observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

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) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported 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 was 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, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, 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, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test determined 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 was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose

relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

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 relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

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 percent of a large number of identical experiments, the true ratio of the risk in a treated 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 (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. 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. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

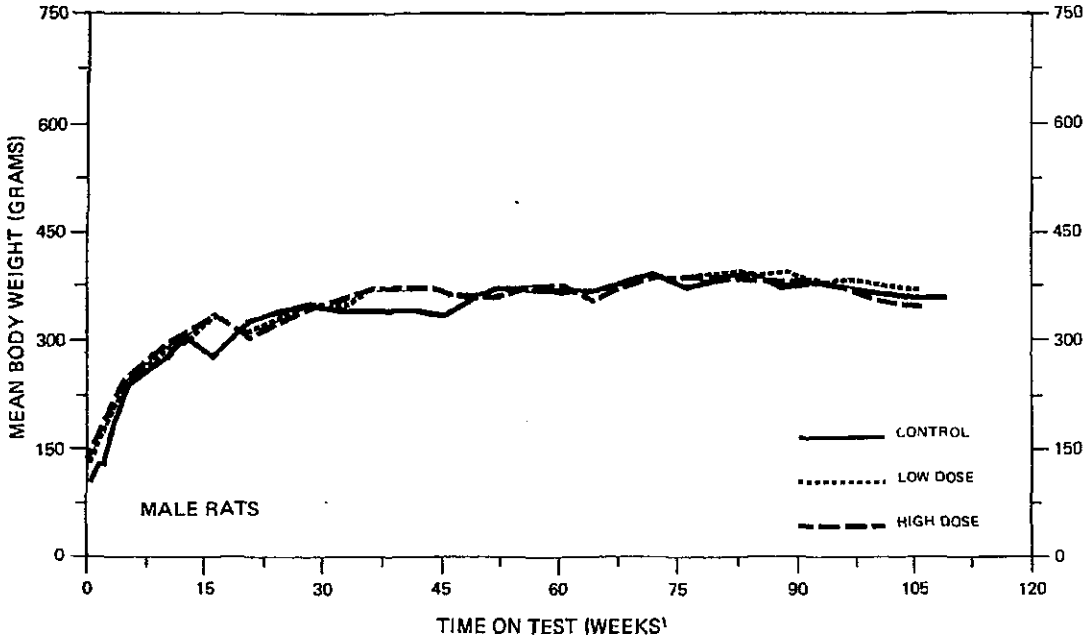
There was no appreciable depression in mean body weight when dosed rats were compared with their respective controls (Figure 2).

Subcutaneous masses were observed in 2 high dose, 3 low dose, and 1 control males, and in 12 high dose, 3 low dose, and 2 control females. Crusted cutaneous masses occurred in 4 high dose males, 1 low dose male, 2 low dose females, and 1 control female, while firm nodular growths were detected in 1 high dose, 2 low dose, and 2 control males, and in 1 low dose female. Swelling of the eyes was exhibited by 2 high dose males, 2 high dose females, and 2 low dose females and swelling of the nose by 1 low dose male. Only 1 control female experienced crusted lesions in the vaginal area while 4 low dose and 9 high dose females were so effected. Alopecia was recorded for 1 low dose female, emaciation was observed in 1 male and 1 female control, and 1 female control exhibited abdominal distention.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 1,5-naphthalenediamine-dosed groups are shown in Figure 3. There was no significant positive association between dosage and mortality for either male or female rats.

Adequate numbers of male rats were at risk from late-developing tumors with 74 percent (37/50) of the high dose, 80 percent (40/50) of the low dose and 68 percent (17/25) of the control surviving on



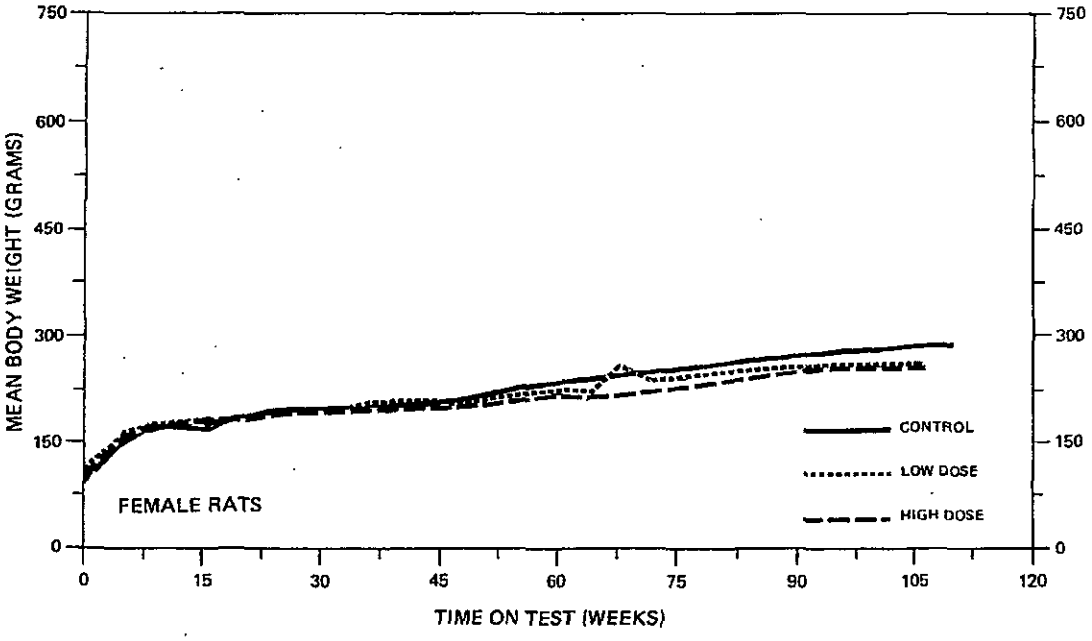
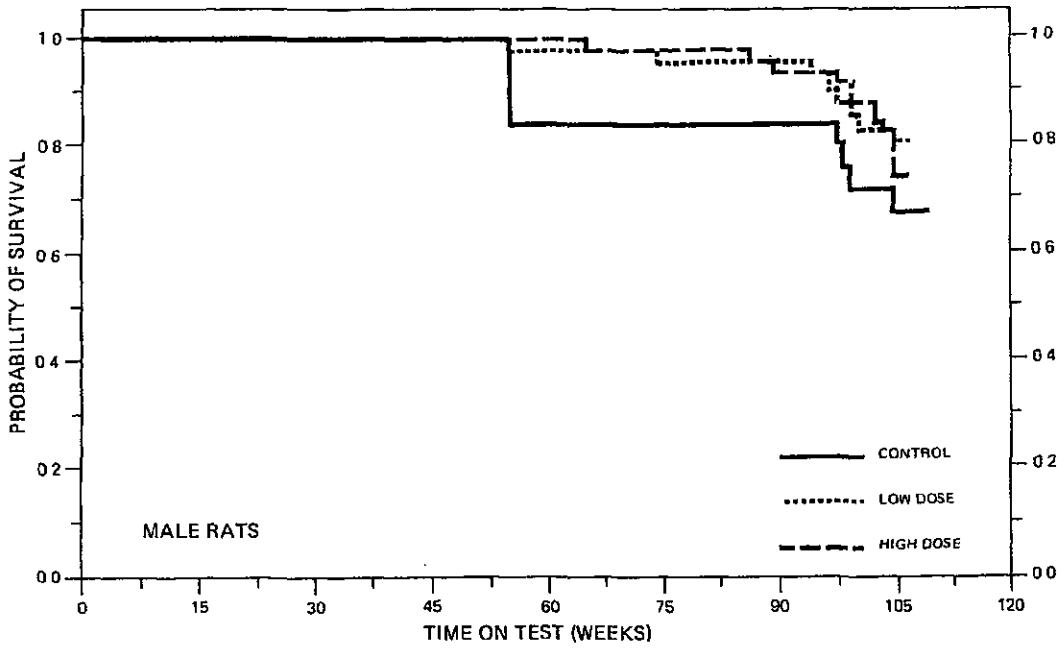


FIGURE 2
GROWTH CURVES FOR 1,5-NAPHTHALENEDIAMINE CHRONIC STUDY RATS



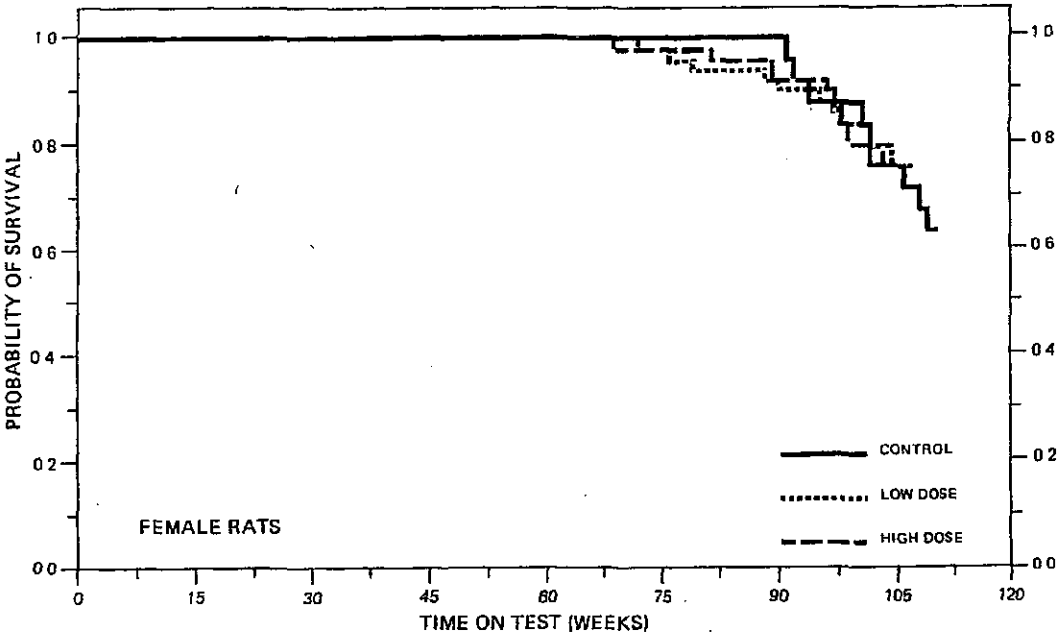


FIGURE 3
SURVIVAL COMPARISONS OF 1,5-NAPHTHALENEDIAMINE CHRONIC STUDY RATS

test until the termination of the study. No lesions were reported for the 4 control rats that died in week 55.

With 76 percent (38/50) of the high dose, 76 percent (38/50) of the low dose and 64 percent (16/25) of the control rats surviving on test until the termination of the study, adequate numbers of females were at risk from late-developing tumors.

C. Pathology

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).

The incidence of liver neoplasms in male and female rats administered 1,5-naphthalenediamine in the diet appeared to be increased relative to controls. In female rats, tumors of the clitoral gland, uterus, and C-cell neoplasms of the thyroid appeared to be related to compound administration. The incidences of these tumors are as follows:

	MALES			FEMALES		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
LIVER						
(Number of animals with tissues examined histopathologically)	(25)	(49)	(49)	(24)	(50)	(49)
Neoplastic Nodule	1	3	2	0	3	4
Hepatocellular Carcinoma	0	4	2	0	1	0
PREPUTIAL/CLITORAL GLAND						
(Number of animals necropsied)	(25)	(49)	(50)	(24)	(50)	(50)
Carcinoma	0	0	1	1	3	8
Adenoma	0	0	1	0	0	5

	MALES			FEMALES		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
UTERUS AND ENDOMETRIUM						
(Number of animals with tissues examined histopathologically)	-	-	-	(24)	(49)	(48)
Adenocarcinoma				1	2	4
Endometrial Stromal Polyp				2	14	20
Endometrial Stromal Sarcoma				1	2	2
THYROID						
(Number of animals with tissues examined histopathologically)	(21)	(47)	(47)	(21)	(49)	(48)
C-Cell Adenoma	0	2	5	0	7	3
C-Cell Carcinoma	2	3	3	1	5	1

Neoplasms of the clitoral (preputial) gland were presented grossly as round, fluctuant cystic subcutaneous lesions in the genital area, which on section were filled with pasty green material. On microscopic examination, the cyst contents consisted of desquamated epithelial cells, frequently mixed with leukocytes from secondary inflammation. The inner portion of the cyst wall was lined by hyperkeratinized squamous epithelium often thrown into papillary folds. Peripheral to this was a zone of large, round glandular cells at least a few of which had coarse, brightly eosinophilic cytoplasmic granules. If the peripheral border appeared smooth and intact, the lesion was classified as an adenoma. If there was disorganization of the glandular structure and invasion into the surrounding stroma, the tumor was called a carcinoma.

Thyroid C-cell tumors were observed in dosed female rats at incidences increased relative to controls (4/48 [8 percent] high dose, 12/49 [24 percent] low dose, 1/21 [5 percent] controls). C-cell adenomas were discrete masses of these cells, often containing small cysts lined by flat epithelium and containing colloid-like material. In C-cell carcinomas, the tumor cells often assumed a spindle shape and tended to invade surrounding tissue.

Uterine horns containing neoplasms were usually grossly enlarged. The neoplasms themselves were varicolored, polypoid, frequently gelatinous masses projecting into the uterine cavity. Endometrial stromal polyps had a fibrous connective tissue core richly supplied with large vessels. The surface of the polyps was covered with well-differentiated endometrium which often formed glands in the superficial portion of the polyps. These tumors frequently became necrotic at the tip and exhibited hemorrhage and secondary inflammation. In a few rats, the connective tissue stroma of these lesions underwent malignant transformation characterized by increased cellularity, mitoses, and formation of plump, pleomorphic nuclei. Such tumors were classified as stromal sarcomas. A uterine adenocarcinoma was a collection of fairly well-differentiated glands arranged back-to-back with no obvious intervening stroma. Nuclei of the glands were markedly pleomorphic with frequent mitoses. There was invasion into the myometrium and sometimes into extra uterine structures.

There were instances in this study, as noted in the summary tables, where neoplastic lesions occurred only in dosed animals, or with increased frequency when compared to the control group. No pulmonary neoplasms were found in the controls; alveolar/bronchiolar tumors were seen in dosed rats of both sexes. There was only one urinary tract neoplasm in a female control; a few more occurred in dosed rats, both male and female. No gliomas of the brain were seen in controls; a few gliomas were found in dosed rats of both sexes. These neoplasms occurred in such small numbers that a conclusive interpretation as to their significance is not possible.

Rats in all groups exhibited a variety of nonneoplastic inflammatory and degenerative changes, and none were associated with administration of the compound.

Based upon the results of this pathologic examination, 1,5-naphthalenediamine was carcinogenic to female Fischer 344 rats since feeding of the compound was associated with adenomas and carcinomas of the clitoral gland. In addition, 1,5-naphthalenediamine feeding appeared to be associated with increased incidences of thyroid, liver and uterine neoplasms in female rats and liver neoplasms in male rats.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 1,5-NAPHTHALENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	1/25(0.04)	3/49(0.06)	2/50(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.531	1.000
Lower Limit	---	0.133	0.056
Upper Limit	---	78.493	56.712
Weeks to First Observed Tumor	99	106	102
Skin: Squamous-Cell Papilloma[b]	2/25(0.08)	1/49(0.02)	1/50(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.255	0.250
Lower Limit	---	0.005	0.004
Upper Limit	---	4.707	4.616
Weeks to First Observed Tumor	109	106	106
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	0/25(0.00)	3/49(0.06)	4/47(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.315	0.508
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	104	106

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	1/25(0.04)	10/49(0.20)	10/50(0.20)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	5.102	5.000
Lower Limit	---	0.801	0.787
Upper Limit	---	212.137	213.351
Weeks to First Observed Tumor	109	100	97
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	1/25(0.04)	7/49(0.14)	4/49(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	3.571	2.041
Lower Limit	---	0.503	0.218
Upper Limit	---	156.046	96.949
Weeks to First Observed Tumor	109	106	104
Pituitary: Adenoma NOS, Chromophobe Adenoma, Acidophil Adenoma, or Basophil Adenoma[b]	2/22(0.09)	7/44(0.16)	11/44(0.25)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.750	2.750
Lower Limit	---	0.376	0.683
Upper Limit	---	16.365	24.081
Weeks to First Observed Tumor	98	96	65

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Adrenal: Pheochromocytoma or Malignant Pheochromocytoma[b]	2/24(0.08)	4/48(0.08)	5/48(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.000	1.250
Lower Limit	---	0.157	0.226
Upper Limit	---	10.563	12.529
Weeks to First Observed Tumor	109	106	102
Thyroid: C-Cell Carcinoma[b]	2/21(0.10)	3/47(0.06)	3/47(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.670	0.670
Lower Limit	---	0.084	0.084
Upper Limit	---	7.650	7.650
Weeks to First Observed Tumor	97	100	106
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	2/21(0.10)	5/47(0.11)	8/47(0.17)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.117	1.787
Lower Limit	---	0.205	0.405
Upper Limit	---	11.249	16.445
Weeks to First Observed Tumor	97	100	104

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pancreatic Islets: Islet-Cell Adenoma or Islet-Cell Carcinoma[b]	1/25(0.04)	2/48(0.04)	5/45(0.11)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.042	2.778
Lower Limit	---	0.058	0.340
Upper Limit	---	60.184	128.213
Weeks to First Observed Tumor	98	106	104
Testis: Interstitial-Cell Tumor[b]	21/25(0.84)	44/49(0.90)	45/49(0.92)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.069	1.093
Lower Limit	---	0.890	0.912
Upper Limit	---	1.325	1.324
Weeks to First Observed Tumor	97	94	65

[a]Treated groups received doses of 0.05 or 0.10 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 1,5-NAPHTHALENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	3/24(0.13)	7/50(0.14)	1/50(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.120	0.160
Lower Limit	---	0.287	0.003
Upper Limit	---	6.292	1.890
Weeks to First Observed Tumor	94	76	103
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	0/24(0.00)	4/50(0.08)	4/49(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.458	0.467
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	102	106
Pituitary: Adenoma NOS, Chromophobe Adenoma, Acidophil Adenoma, or Basophil Adenoma[b]	6/21(0.29)	10/50(0.20)	17/47(0.36)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.700	1.266
Lower Limit	---	0.275	0.577
Upper Limit	---	2.090	3.426
Weeks to First Observed Tumor	91	98	98

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Carcinoma NOS, Adenoma NOS, Chromophobe Adenoma, Chromophobe Carcinoma, Acidophil Adenoma, or Basophil Adenoma[b]	6/21(0.29)	11/50(0.22)	18/47(0.38)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.770	1.340
Lower Limit	---	0.312	0.618
Upper Limit	---	2.262	3.606
Weeks to First Observed Tumor	91	88	98
Adrenal: Cortical Adenoma or Cortical Carcinoma[b]	0/24(0.00)	3/50(0.06)	1/49(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.297	0.027
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	106	106
Adrenal: Pheochromocytoma[b]	1/24(0.04)	0/50(0.00)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	1.469
Lower Limit	---	0.000	0.127
Upper Limit	---	8.966	75.534
Weeks to First Observed Tumor	110	---	106

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Carcinoma[b]	1/21(0.05)	5/49(0.10)	1/48(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.143	0.438
Lower Limit	---	0.266	0.006
Upper Limit	---	99.147	33.659
Weeks to First Observed Tumor	109	106	106
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	1/21(0.05)	12/49(0.24)	4/48(0.08)
P Values[c]	N.S.	P = 0.046	N.S.
Departure from Linear Trend[e]	P = 0.009	---	---
Relative Risk (Control)[d]	---	5.143	1.750
Lower Limit	---	0.855	0.192
Upper Limit	---	215.370	83.548
Weeks to First Observed Tumor	109	104	103
Thyroid: Papillary Carcinoma, Follicular-Cell Carcinoma, or Papillary Cystadenocarcinoma NOS[b]	1/21(0.05)	1/49(0.02)	3/48(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.429	1.313
Lower Limit	---	0.006	0.115
Upper Limit	---	32.983	67.452
Weeks to First Observed Tumor	110	106	99

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Papillary Carcinoma, Follicular-Cell Carcinoma, Papillary Cystadenocarcinoma NOS, or Papillary Cystadenoma[b]	1/21(0.05)	2/49(0.04)	4/48(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.857	1.750
Lower Limit	---	0.648	0.191
Upper Limit	---	49.555	84.310
Weeks to First Observed Tumor	110	106	81
Mammary Gland: Fibroadenoma[b]	4/24(0.17)	5/50(0.10)	13/50(0.26)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.600	1.560
Lower Limit	---	0.145	0.556
Upper Limit	---	2.812	6.019
Weeks to First Observed Tumor	109	102	98
Mammary Gland: Fibroadenoma, Adenocarcinoma NOS, or Papillary Adenocarcinoma[b]	4/24(0.17)	5/50(0.10)	14/50(0.28)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.600	1.680
Lower Limit	---	0.145	0.609
Upper Limit	---	2.807	6.412
Weeks to First Observed Tumor	109	102	98

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Clitoral Gland: Carcinoma NOS[b]	1/24(0.04)	3/50(0.06)	8/50(0.16)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.440	3.840
Lower Limit	---	0.125	0.566
Upper Limit	---	75.487	168.221
Weeks to First Observed Tumor	110	106	69
Clitoral Gland: Adenoma NOS or Carcinoma NOS[b]	1/24(0.04)	3/50(0.06)	13/50(0.26)
P Values[c]	P = 0.003	N.S.	P = 0.021
Relative Risk (Control)[d]	---	1.440	6.240
Lower Limit	---	0.125	1.043
Upper Limit	---	74.077	258.268
Weeks to First Observed Tumor	110	106	69
Uterus: Endometrial Stromal Polyp[b]	2/24(0.08)	14/49(0.29)	20/48(0.42)
P Values[c]	P = 0.003	P = 0.043	P = 0.003
Relative Risk (Control)[d]	---	3.429	5.000
Lower Limit	---	0.892	1.385
Upper Limit	---	29.588	41.202
Weeks to First Observed Tumor	102	88	96

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Uterus and Endometrium: Adenocarcinoma NOS[b]	1/24(0.04)	2/49(0.04)	4/48(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.980	2.000
Lower Limit	---	0.054	0.216
Upper Limit	---	56.627	96.367
Weeks to First Observed Tumor	110	104	106
Zymbal's Gland: Sebaceous Adenocarcinoma[b]	0/24(0.00)	0/50(0.00)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.296
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	89

[a]Treated groups received doses of 0.05 or 0.10 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

every type of tumor in either sex where at least two such tumors were observed in at least one of the control or 1,5-naphthalenediamine-dosed groups and where such tumors were observed in at least 5 percent of the group.

For female rats an increased incidence of endometrial stromal polyps was observed in both the high and low dose groups compared to the control group. The Cochran-Armitage test indicated a significant ($P = 0.003$) positive association between compound administration and tumor incidence. The Fisher exact tests supported this result with a significant ($P = 0.003$) comparison of the high dose group to the control; for the low dose comparison the probability level was $P = 0.043$, a marginal result which was not significant under the Bonferroni criterion. Based on these results, the administration of 1,5-naphthalenediamine was associated with an elevated incidence of endometrial stromal polyps in female rats.

A number of adenomas NOS and carcinomas NOS of the clitoral gland were observed in female rats. The Cochran-Armitage test indicated a significant ($P = 0.003$) positive association between dose and the combined incidence of adenomas NOS or carcinomas NOS of the clitoral gland. The Fisher exact test comparing high dose to control was also significant ($P = 0.021$). In historical data collected by this laboratory for the NCI Carcinogenesis Testing Program, 4/249 (2 percent) of the untreated female Fischer 344 rats had one of these tumors, compared to the 13/50 (26 percent) observed in the high dose group in

this bioassay. Based upon these statistical results, the administration of 1,5-naphthalenediamine was associated with an elevated incidence of clitoral gland neoplasms in female rats.

For females the Fisher exact test comparing control to low dose for the combined incidence of C-cell adenomas or C-cell carcinomas of the thyroid had a probability level of $P = 0.046$, a marginal result which was not significant under the Bonferroni criterion.

Based on these statistical tests, it is concluded that 1,5-naphthalenediamine was carcinogenic for female rats, producing tumors of the clitoral gland and uterus.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

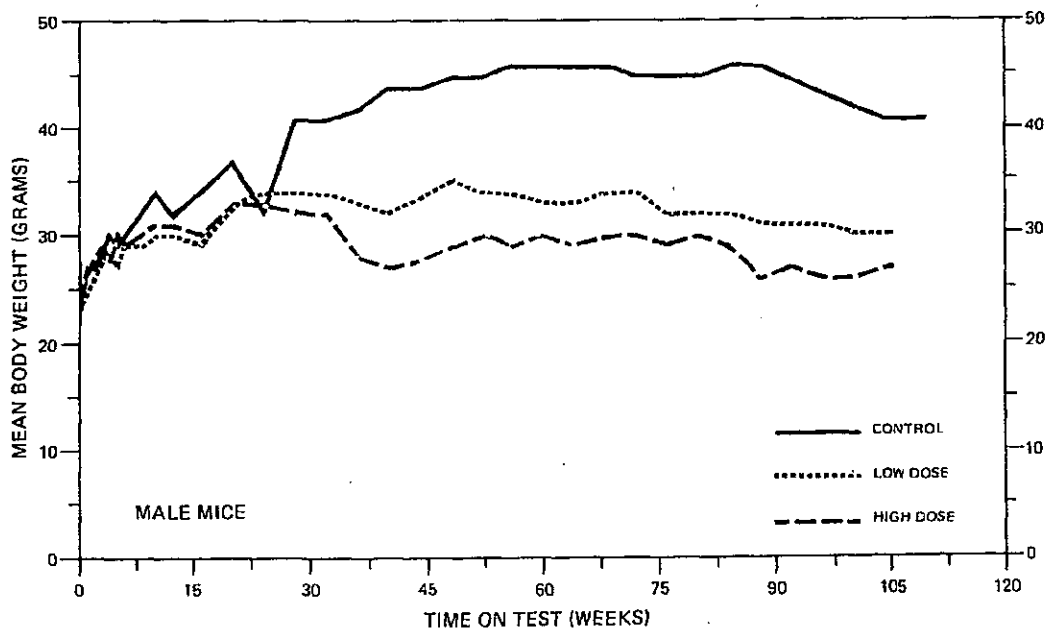
Mean body weight depression was readily apparent in dosed male mice when compared to controls. A similar but less pronounced trend was evident in dosed females (Figure 4).

One low dose male had a soft subcutaneous mass on the leg and two males in this group had palpable abdominal masses. Firm nodular growths developed in one low dose male and two high dose females. Alopecia was observed in 27 control males, 16 low dose males, 4 high dose males, 25 control females, and 3 low dose females. Two low dose and two high dose males experienced noticeable swelling of the eyes. Abdominal distention was observed in one control male and one control female mouse.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 1,5-naphthalenediamine-dosed groups are shown in Figure 5. There was no significant positive association between dosage and mortality for either male or female mice.

Adequate numbers of male mice were at risk from late-developing tumors with 58 percent (29/50) of the high dose, 78 percent (39/50) of the low dose and 66 percent (33/50) of the controls surviving on test until the termination of the study. The 6 control male mice that died in week 11 were autolyzed, as were 2 of the 4 high dose male mice that died in week 41.



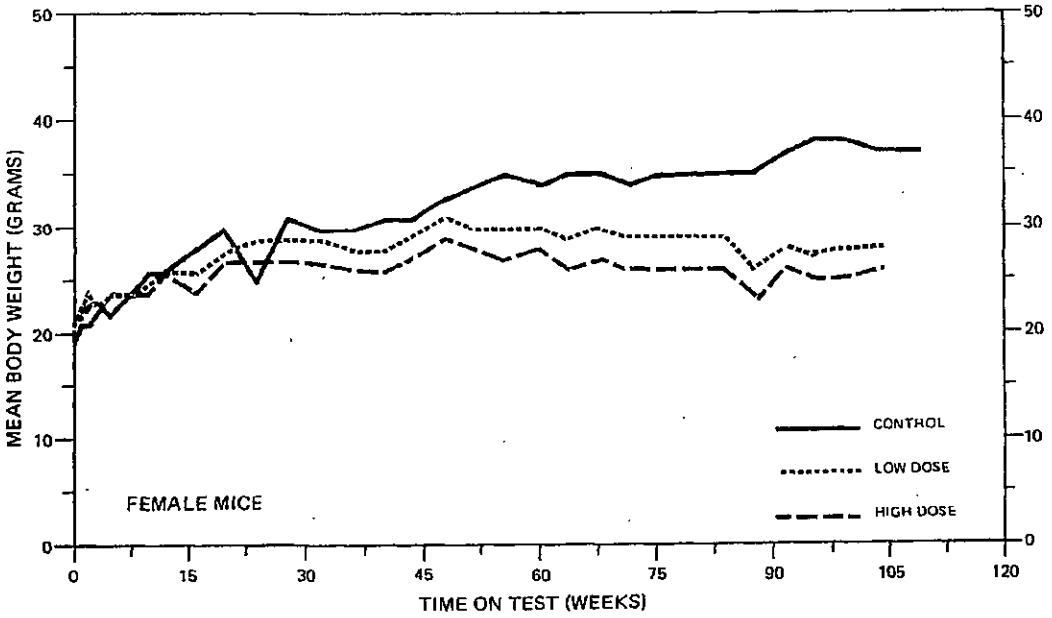
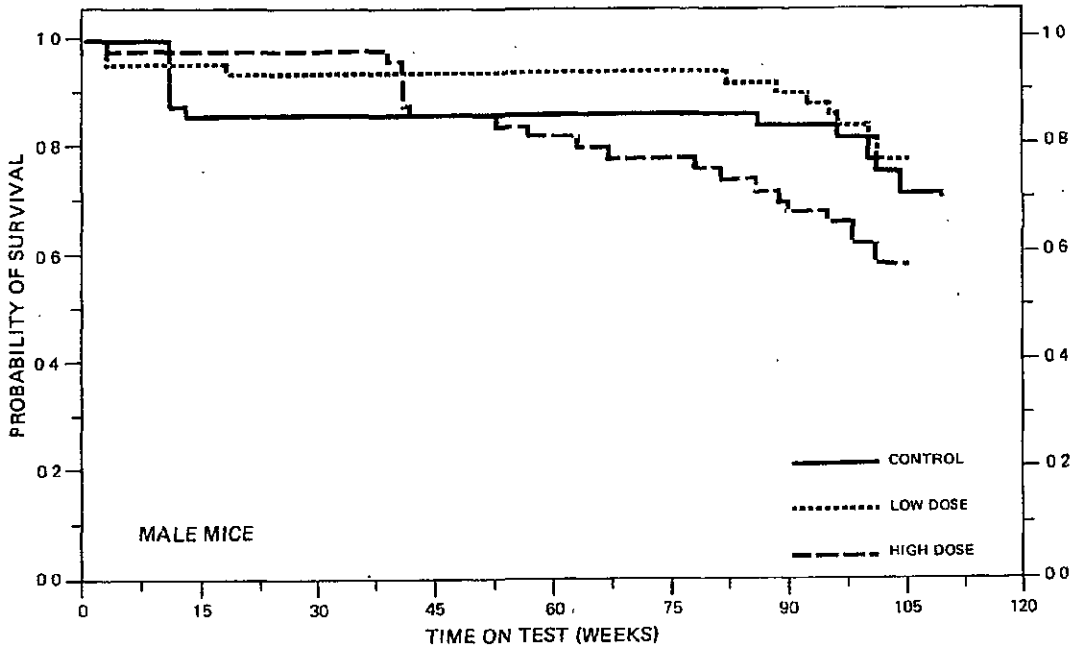


FIGURE 4
GROWTH CURVES FOR 1,5-NAPHTHALENEDIAMINE CHRONIC STUDY MICE



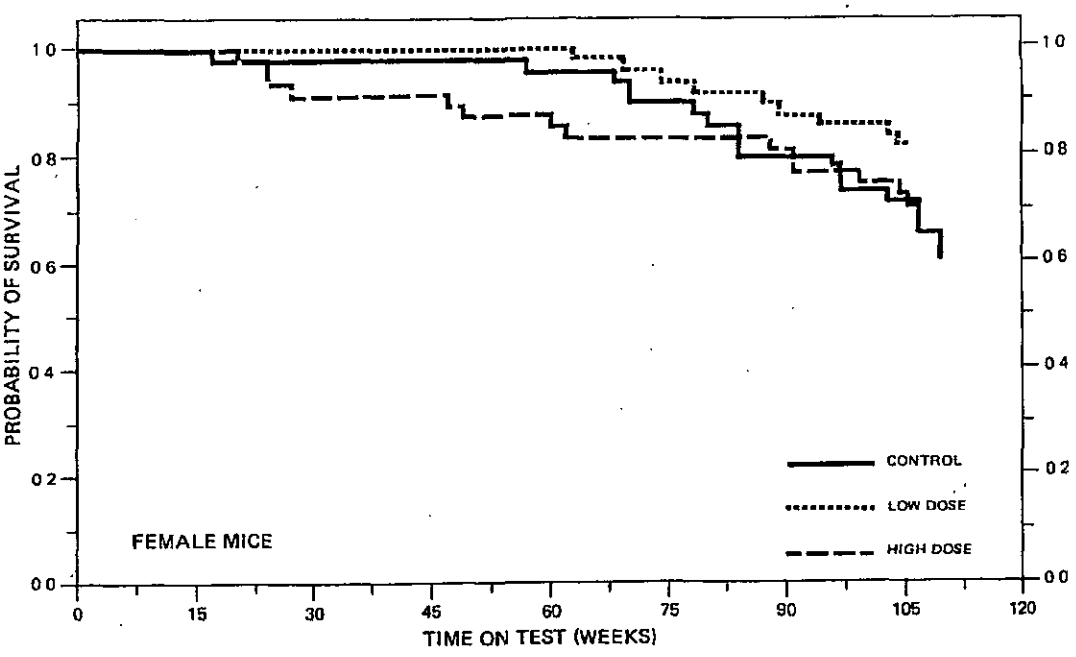


FIGURE 5
SURVIVAL COMPARISONS OF 1,5-NAPHTHALENEDIAMINE CHRONIC STUDY MICE

For female mice, with 68 percent (34/50) of the high dose, 82 percent (41/50) of the low dose and 60 percent (30/50) of the control mice surviving on test until the termination of the study, adequate numbers were at risk from late-developing tumors.

C. Pathology

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).

Dietary administration of 1,5-naphthalenediamine produced an increase in hepatocellular neoplasms in female mice, and it produced a dose-related increase in thyroid neoplasms and compound-related nonneoplastic thyroid lesions in both sexes. The compound-related lesions are summarized below:

	MALES			FEMALES		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
LIVER						
(Number of animals with tissues examined histopathologically)	(39)	(45)	(43)	(46)	(49)	(46)
Hepatocellular Carcinoma	12	10	7	1	25	16
Hepatocellular Adenoma	0	3	6	0	3	11
THYROID						
(Number of animals with tissues examined histopathologically)	(38)	(46)	(43)	(44)	(49)	(45)
Follicular-Cell Adenoma (Papillary or Follicular-Cell Adenoma, Papillary Cystadenoma)	0	8	16	2	17	14
Follicular-Cell Carcinoma	0	1	1	2	0	1
Follicular-Cell Hyperplasia	2	12	9	2	1	4
C-Cell Adenoma	0	2	0	0	1	2
C-Cell Carcinoma	0	0	4	0	1	6

In male mice, dietary administration of the compound did not increase the incidence of hepatocellular neoplasms, whereas dosed females showed a striking increase in hepatocellular carcinomas and hepatocellular adenomas.

Grossly, hepatocellular neoplasms appeared as smooth, nodular, rounded masses distorting the normal shape of the liver. Color varied, many neoplasms appearing pale tan or dark red. Microscopically, hepatocellular carcinomas were expansive masses of hepatocytes exhibiting loss of normal architectural pattern, the cells being arranged in sheets or trabeculae instead of the normal lobules. Nuclei were frequently uniform, although variable amounts of pleomorphism did occur. The cytoplasm was either basophilic or acidophilic, sometimes varying from one region of the tumor to another, and was frequently pale. Lesions classified as hepatocellular adenomas were smaller, usually better differentiated, and were less pleomorphic than the hepatocellular carcinomas.

The criteria for classification of thyroid neoplasms in mice were the same as those used to classify thyroid neoplasms in rats. The nonneoplastic thyroid lesions found in dosed mice were similar to those in the rats but occurred in higher incidences. Hyperplasia of follicular cells (focal, papillary or adenomatous) were found in 2/38 (5 percent) control, 12/46 (26 percent) low dose, and 9/43 (21 percent) high dose male mice. Abundant golden brown pigment was seen in follicular epithelium, colloid, and macrophages. In the mice,

there were frequent foci of lymphocytes in the thyroid parenchyma and occasional cystic areas filled with amorphous material containing long clefts suggesting cholesterol crystals.

Three transitional-cell papillomas occurred in the bladder or urethra of dosed mice (two high dose males and one high dose female), but none occurred in controls.

Based upon the results of this pathologic examination, 1,5-naphthalenediamine was carcinogenic to B6C3F1 mice, producing hepatocellular neoplasms in females and thyroid neoplasms in both sexes.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of tumor in either sex where at least two such tumors were observed in at least one of the control or 1,5-naphthalenediamine-dosed groups and where such tumors were observed in at least 5 percent of the group.

For both male and female mice elevated incidences of thyroid tumors were observed in the dosed groups. In female mice the Cochran-Armitage test indicated a significant ($P = 0.005$) positive association between dietary concentration and the incidence of C-cell carcinomas. This was supported by a significant ($P = 0.014$) Fisher exact test for the high dose group. For males the Cochran-Armitage test result was also significant ($P = 0.017$), but the Fisher exact tests were