPSIED	50	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	48
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(48)
TRICHOEPITHELIOMA	1 (2%)		
*SUBCUT TISSUE	(50)	(49)	(48)
SARCOMA, NOS	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA	4 (8%)	4 (8%)	3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
ADENOSQUAMOUS CARCINOMA, METASTA		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(48)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)	3 (6%)	2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (4%)	1 (2%)	1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE	3 (6%)	1 (2%)	3 (6%)
LYMPHOCYTIC LEUKEMIA	1 (2%)		3 (6%)
#SPLEEN	(50)	(49)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
#LIVER	(50)	(49)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#STOMACH	(50)	(49)	(48)
MAST-CELL SARCOMA			1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#PEYERS PATCH	(50)	(49)	(48)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)		
#DUODENUM	(50)	(49)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(50)	(49)	(48)
HEMANGIOMA	1 (2%)		1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(48)
HEPATOCELLULAR ADENOMA	2 (4%)		1 (2%)
#GASTRIC MUCOSA	(50)	(49)	(48)
ADENOMA, NOS			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(47)	(47)
CHROMOPHOBE ADENOMA		1 (2%)	
ACIDOPHIL ADENOMA		1 (2%)	
#THYROID	(50)	(46)	(48)
FOLLICULAR-CELL ADENOMA		1 (2%)	2 (4%)
C-CELL ADENOMA	1 (2%)		
#PANCREATIC ISLETS	(50)	(49)	(48)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(49)	(48)
ADENOCARCINOMA, NOS		2 (4%)	1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ADENOSQUAMOUS CARCINOMA		1 (2%)	1 (2%)
MIXED TUMOR, MALIGNANT		4 (8%)	5 (10%)
*VAGINA	(50)	(49)	(48)
SARCOMA, NOS	1 (2%)		
#OVARY	(50)	(49)	(48)
HILAR-CELL TUMOR	1 (2%)		
LIPOMA	1 (2%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(49)	(48)
ADENOMA, NOS	2 (4%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(50)	(49)	(48)
LIPOMA	1 (2%)		
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	4	6	5
MORIBUND SACRIFICE	2	7	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		2	
TERMINAL SACRIFICE	44	35	38
ANIMAL MISSING			2

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	21	19	23
TOTAL PRIMARY TUMORS	27	22	27
TOTAL ANIMALS WITH BENIGN TUMORS	14	7	8
TOTAL BENIGN TUMORS	14	8	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	13	13	18
TOTAL MALIGNANT TUMORS	13	14	19
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1
TOTAL SECONDARY TUMORS		1	1
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 C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS ADMINISTERED RESERPINE IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED RESERPINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)	4 (8%)	3 (6%)
INFLAMMATION, SUPPURATIVE			2 (4%)
ABSCESS, CHRONIC			1 (2%)
HYPERPLASIA, BASAL CELL		1 (2%)	
*SUBCUT TISSUE	(49)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)		
INFLAMMATION, SUPPURATIVE			1 (2%)
ABSCESS, CHRONIC			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(49)	(49)	(49)
ATELECTASIS	1 (2%)		
INFLAMMATION, MULTIFOCAL	1 (2%)		
INFLAMMATION, SUPPURATIVE	1 (2%)	1 (2%)	
INFLAMMATION, NECROTIZING	2 (4%)	1 (2%)	
PNEUMONIA, CHRONIC MURINE	2 (4%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)		1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(49)	(47)	(48)
MYELOFIBROSIS		1 (2%)	
#SPLEEN	(49)	(47)	(50)
FIBROSIS, FOCAL	1 (2%)	2 (4%)	
NECROSIS, FOCAL	1 (2%)		
HYPERPLASIA, LYMPHOID	1 (2%)		
#LUNG/BRONCHUS	(49)	(49)	(49)
HYPERPLASIA, LYMPHOID	2 (4%)	2 (4%)	2 (4%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
#MESENTERIC L. NODE	(49)	(49)	(50)
LYMPHANGIECTASIS			1 (2%)
#HEART/ATRIUM	(49)	(49)	(49)
THROMBUS, ORGANIZED	1 (2%)		1 (2%)
#AURICULAR APPENDAGE	(49)	(49)	(49)
THROMBUS, ORGANIZED		2 (4%)	1 (2%)
#MYOCARDIUM	(49)	(49)	(49)
INFLAMMATION, INTERSTITIAL	26 (53%)	17 (35%)	25 (51%)
*AORTA	(49)	(50)	(50)
INFLAMMATION, CHRONIC	1 (2%)		
*MESENTERY	(49)	(50)	(50)
PERIARTERITIS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(49)	(49)	(50)
CONGESTION, PASSIVE			1 (2%)
CYTOPLASMIC VACUOLIZATION	2 (4%)	2 (4%)	1 (2%)
BASOPHILIC CYTO CHANGE		1 (2%)	
CYTOLOGIC ALTERATION, NOS			1 (2%)
#BILE DUCT	(49)	(49)	(50)
HYPERPLASIA, NOS	1 (2%)		2 (4%)
#PANCREAS	(49)	(47)	(49)
INFLAMMATION, INTERSTITIAL	3 (6%)	2 (4%)	3 (6%)
INFLAMMATION, CHRONIC	1 (2%)		
#STOMACH	(49)	(49)	(50)
INFLAMMATION, NECROTIZING	2 (4%)		
#GASTRIC MUCOSA	(49)	(49)	(50)
MINERALIZATION	1 (2%)		
#GASTRIC SUBMUCOSA	(49)	(49)	(50)
EDEMA, NOS	1 (2%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC SUPPURATIV	1 (2%)		
#SMALL INTESTINE	(49)	(44)	(49)
INFLAMMATION, FOCAL GRANULOMATOU	1 (2%)		
*RECTAL SUBMUCOSA	(49)	(50)	(50)
HEMORRHAGE		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(49)	(47)	(49)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC	35 (71%)	12 (26%)	21 (43%)
ENDOCRINE SYSTEM			
#PITUITARY	(49)	(49)	(47)
HYPERPLASIA, CHROMOPHOBE-CELL		1 (2%)	
#ADRENAL	(48)	(49)	(48)
HYPERPLASIA, FOCAL			1 (2%)
#ADRENAL CORTEX	(48)	(49)	(48)
LIPOIDOSIS	1 (2%)		
EOSINOPHILIC CYTO CHANGE	1 (2%)		
HYPERPLASIA, NODULAR		1 (2%)	
#ADRENAL MEDULLA	(48)	(49)	(48)
NECROSIS, DIFFUSE		1 (2%)	
HYPERPLASIA, FOCAL	3 (6%)	4 (8%)	3 (6%)
#THYROID	(49)	(49)	(50)
HYPERPLASIA, C-CELL		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(50)	(50)
CYSTIC DUCTS	3 (6%)	1 (2%)	
*MAMMARY LOBULE	(49)	(50)	(50)
HYPERPLASIA, NOS	1 (2%)		
*PREPUCE	(49)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
#PROSTATE	(48)	(45)	(47)
INFLAMMATION, SUPPURATIVE	6 (13%)	4 (9%)	2 (4%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC SUPPURATIV	1 (2%)		
FIBROSIS	1 (2%)		
NERVOUS SYSTEM			
#CEREBRUM	(49)	(49)	(49)
MALACIA	1 (2%)		
#BRAIN	(49)	(49)	(49)
HEMORRHAGE	3 (6%)		
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
#CEREBELLUM	(49)	(49)	(49)
HEMORRHAGE		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE/RETINA	(49)	(50)	(50)
ATROPHY, NOS		1 (2%)	
*EYELID	(49)	(50)	(50)
INFLAMMATION, FOCAL		1 (2%)	
INFLAMMATION, SUPPURATIVE			1 (2%)
*MIDDLE EAR	(49)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(49)	(50)	(50)
STEATITIS	3 (6%)	2 (4%)	3 (6%)
ALL OTHER SYSTEMS			
NONE			

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			1
AUTOLYSIS/NO NECROPSY	1		

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED RESERPINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(50)
ULCER, FOCAL	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC SUPPURATIV		1 (2%)	
RESPIRATORY SYSTEM			
#TRACHEA	(49)	(48)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
#LUNG	(48)	(48)	(50)
INFLAMMATION, MULTIFOCAL		1 (2%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	
PNEUMONIA, CHRONIC MURINE	1 (2%)		
INFLAMMATION, CHRONIC SUPPURATIV		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(50)	(49)	(50)
HYPERPLASIA, RETICULUM CELL		1 (2%)	
#SPLEEN	(49)	(48)	(49)
HEMATOPOIESIS	2 (4%)	4 (8%)	1 (2%)
#LUNG/BRONCHUS	(48)	(48)	(50)
HYPERPLASIA, LYMPHOID	1 (2%)		1 (2%)
CIRCULATORY SYSTEM			
#HEART/ATRIUM	(49)	(49)	(50)
THROMBUS, ORGANIZED		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#MYOCARDIUM	(49)	(49)	(50)
INFLAMMATION, INTERSTITIAL	8 (16%)	11 (22%)	10 (20%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(50)
CYTOPLASMIC VACUOLIZATION		1 (2%)	5 (10%)
BASOPHILIC CYTO CHANGE	5 (10%)		2 (4%)
#PANCREAS	(50)	(48)	(49)
INFLAMMATION, INTERSTITIAL			1 (2%)
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(47)
PYELONEPHRITIS, NOS	1 (2%)		
INFLAMMATION, CHRONIC	2 (4%)		1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(48)	(45)
HYPERPLASIA, CHROMOPHOBE-CELL	1 (2%)	1 (2%)	2 (4%)
#ADRENAL	(49)	(48)	(49)
HEMORRHAGE			1 (2%)
CYTOPLASMIC VACUOLIZATION			1 (2%)
#ADRENAL CORTEX	(49)	(48)	(49)
CYTOPLASMIC VACUOLIZATION	1 (2%)	1 (2%)	1 (2%)
#THYROID	(48)	(48)	(48)
HYPERPLASIA, C-CELL	1 (2%)	3 (6%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(49)	(50)
CYSTIC DUCTS	10 (20%)	17 (35%)	12 (24%)
*MAMMARY LOBULE	(50)	(49)	(50)
HYPERPLASIA, NOS	1 (2%)		1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*PREPUTIAL GLAND	(50)	(49)	(50)
METAPLASIA, SQUAMOUS		1 (2%)	
#UTERUS	(50)	(49)	(49)
INFLAMMATION, SUPPURATIVE		1 (2%)	
#UTERUS/ENDOMETRIUM	(50)	(49)	(49)
INFLAMMATION, SUPPURATIVE	8 (16%)	5 (10%)	5 (10%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (2%)		
HYPERPLASIA, NOS	7 (14%)	6 (12%)	
HYPERPLASIA, EPITHELIAL			1 (2%)
HYPERPLASIA, FOCAL	1 (2%)		
HYPERPLASIA, CYSTIC	12 (24%)	4 (8%)	5 (10%)
#OVARY	(50)	(49)	(50)
INFLAMMATION, SUPPURATIVE			2 (4%)
INFLAMMATION, CHRONIC SUPPURATIV	2 (4%)	1 (2%)	1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(50)	(49)	(50)
STEATITIS	4 (8%)	1 (2%)	2 (4%)
ABSCESS, CHRONIC	1 (2%)		
ALL OTHER SYSTEMS			
DIAPHRAGM			
STEATITIS			1

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	1	2
AUTOLYSIS/NO NECROPSY		1	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED RESERPINE IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED RESERPINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(49)
EPIDERMAL INCLUSION CYST			1 (2%)
*SUBCUT TISSUE	(50)	(50)	(49)
INFLAMMATION, FOCAL		1 (2%)	
ABSCESS, CHRONIC		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(46)
INFLAMMATION, NECROTIZING	1 (2%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)		
#LUNG/ALVEOLI	(50)	(50)	(46)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, ACUTE FOCAL	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(49)
HYPERPLASIA, LYMPHOID	1 (2%)		
#SPLEEN	(50)	(50)	(47)
FIBROSIS, FOCAL			1 (2%)
HYPERPLASIA, LYMPHOID	2 (4%)		
HEMATOPOIESIS	1 (2%)		4 (9%)
#MESENTERIC L. NODE	(50)	(50)	(48)
HYPERPLASIA, LYMPHOID	1 (2%)		
#RENAL LYMPH NODE	(50)	(50)	(48)
HYPERPLASIA, LYMPHOID		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#INGUINAL LYMPH NODE	(50)	(50)	(48)
INFLAMMATION, SUPPURATIVE			1 (2%)
HYPERPLASIA, LYMPHOID			1 (2%)
#LIVER	(50)	(50)	(48)
HEMATOPOIESIS		1 (2%)	
#PEYERS PATCH	(50)	(50)	(48)
HYPERPLASIA, LYMPHOID		1 (2%)	
#KIDNEY	(50)	(50)	(48)
HYPERPLASIA, LYMPHOID	1 (2%)		
CIRCULATORY SYSTEM			
#HEART/ATRIUM	(49)	(50)	(47)
THROMBUS, ORGANIZED	1 (2%)		
#MYOCARDIUM	(49)	(50)	(47)
INFLAMMATION, INTERSTITIAL	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(48)
NECROSIS, COAGULATIVE	1 (2%)		1 (2%)
CYTOPLASMIC VACUOLIZATION	1 (2%)	2 (4%)	
BASOPHILIC CYTO CHANGE			1 (2%)
ANGIECTASIS	1 (2%)		1 (2%)
#PANCREATIC ACINUS	(50)	(49)	(48)
ATROPHY, FOCAL	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(48)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		
#KIDNEY/CAPSULE	(50)	(50)	(48)
INFLAMMATION, SUPPURATIVE			1 (2%)
ENDOCRINE SYSTEM			
NONE			

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(50)	(49)
DILATATION, NOS	1 (2%)		
CYSTIC DUCTS	1 (2%)	1 (2%)	
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC SUPPURATIV	1 (2%)		
*SEMINAL VESICLE	(50)	(50)	(49)
HEMORRHAGE			1 (2%)
INFLAMMATION, MULTIFOCAL		1 (2%)	
INFLAMMATION, CHRONIC		10 (20%)	6 (12%)
INFLAMMATION, CHRONIC FOCAL			2 (4%)
FIBROSIS		12 (24%)	5 (10%)
FIBROSIS, FOCAL		1 (2%)	
HYPERPLASIA, EPITHELIAL		4 (8%)	4 (8%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(49)
PHTHISIS BULBI	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*KNEE JOINT	(50)	(50)	(49)
EXOSTOSIS		1 (2%)	
BODY CAVITIES			
*MESENTERY	(50)	(50)	(49)
STEATITIS		1 (2%)	1 (2%)
ALL OTHER SYSTEMS			
NONE			

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	15	9	16
ANIMAL MISSING/NO NECROPSY			1

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED RESERPINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			2
ANIMALS NECROPSIED	50	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	48
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(49)	(48)
HEMATOMA, NOS			1 (2%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(48)
HYPERPLASIA, LYMPHOID	6 (12%)	1 (2%)	2 (4%)
#BONE MARROW	(50)	(48)	(48)
MYELOFIBROSIS	5 (10%)		
#SPLEEN	(50)	(49)	(48)
HYPERPLASIA, LYMPHOID	3 (6%)	1 (2%)	1 (2%)
HEMATOPOIESIS			1 (2%)
#MESENTERIC L. NODE	(50)	(49)	(48)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
#RENAL LYMPH NODE	(50)	(49)	(48)
HYPERPLASIA, LYMPHOID	1 (2%)		
#LUNG/BRONCHUS	(50)	(49)	(48)
HYPERPLASIA, LYMPHOID			1 (2%)
#LUNG	(50)	(49)	(48)
HYPERPLASIA, LYMPHOID	3 (6%)	1 (2%)	2 (4%)
#LIVER	(50)	(49)	(48)
HYPERPLASIA, LYMPHOID			1 (2%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIESIS		1 (2%)	
#KIDNEY/PELVIS	(50)	(49)	(48)
HYPERPLASIA, LYMPHOID		1 (2%)	
CIRCULATORY SYSTEM			
*PULMONARY ARTERY	(50)	(49)	(48)
HYPERTROPHY, NOS	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(48)
NECROSIS, COAGULATIVE			1 (2%)
CYTOPLASMIC VACUOLIZATION		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(48)
INFLAMMATION, CHRONIC	1 (2%)		
ENDOCRINE SYSTEM			
#RIGHT ADRENAL GLAND	(50)	(49)	(48)
ATROPHY, NOS	1 (2%)		
#LEFT ADRENAL GLAND	(50)	(49)	(48)
HYPERTROPHY, COMPENSATORY	1 (2%)		
#ADRENAL CORTEX	(50)	(49)	(48)
HYPERPLASIA, FOCAL		1 (2%)	
#THYROID	(50)	(46)	(48)
CYSTIC FOLLICLES	2 (4%)	1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(49)	(48)
CYSTIC DUCTS		4 (8%)	6 (13%)
#UTERUS	(50)	(49)	(47)
DECIDUAL ALTERATION, NOS		1 (2%)	1 (2%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#UTERUS/ENDOMETRIUM	(50)	(49)	(47)
EDEMA, NOS	1 (2%)		
INFLAMMATION, SUPPURATIVE	1 (2%)		
HYPERPLASIA, NOS		2 (4%)	
HYPERPLASIA, CYSTIC	42 (84%)	18 (37%)	14 (30%)
#OVARY	(50)	(49)	(48)
FOLLICULAR CYST, NOS	1 (2%)	1 (2%)	
INFLAMMATION, CHRONIC SUPPURATIV			1 (2%)
HYPERPLASIA, STROMAL		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(50)	(49)	(48)
INFLAMMATION, OSSIFYING	1 (2%)		
PHTHISIS BULBI	1 (2%)		
*HARDERIAN GLAND	(50)	(49)	(48)
FIBROSIS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	13	10

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL MISSING/NO NECROPSY			2
AUTOLYSIS/NO NECROPSY		1	

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

APPENDIX E

ANALYSIS OF RESERPINE

APPENDIX E

Analysis of Reserpine
Midwest Research Institute

A. Elemental Analysis

Element:	C	H	N
Theory:	65.11	6.62	4.60
Found:	65.22	6.48	4.54
	65.01	6.57	4.59

B. Physical Properties

1. Melting point — Literature: 264-267°C decomp. (Neuss et al., 1954).
   Found: 260-267°C decomp.

2. Optical rotation — Literature: $[a]_D^{26}$ = -115 for a 1.03% solution in chloroform (Neuss et al., 1954).
   Found: $[a]_D^{30}$ = -114.94 for a 1.03% solution in chloroform.

C. Thin-Layer Chromatography

Plate used: Silica gel F-254
Visualization: Ultraviolet, 254 and 365 nm and ferricyamide-ferric chloride
Reference Standard: Reserpine (USP Reference Standard)

$$R_{st} = \frac{R_f(\text{sample})}{R_f(\text{standard})}$$

An R_{st} = 1.0 signifies identical R_f values for test sample and standard.

System I: Chloroform:methanol (42:8)
Results: R_f 0.89 (major), 0.80 (trace), 0.13 (trace)
R_{st} 1.00 (major), 0.90 (trace), 0.15 (trace)

System II: Benzene:diethylamine (95:5)
Results: R_f 0.25 (major), 0.03 (trace), origin (trace)
R_{st} 1.00 (major), 0.12 (trace), origin (trace)

D. High-Pressure Liquid Chromatography

Instrument:	ALC 202 with Solvent Programmer 660
Detector:	U.V., 254 nm
Column:	Micropak Porasil, 30 cm x 4 mm ID
Solvent:	5 to 25% isopropanol in chloroform
Results:	Major peak at 7.8 minutes (identical to USP standard reserpine), with trace impurity (< 0.5% of major peak) at 11.6 minutes

E. Nonaqueous Titration of Secondary Amine Group with Perchloric Acid

101.0±0.7 (δ)%

F. Visible Spectrometric Analysis (The Pharmacopeia, 1970) (Cary 118), compared with USP Reserpine reference standard

97.8±1.0 (δ)% (USP limits: 97% to 101%)

G. Spectral Data

1. Infrared: The infrared absorption spectrum (Figure 5) was consistent with the spectrum given in the literature (Sadtler Standard Spectra).

2. Ultraviolet/ Visible: (Cary 118)

Determined	Calculated from literature spectrum (Hayden et al., 1962)
$\epsilon max_{295\ nm} = 1.013 \pm 0.004(\delta) \times 10^4$	$\epsilon max_{295\ nm} = \sim 1.00 \times 10^4$
$\epsilon max_{268\ nm} = 1.706 \pm 0.008(\delta) \times 10^4$	$\epsilon max_{267\ nm} = \sim 1.64 \times 10^4$
No absorbance from 350 to 800 nm at 1 mg/ml	
Solvent: $CHCl_3$ MeOH (3.6:1.4)	Solvent: $CHCl_3$ MeOH (3.6:1.4) (Neuss et al, 1954)
	$\epsilon max_{295} = 10,200$
	$\epsilon max_{267} = 17,000$
	$\epsilon max_{216} = 61,700$
	Solvent: Methanol

3. Nuclear Magnetic Resonance

Instrument: Varian HA-100

Solvent: $CDCl_3$ with internal TMS

Assignments: (see Figure 6)

(a) 1.60 to 3.20 δ

(b) 3.42 δ

(c) 3.72 to 3.85 δ

(d) 4.20 to 4.45 δ

(e) 4.88 δ

(f) 6.72 δ

(g) 7.28 δ

(h) 7.98 δ

Integration Ratios:

(a) 15.73

(b) 3.41

(c) 15.24

(d) 2.93

(e) 0.98

(f) 2.20

(g) 2.20

(h) 0.98

No literature reference found

Identical to spectrum of USP reference standard

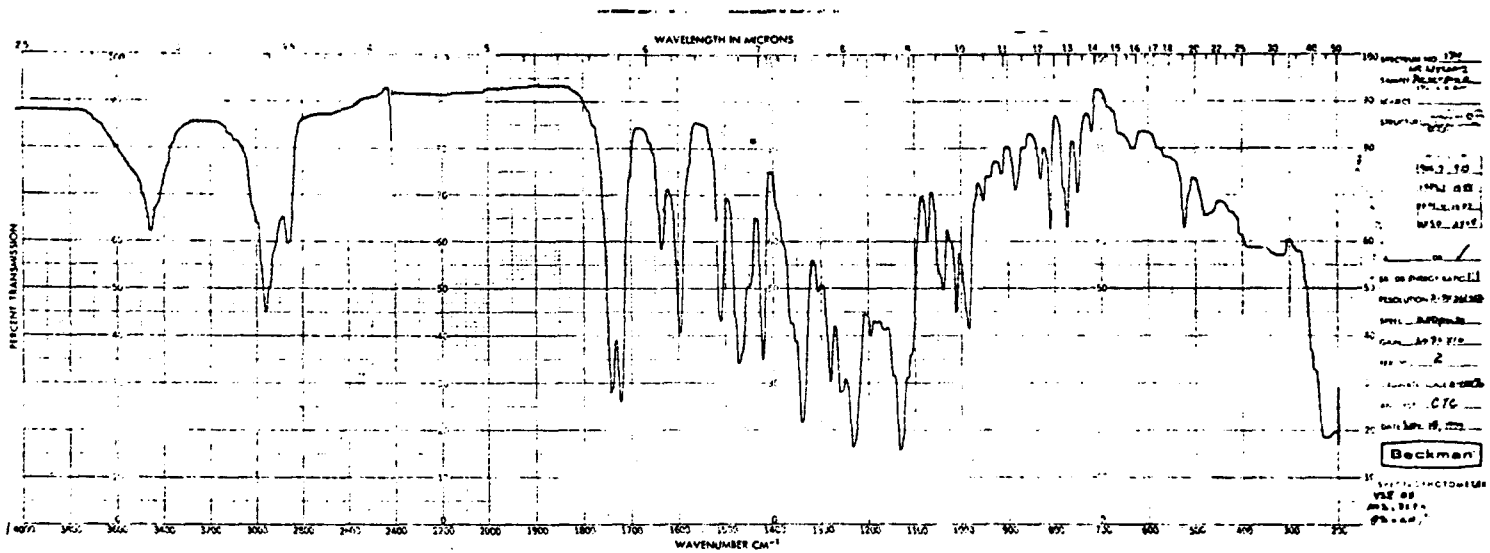

FIGURE 5. INFRARED ABSORPTION SPECTRUM OF RESERPINE

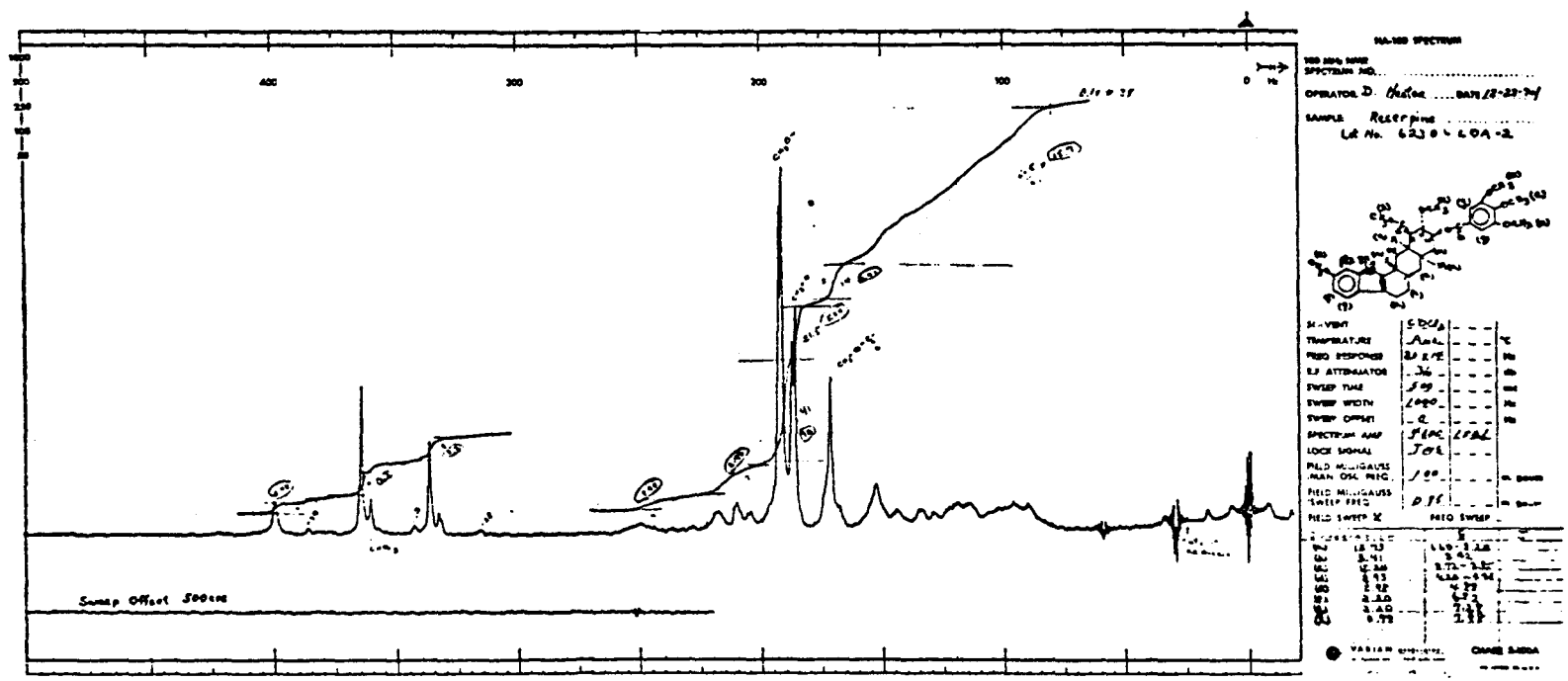

FIGURE 6. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF RESERPINE

APPENDIX F

ANALYSES OF FORMULATED DIETS FOR CONCENTRATIONS OF RESERPINE

Appendix F
Analyses of Formulated Diets for Concentrations of Reserpine

An 8-g sample of the diet mixture was shaken with 100 ml of chloroform. Twenty-five milliliters of the chloroform extract was taken and analyzed by the procedure for analysis of reserpine cited in the Official Methods of Analysis of the Association of Official Analytical Chemists (1975).

Theoretical Dietary Level	No. of Samples	Sample Analytical Mean (%)	Coefficient of Variation (%)	Range (%)
10 ppm	10	0.0011	13.4	0.0014-0.0009
5 ppm	7	0.00046	22.9	0.00058-0.00031

APPENDIX G

ANALYSES OF FORMULATED DIETS FOR STABILITY OF RESERPINE

Appendix G

Analyses of Formulated Diets for Stability of Reserpine

	Theoretical Concentration (ppm)	Concentration Found (ppm)	Storage Temperature	Mix Date	Analysis Date	Elapsed Time
A.[a]	10.0	11.0	5°C	10-01-75	08-26-76	~ 11 mos.
	10.0	9.5	5°C	11-15-75	08-26-76	~ 9.5 mos.
	10.0	14.0	5°C	12-27-75	08-26-76	~ 8 mos.
	10.0	10.4	5°C	02-24-76	08-26-76	~ 6 mos.
	10.0	8.6	5°C	03-21-76	08-26-76	~ 5 mos.
	10.0	5.6	5°C	05-11-76	08-26-76	~ 3.5 mos.
	10.0	8.5	5°C	06-24-76	08-26-76	~ 2 mos.
	10.0	9.5	5°C	07-21-76	11-30-76	~ 4 mos.
	10.0	12.0	5°C	08-28-76	11-30-76	~ 3 mos.
	10.0	12.0	5°C	09-06-76	11-30-76	~ 3 mos.
	10.0	12.0	5°C	03-10-77	03-22-77	~ 2 wks.
	10.0	9.4	5°C	04-14-77	04-22-77	~ 1 wk.
	10.0	12.1	5°C	06-07-77	06-28-77	3 wks.
B.[b]	10.0	13.0	22°C	09-15-77	09-21-77	~ 1 wk.
	10.0	10.0	22°C	09-15-77	09-21-77	~ 1 wk.
	10.0	12.0	22°C	09-15-77	09-21-77	~ 1 wk.
C.[c]	5.0	4.7	5°C	10-01-75	08-26-76	~ 11 mos.
	5.0	3.5	5°C	11-15-75	08-26-76	~ 9.5 mos.
	5.0	5.6	5°C	12-27-75	08-26-76	~ 8 mos.
	5.0	3.1	5°C	02-24-76	08-26-76	~ 6 mos.
	5.0	3.7	5°C	04-29-76	08-26-76	~ 4 mos.
	5.0	4.3	5°C	10-18-76	11-30-76	~ 1.5 mos.
	5.0	5.5	5°C	02-24-77	03-22-77	~ 1 mos.
	5.0	5.8	5°C	06-07-77	06-28-77	3 wks.
D.[d]	5.0	5.7	22°C	09-15-77	09-21-77	~ 1 wk.
	5.0	5.3	22°C	09-15-77	09-21-77	~ 1 wk.
	5.0	7.2	22°C	09-15-77	09-21-77	~ 1 wk.

(a) The mean $\pm$ standard deviation is 10.4 ± 2
(b) The mean $\pm$ standard deviation is 11.7 ± 1
(c) The mean $\pm$ standard deviation is 4.5 ± 1
(d) The mean $\pm$ standard deviation is $6.1 \pm .8$

Appendix H

ANALYSES OF THE INCIDENCE OF ADRENAL LESIONS IN MALE RATS
AS REPORTED BY CAPEN, C. C., HOLLAND, J. M.,
and NIELSON, S. W.

Appendix H

Analyses of the Incidence of Adrenal Lesions in Male Rats as Reported by Capen, C. C., Holland, J. M., and Nielson, S. W. (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal Medulla: Hyperplasia (Nodular) (b)	7/48 (15)	10/49 (20)	10/48 (21)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (e)		1.399	1.429
Lower Limit		0.526	0.537
Upper Limit		3.979	4.056
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant (b)	1/48 (2)	9/49 (18)	13/49 (27)
P Values (c,d)	P=0.001	P=0.008	P less than 0.001
Relative Risk (Matched Control) (e)		8.816	13.000
Lower Limit		1.299	2.088
Upper Limit		376.985	536.977
Adrenal Medulla: Neuroblastoma (b)	0/48 (0)	1/49 (2)	0/48 (0)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (e)		Infinite	--
Lower Limit		0.053	--
Upper Limit		Infinite	--
Adrenal: Hyperplasia (Nodular) or Pheochromocytoma or Pheochromocytoma, Malignant (b)	8/48 (17)	20/49 (41)	23/48 (49)
P Values (c,d)	P=0.001	P=0.008	P = 0.001
Relative Risk (Matched Control) (e)		2.449	2.875
Lower Limit		1.158	1.401
Upper Limit		5.737	6.549

Analyses of the Incidence of Adrenal Lesions in Male Rats as Reported by Capen, C. C., Holland, J. M., and Nielson, S. W. (a)

(a) Dosed groups received 5 or 10 ppm.
(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 matched-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.

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

May 1, 1979

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 of the Institute's bioassay program to identify and 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, and State health officials. 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 Reserpine.

A representative of CIBA-GEIGY presented a public statement regarding the report on the bioassay study of Reserpine. He said that the conclusion of an increased tumor formation in treated male rats and treated mice of both sexes were not substantiated by data in the tumor summary tables. He commented on several specific tumor incidences and types, which he regarded as inconsistencies or omissions in the report. A pathologist, representing CIBA-GEIGY, noted a number of discrepancies between his pathologic findings and those given in the report. He particularly challenged the accuracy and completeness of the findings with respect to rat adrenal tumors; the CIBA-GEIGY analysis indicating no significant increase of primary medullary tumors. In regard to seminal vesicle tumors in mice, he explained that the tumors were probably metastatic in origin and not Reserpine-related. Finally, he said that the mammary gland tumors in dosed female mice were not associated with treatment. In continuing the presentation, the company's original spokesman stated that CIBA-GEIGY did not agree that the study would indicate that Reserpine posed a possible risk to human beings. He cited an 1977 FDA Toxicology Advisory Committee report on anti-psychotic drugs in support of their contention that potential risk of mammary cancer cannot be extrapolated from rodents to man. He recommended that a panel of distinguished pathologists be commissioned to review the discrepancies between the NCI and CIBA-GEIGY findings.

Based on the information available in the NCI bioassay report, the primary reviewer agreed with the conclusion that Reserpine, under the conditions of test, produced an increased incidence of tumors at selected sites in treated rats and mice. She summarized the experimental design and said that she considered the study well conducted. Despite the difficulty in discerning between benign and malignant pheochromocytomas, there appeared to be a real increase of this tumor type in treated male rats. The primary reviewer wondered about the relationship between the inflammatory lesions, hyperplasia, and undifferentiated carcinomas of the seminal vesicles observed in treated male mice.

The secondary reviewer agreed with the previous assessment of the study. He suggested that a pathology review would be appropriate, although it should be independent of the Subgroup's consideration of the bioassay report. Findings of the pathology review could be appended later to the bioassay report or sent to the proper regulatory agency. He added that the pathology review called for by CIBA-GEIGY should be paid for by industry.

Based on the report, the third reviewer said that he supported the NCI's staff conclusion on the carcinogenicity of the drug. He also agreed with the suggestion regarding the pathology review. He suggested that an analysis of the conflicting epidemiologic studies, and perhaps a new one, would be advisable.

In regard to the seminal vesicles tumors, a staff pathologist said that the inflammatory lesions and epithelial hyperplasia in treated male mice made him more confident that the tumors were primary in origin. He did not recall seeing any of these tumors in several thousand mice which he personally had examined. Another staff pathologist showed and described photomicrographs of lesions observed in the seminal vesicles. He said that, to his knowledge, there were no other tumors in the mice that would suggest the tumors in the seminal vesicles were metastatic in origin. He emphasized the extremely rare nature of the tumor.

One Subgroup member questioned if the study was proper for testing a substance that may exert its effect through hormonal mechanisms. Another member speculated that Reserpine may be an endocrine-affecting substance. Until the mechanism of action of Reserpine is known, a third member argued that the drug should be regarded as posing a potential risk to humans, especially since it is carcinogenic in two species.

In commenting on the CIBA-GEIGY presentation, one Subgroup member said that the company's assertion concerning no tumor increase was misleading. CIBA-GEIGY referred to total tumors, whereas the appropriate method for comparison is on a site-by-site basis. He also dismissed the significance of CIBA-GEIGY's allusions to a reduced tumor incidence at certain sites among treated animals. The Subgroup member objected to the recommendation that another group of pathologists review areas

of disagreement. He considered such a review unnecessary and more appropriate as part of the regulatory process.

A discussion ensued as to the need to retest Reserpine, possibly using an experimental design more appropriate for assessing endocrine-related tumors. One Subgroup member thought another study would be proper because of the therapeutic value of the drug and the number of people exposed. Another Subgroup member said that any new study should not be the NCI's responsibility. It was recommended that additional studies should be paid for by industry.

After some discussion regarding the framing of a motion, it was moved that the report on the bioassay of Reserpine be accepted as written. The motion was seconded and approved unanimously. As a second motion, it was moved that areas of pathology concern be reviewed by an independent group of pathologists. This motion was seconded and approved by a vote of three to one.

Clearinghouse Members Present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
David B. Clayson, University of Nebraska Medical Center
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center
Sheldon Samuels, AFL-CIO
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center
Kenneth Wilcox, Michigan State Health Department

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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 for other reasons. Thus, certain comments and criticisms reflected in the review may no longer be applicable.

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