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BIOASSAY OF 4-AMINO-2-NITROPHENOL FOR POSSIBLE CARCINOGENICITY

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

BIOASSAY OF

4-AMINO-2-NITROPHENOL

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

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REPORT ON BIOASSAY OF 4-AMINO-2-NITROPHENOL FOR POSSIBLE CARCINOGENICITY

Availability

4-Amino-2-nitrophenol (CAS 99-56-9) has been tested for cancer-causing activity with rats and mice in the Bioassay Program, Division of Cancer Cause and Prevention, National Cancer Institute. A report is available to the public.

Summary: A bioassay of 4-amino-2-nitrophenol for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F1 mice. Applications of the chemical include use as an ingredient of hair dyes and as an intermediate in the manufacture of dyes.

It is concluded that under the conditions of the bioassay, 4-amino-2-nitrophenol was carcinogenic for male Fischer 344 rats, inducing transitional-cell carcinomas of the urinary bladder; the transitional-cell carcinomas of the urinary bladder observed in three dosed female rats may also have been associated with administration of the 4-amino-2-nitrophenol. The test chemical was not carcinogenic for male or female B6C3F1 mice at the doses tested.

Single copies of the report are available from the Office of Cancer Communications, National Cancer Institute, Building 31, Room 10A21, National Institutes of Health, Bethesda, Maryland 20014.

Dated: October 3, 1978

Director
National Institutes of Health

(Catalogue of Federal Domestic Assistance Program Number 13.393, Cancer Cause and Prevention Research)

BIOASSAY OF
4-AMINO-2-NITROPHENOL
FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
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FOREWORD: This report presents the results of the bioassay of 4-amino-2-nitrophenol conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: The bioassay of 4-amino-2-nitrophenol was conducted by Litton Bionetics, Inc., Kensington, Maryland, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., the prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design and doses were chosen by Drs. E. K. Weisburger[1], J. H. Weisburger[1,2], and N. P. Page[1,3], the NCI project officers; Dr. F. M. Garner[4] was the principal investigator, and Mr. S. Johnson[4], co-principal investigator. The administration of the test chemical and the observation of the animals were supervised by Dr. Garner, and technical assistance with the bioassay was provided by Mr. R. Cypher[4], Mr. H. D. Thornett[4], and Mr. D. J. Howard[4]. Ms. J. Blalock[4] was responsible for data assembly.

Histopathologic examination was performed by Drs. P. K. Hildebrandt[4], N. J. Wosu[4], F. M. Garner and Dr. B. C. Zook[4]. Dr. R. Montali[4] reviewed the diagnoses and prepared the interpretive pathology narrative.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[5]. The statistical analyses were performed by Dr. J. R. Joiner[6], using methods selected for the bioassay program by Dr. J. J. Gart[7]. Chemicals used in this bioassay were analyzed under the direction of Dr. E. Murrill[8], dosed feed mixtures were analyzed by Mr. H. Paulin[4], and the results of the analyses were reviewed by Dr. S. S. Olin[6]. The chemical structure was supplied by NCI.

This report was prepared at Tracor Jitco[6] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. L. A. Campbell, Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Dr. J. F. Robens, toxicologist; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. L. A. Waitz, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley and Ms. P. J. Graboske.

The following other scientists at NCI were responsible for evaluating the bioassay, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Dawn G. Goodman, Dr. Richard A. Griesemer, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Robert A. Squire[9], Dr. Sherman Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

A bioassay of 4-amino-2-nitrophenol for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F1 mice.

Groups of 50 rats and 50 mice of each sex were administered 4-amino-2-nitrophenol at one of two doses, either 1,250 or 2,500 ppm, for 103 weeks. Matched controls consisted of groups of 20 untreated rats and 20 untreated mice of each sex. All dosed and matched-control groups of each species and sex were killed at 105 weeks.

Mean body weights of dosed rats of each sex were not appreciably affected by administration of the 4-amino-2-nitrophenol, and mean body weights of dosed mice of each sex were only slightly lower than those of corresponding matched controls. Survival of neither rats nor mice was affected by the test chemical, and sufficient numbers of animals in dosed and control groups were at risk for development of late-appearing tumors. Since both male and female mice receiving 4-amino-2-nitrophenol had little or no depression in mean weights and their survival was comparable to that of controls, they may have been able to tolerate a higher dose.

In rats, transitional-cell carcinomas of the urinary bladder showed a dose-related trend in the males ($P < 0.001$) and occurred at a significantly higher incidence ($P = 0.018$) in the high-dose males than in the matched-control males (controls 0/15, low-dose 0/46, high-dose 11/39 [28%]). Carcinomas of the bladder also occurred in one low-dose female and two high-dose females, but in none of the control females. Transitional-cell papillomas of the bladder occurred in two additional high-dose males, and transitional-cell hyperplasia of the bladder occurred in four additional high-dose males, but neither lesion occurred in control males. No tumors of the bladder were found among 220 male and 220 female historical-control rats at this laboratory.

In mice, no tumors occurred in dosed groups of males or females

at incidences that were significantly higher than those in the corresponding matched-control groups.

Deposition of pigment occurred in the lamina propria of the small intestine in at least 91% of the animals in the dosed groups of rats and in at least 89% of the animals in the dosed groups of mice, but in none of the control groups of either species.

It is concluded that under the conditions of the bioassay, 4-amino-2-nitrophenol was carcinogenic for male Fischer 344 rats, inducing transitional-cell carcinomas of the urinary bladder; the transitional-cell carcinomas of the urinary bladder observed in three dosed female rats may also have been associated with administration of the 4-amino-2-nitrophenol. The test chemical was not carcinogenic for male or female B6C3F1 mice at the doses tested.

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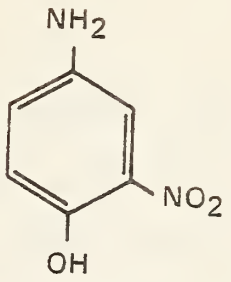
I. INTRODUCTION

4-Amino-2-nitrophenol (CAS 119-34-6; NCI C03963) is used as an industrial dye intermediate, and as a constituent of "semi-permanent" hair dyes.



4-AMINO-2-NITROPHENOL

This compound is designated CI76555 by the Society of Dyers and Colourists, who describe its industrial use as an oxidation base (Oxidation Base 25) in applications involving furs. These bases are applied in conjunction with an oxidizing agent, such as hydrogen peroxide, for the development of the color (Society of Dyers and Colourists, 1971).

In contrast, in hair dyes for humans, 4-amino-2-nitrophenol is formulated without an oxidizing agent. It is used in concentrations estimated at 0.1-1.0% in the "semi-permanent" hair dyes, which are applied as shampoos and remain on the hair for 20-40 minutes before they are rinsed out (Wall, 1972; Corbett and Menkart, 1973; Burnett et al., 1976; FDA, 1977).

4-Amino-2-nitrophenol was one of a group of hair dye constituents selected for the Carcinogenesis Testing Program because several of these chemicals had been shown to be mutagenic in bacterial test systems (work later published by Ames et al., 1975).

II. MATERIALS AND METHODS

A. Chemical

Two batches of 4-amino-2-nitrophenol were obtained from the Aldrich Chemical Company, Milwaukee, Wisconsin. Lot No. 071137 was used in the subchronic studies; Lot No. 100737 was used in the chronic studies.

The identity of both lots was established through elemental analyses (C, H, N), and spectral data (ultraviolet, infrared, and nuclear magnetic resonance). Lot No. 071137 had a purity of 99.0 $\pm$ 0.3% as determined by perchloric acid titration of the amine function, and Lot No. 100737 had a purity of 99.6 $\pm$ 0.3% by the same method. Vapor-phase chromatography and thin-layer chromatography showed several trace impurities in Lot No. 071137 and one impurity in Lot No. 100737. Lot No. 071137 contained 0.18 $\pm$ 0.01% water, and Lot No. 100737, less than 0.2% water, as determined by Karl Fischer analysis. Melting ranges were similar for each lot (Lot No. 100737: 128-130°C [visual, capillary]; Lot No. 671137: 126.5-129°C [visual, capillary]) and corresponded to values in the literature (127-128°C) (Verkade et al., 1946).

After the completion of the bioassay, a sample from Lot No. 100737 was reanalyzed. The infrared spectrum of this lot was

identical with that obtained in the original analysis, and perchloric acid titration indicated 98.7 ± 0.2% purity.

The bulk chemical was stored at 4°C.

B. Dietary Preparation

A 6-kilogram diet was prepared three times per week for the rats and two times per week for the mice. To obtain each dietary concentration, the appropriate weight of the compound was mixed with a small portion of Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) in a mortar. This premix was then added to the remaining feed and blended for 15 minutes in a Patterson-Kelly twin-shell blender equipped with an intensifier bar. Dosed feed preparations were stored at 4°C for up to 1 week.

As a quality control measure, selected samples from 14 freshly prepared diets were analyzed during the chronic studies. The compound was extracted from feed with 0.1 N ammonium hydroxide in 1:1 water:methanol, diluted with methanol, centrifuged, and the absorbance of the supernatant read at 445 nm. Concentrations were found to be within 25% of theoretical concentrations.

C. Animals

Fischer 344 rats and B6C3F1 mice were obtained from the Frederick

Cancer Research Center, Frederick, Maryland, under a contract with the Division of Cancer Treatment, NCI.

The animals were 28 days of age when received at the laboratory and were quarantined for 2 weeks prior to the start of the bioassay. Any animals with clinical signs of disease and any runts were destroyed. The remaining animals were segregated into equal weight groups and assigned to control or dosed groups in such a way that the mean weights of animals in each cage within a particular group were approximately the same.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature was maintained at 21-25°C and the relative humidity at 45-55%. There were 15 changes of room air per hour, and the incoming and exhaust air was filtered through high efficiency particulate air (HEPA) filters. The animal rooms were positively pressurized with respect to the exit hall and negatively pressurized with respect to the entrance hall. Rooms were illuminated with cool white fluorescent lighting for 8 hours per day.

Rats were housed four per cage and mice five per cage in solid polycarbonate cages (Lab Products, Inc., Garfield, N. J.). Each cage was covered with a wire mesh screen and a sheet of filter

paper, and contained a heat-treated hardwood chip bedding (Absorb-Dri®, Lab Products, Inc., Garfield, N. J.) in the bottom. Cages were washed and furnished with fresh bedding two times per week. Water bottles, sipper tubes and stoppers were also washed twice per week while feed hoppers were washed once per week. All of this equipment was cleaned at 82°C with detergent, rinsed, and steamed.

Control animals were fed Wayne® Lab Blox animal meal, and dosed animals received the same product mixed with the test chemical. The feed hoppers were filled three times per week. Acidified tap water (pH 2.5) was available ad libitum in water bottles.

Rats and mice were housed in separate rooms. Control and dosed animals were housed in the same room as the respective dosed animals. Animals fed 4-amino-2-nitrophenol were maintained in the same rooms as animals of the same species being administered one of the following chemicals:

Rats

Feed Studies

(CAS 624-18-0) p-phenylenediamine dihydrochloride
(CAS 18662-53-8) nitrilotriacetic acid, trisodium salt
(CAS 101-61-1) 4,4′-methylene bis(N,N′-dimethylaniline)
(CAS 105-11-3) p-quinone dioxime

Gavage Studies

(CAS 4377-33-7) 2-(chloromethyl)pyridine hydrochloride
(CAS 100-42-5) styrene

Mice

Feed Studies

(CAS 76-78-9) triphenyltin hydroxide
(CAS 91-93-0) 3,3'-dimethoxybenzidine diisocyanate
(CAS 77-65-6) alpha-bromo-alpha-ethylbutyryl carbamide
(CAS 105-55-5) N,N'-diethylthiourea
(CAS 1596-84-5) succinic acid, 2,2-dimethylhydrazide
(CAS 126-31-8) iodomethanesulfonic acid, sodium salt
(CAS 105-11-3) p-quinone dioxime
(CAS 150-38-9) ethylenediaminetetraacetic acid, trisodium salt trihydrate

Gavage Studies

(CAS 434-13-9) lithocholic acid

E. Subchronic Studies

Subchronic studies were conducted with Fischer 344 rats and B6C3F1 mice of each sex to estimate the maximum tolerated doses of 4-amino-2-nitrophenol, on the basis of which two concentrations (hereinafter referred to as "low" and "high" doses) were selected for the chronic studies.

4-Amino-2-nitrophenol was administered in the diet to rats at one of 11 doses, either 147, 215, 316, 464, 681, 1,000, 1,470, 2,150, 3,160, 4,640, or 6,810 ppm and to mice at one of 11 doses, either 100, 147, 215, 316, 464, 681, 1,000, 1,470, 2,150, 3,160, or 4,640 ppm. Groups of five males and five females were tested at

each dose, and groups of equal size were used as matched controls. The compound was administered for 6 weeks, and the animals were observed for the following 2 weeks.

After the 6 weeks of compound administration, there were no deaths in the rats or the mice, nor were there any changes in the mean body weights of the dosed animals in comparison with the controls, other than an approximate 20% weight depression in female rats fed concentrations of 3,160 ppm and greater. No gross pathologic changes were reported.

The doses selected for the chronic studies in both species were 1,250 and 2,500 ppm.

F. Chronic Studies

The test groups, doses administered, and times on study of the chronic feeding studies are shown in tables 1 and 2.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, and animals that were moribund were killed and necropsied. Rats and mice were weighed at regular intervals. Palpation for masses was carried out at each weighing.

The pathologic evaluation consisted of gross and microscopic

Table 1. 4-Amino-2-Nitrophenol Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals[a]	4-Amino-2-Nitrophenol Dose[b] (ppm)	Time on Study: Dosed (weeks)	Time on Study: Observed[c] (weeks)
Male				
Matched-Control	20	0		105
Low-Dose	50	1,250	103	2
High-Dose	50	2,500	103	2
Female				
Matched-Control	20	0		105
Low-Dose	50	1,250	103	2
High-Dose	50	2,500	103	2

[a]All animals were approximately 6 weeks of age when placed on study.

[b]Rats were fed the diet preparations ad libitum, 7 days per week.

[c]Control diet was fed during the observation period.

Table 2. 4-Amino-2-Nitrophenol Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals[a]	4-Amino-2-Nitrophenol Dose[b] (ppm)	Time on Study	
			Dosed (weeks)	Observed[c] (weeks)
Male				
Matched-Control	20	0		105
Low-Dose	50	1,250	103	2
High-Dose	50	2,500	103	2
Female				
Matched-Control	20	0		105
Low-Dose	50	1,250	103	2
High-Dose	50	2,500	103	2

[a]All animals were approximately 6 weeks of age when placed on study.

[b]Mice were fed the diet preparations ad libitum, 7 days per week.

[c]Control diet was fed during the observation period.

examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. All animals were killed with carbon dioxide. The following tissues were examined microscopically: skin, lymph nodes, mammary gland, salivary gland, bone marrow, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, small intestine, large intestine, liver, gallbladder (mice), pancreas, spleen, kidney, adrenal, urinary bladder, prostate or uterus, testis or ovary, brain, and pituitary. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. An occasional section was subjected to special staining techniques for more definitive diagnosis.

A few tissues from some animals were not examined, particularly from those animals that died early. Also, some animals may have been missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. Thus, the number of animals from which particular organs or tissues were examined microscopically varies, and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

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

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 an animal died naturally or was 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 dosed 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 dosed 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 dosed 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% 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 ($P < 0.025$ 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. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of dosed male and female rats were not appreciably affected by the administration of 4-amino-2-nitrophenol (figure 1). Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. No other clinical signs were reported on the animals administered the test chemical.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered 4-amino-2-nitrophenol in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 2. The result of the Tarone test for dose-related trend in mortality is not significant in either sex.

In male rats, 33/50 (66%) of the high-dose group, 37/50 (74%) of the low-dose group, and 12/20 (60%) of the matched-control group lived to termination of the study. In females, 37/50 (74%) of the high-dose group, 36/49 (74%) of the low-dose group, and 14/20 (70%) of the matched-control group survived to termination of the study.

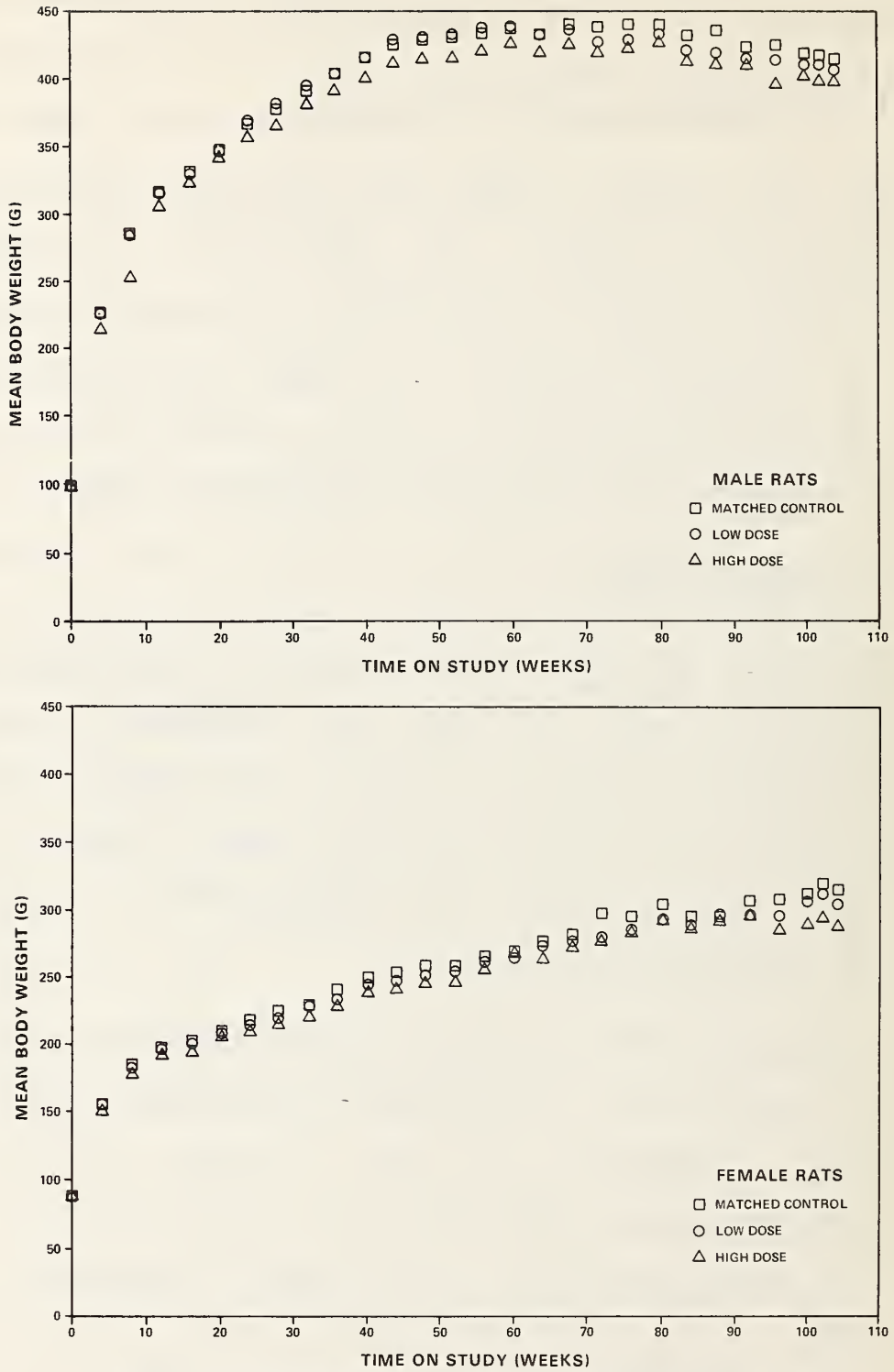
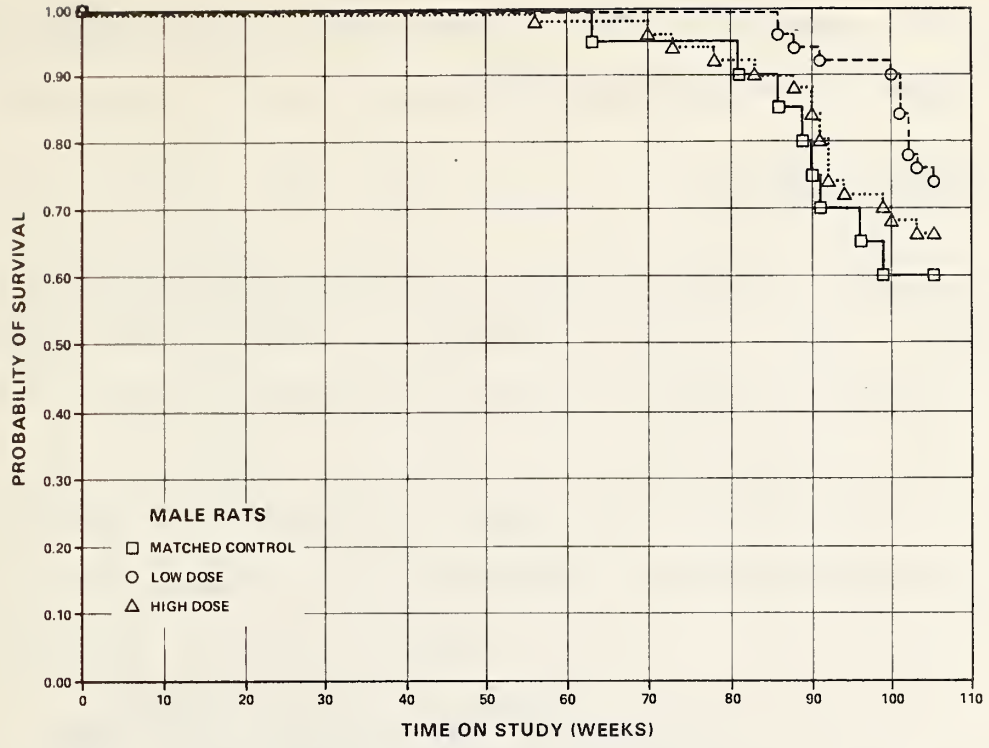


Figure 1. Growth Curves for Rats Fed 4-Amino-2-Nitrophenol in the Diet



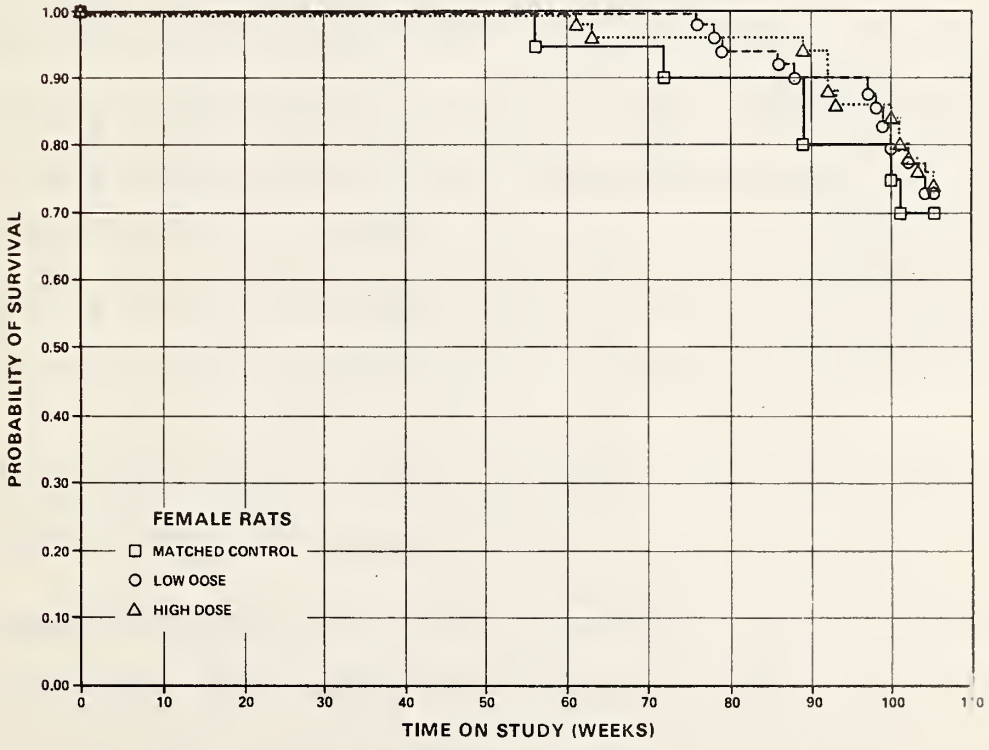


Figure 2. Survival Curves for Rats Fed 4-Amino-2-Nitrophenol in the Diet

Sufficient numbers of rats of each sex were at risk for 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.

Tumors of the urinary bladder occurred only in the rats administered 4-amino-2-nitrophenol, as follows:

	RATS					
	Males			Females		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Number of Animals with Tissues Examined Microscopically	(15)	(46)	(39)	(15)	(42)	(46)
Transitional-cell carcinoma			11(28%)		1(2%)	2(4%)
Transitional-cell papilloma			2(5%)			
Transitional-cell hyperplasia			4(10%)			

Microscopically, the transitional-cell tumors varied from papillary structures packed with hyperchromatic epithelial cells, pleomorphic nuclei, and mitotic figures to subepithelial, dome-shaped solid masses of similar tumor cells. The masses often

protruded into the bladder lumen. There was invasion of the bladder wall, and in one case metastases appeared in the lungs.

Other remaining tumors that occurred in the control and dosed rats were considered spontaneous. Most of them had incidences as expected for this age and Fischer 344 strain of rat. In these cases there were approximate equivalent frequencies and expected sex predilections for tumors and hyperplasias, including testicular interstitial-cell tumors in the males, pituitary chromophobe tumors mainly in females, and C-cell tumors and hyperplasias of the thyroid in both sexes.

There were a few randomly distributed malignant tumors in the dosed rats that did not occur in the controls, including an epidermoid (squamous-cell) carcinoma of the salivary gland (1/47 high-dose males); a fibrosarcoma (1/50 high-dose males); an osteosarcoma (1/50 high-dose females); and a chondrosarcoma (1/50 high-dose females) that metastasized to the lung, but because of the single occurrences they were not considered significant.

There occurred also a variety of nonneoplastic lesions that are commonly observed in Fischer 344 rats.

Pigmentary changes occurred in the lamina propria of the small intestines in 44/45 low-dose males, 43/45 high-dose males, 43/44 low-dose females, and 43/47 high-dose females. The pigment was

dark brown and finely granular. It was within macrophages of the lamina propria and usually oriented more towards the tips of the villi. The pigment was not birefringent under polarized light and was negative for iron (Prussian-blue). It was melanin-like, and appeared to be more abundant in the high-dose group.

There were degenerative and inflammatory conditions usually encountered in aging rats.

The results of the histopathologic examination indicate that the transitional-cell tumors of the urinary bladder occurring in the high-dose male rats and in the low- and high-dose females, the transitional-cell papillomas and hyperplasias occurring in the dosed males, and the pigmentary intestinal changes occurring in all dosed groups were induced by the administration of 4-amino-2-nitrophenol under the conditions of this bioassay.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E 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 rats, the results of the Cochran-Armitage test for positive dose-related trend in the incidence of transitional-cell

carcinomas of the urinary bladder is significant ($P < 0.001$). A departure from linear trend is indicated ($P = 0.030$), because of the relatively steep increase in incidence in the high-dose group. The results of the Fisher exact test show that the incidence in the high-dose group is significantly higher ($P = 0.018$) than that in the matched-control group. The statistical conclusion is that the incidence of transitional-cell carcinomas of the urinary bladder in male rats is associated with the administration of 4-amino-2-nitrophenol. No tumors of the urinary bladder were found in 220 male or 220 female historical controls at this laboratory. The results of the statistical tests on the incidence of this tumor in female rats are not significant; however, transitional-cell carcinomas of the urinary bladder were observed in 1/43 (2%) of the low-dose females and 2/44 (5%) of the high-dose females compared with 0/15 in the control groups.

A significant dose-related trend in the negative direction ($P = 0.043$) is observed in the incidence of thyroid tumors in male rats, due to the higher incidence in the control group than in the dosed groups.

IV. RESULTS - MICE

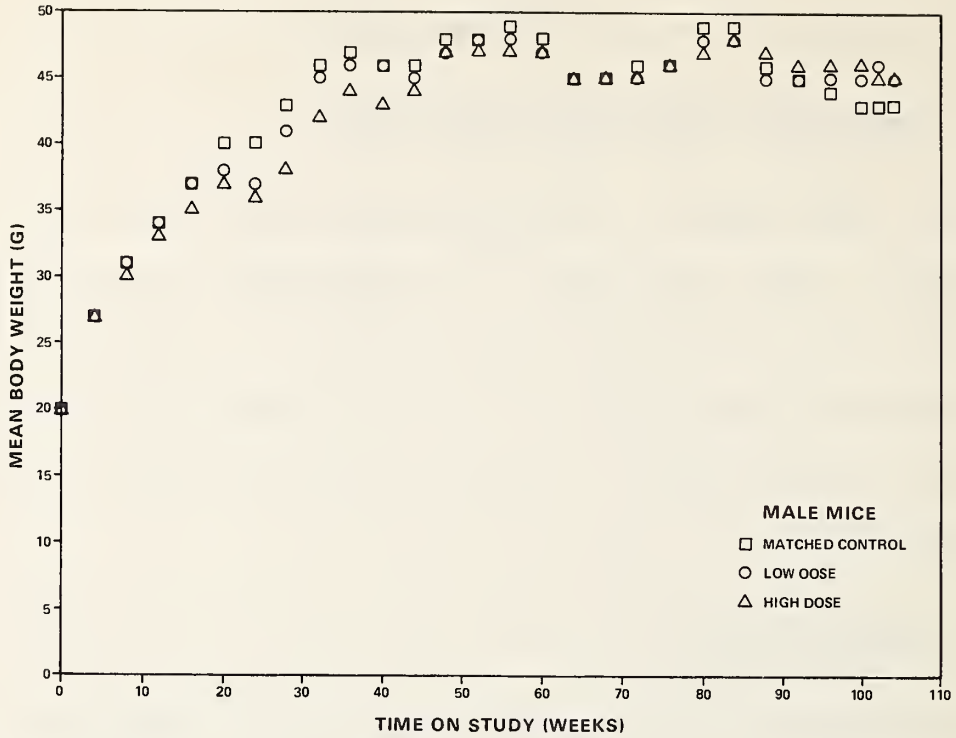
A. Body Weights and Clinical Signs (Mice)

Mean body weights of dosed male and female mice were only slightly lower than those of corresponding matched controls (figure 3). Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. No other clinical signs were reported.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered 4-amino-2-nitrophenol in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 4. The result of the Tarone test for dose-related trend in mortality is not significant in either sex.

In male mice, 43/50 (86%) of the high-dose group, 41/50 (82%) of the low-dose group, and 17/20 (85%) of the matched-control group lived to termination of the study. In females, 44/50 (88%) of the high-dose group, 46/50 (92%) of the low-dose group, and 19/20 (95%) of the matched-control group survived to termination of the study.



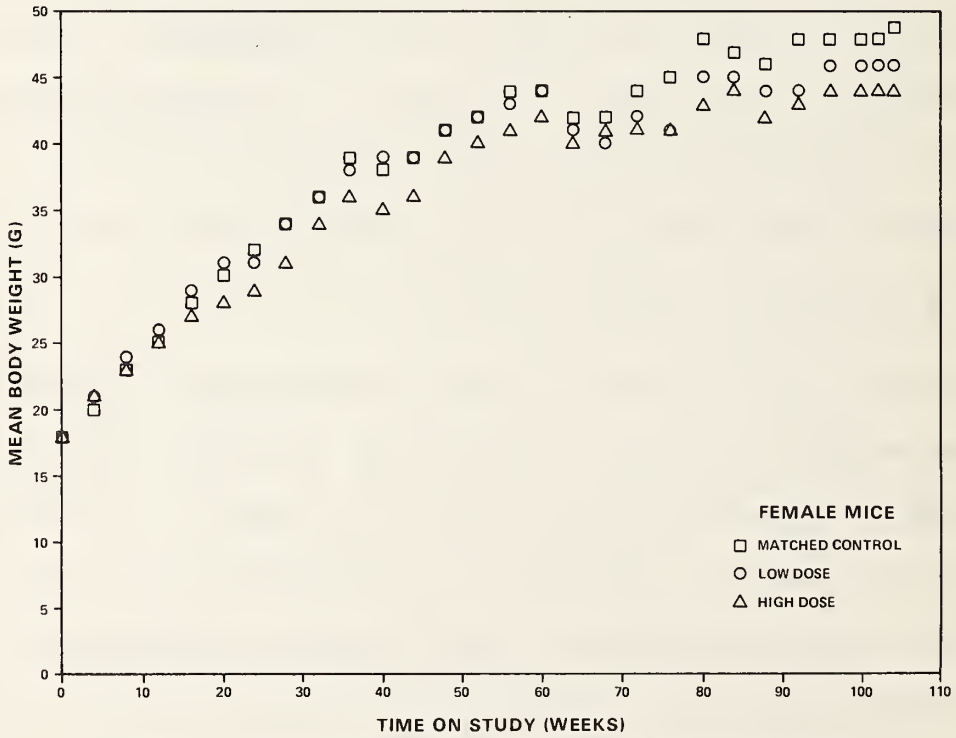
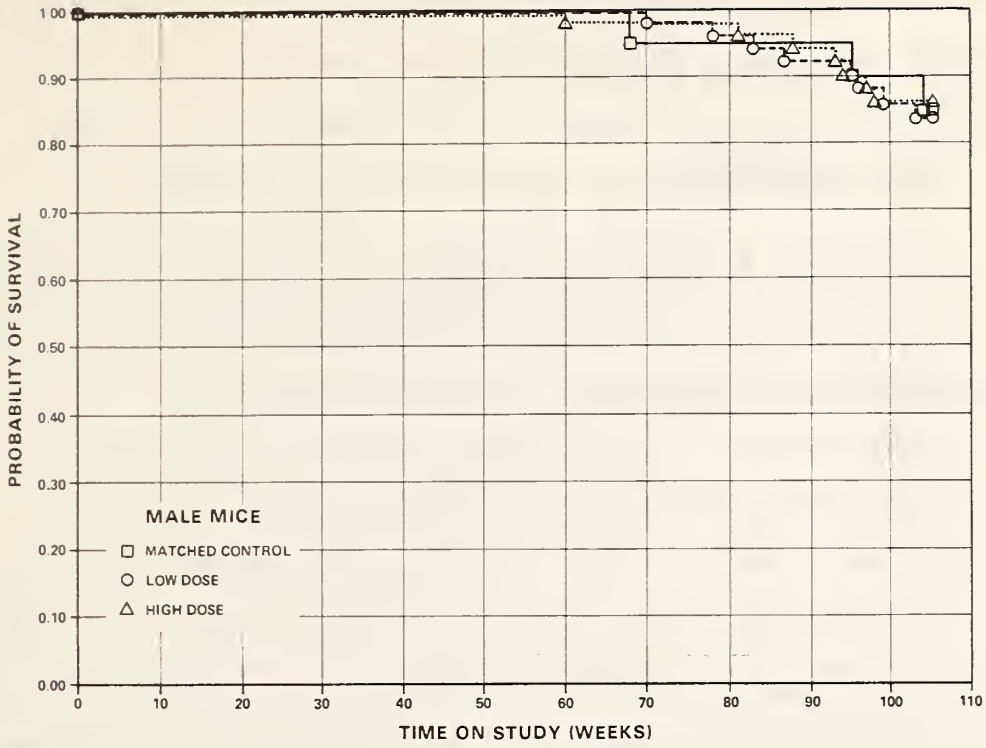


Figure 3. Growth Curves for Mice Fed 4-Amino-2-Nitrophenol in the Diet



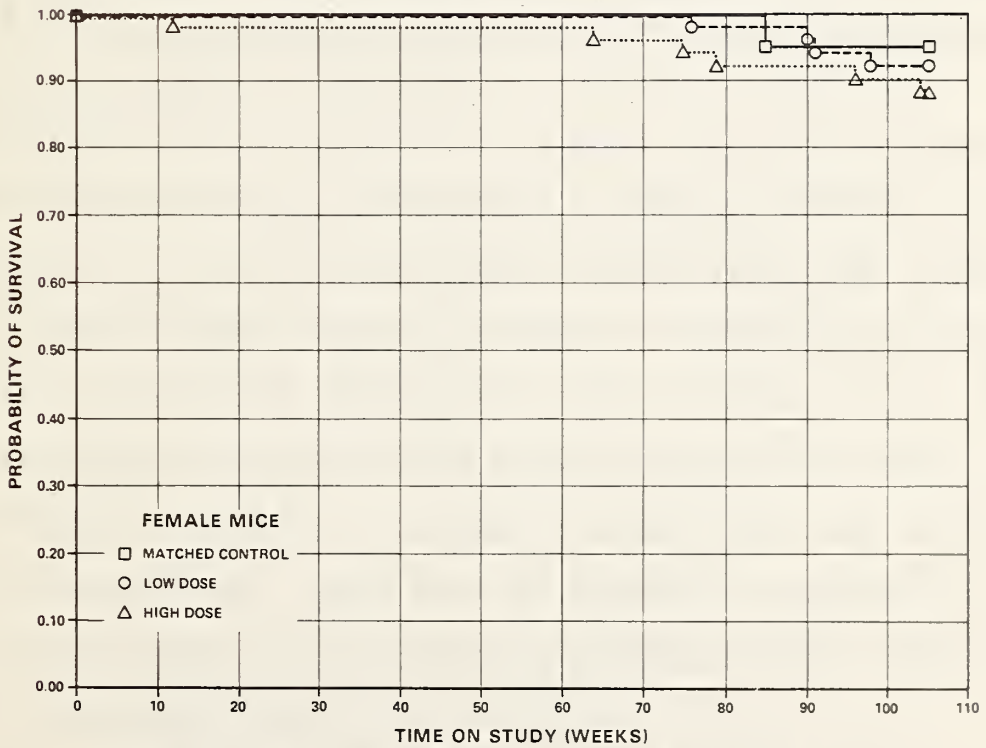


Figure 4. Survival Curves for Mice Fed 4-Amino-2-Nitrophenol in the Diet

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

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.

All of the tumors and hyperplasias that occurred in the mice were spontaneous types which occurred in approximately equal incidences in the control and dosed groups. There was a slight increase of hepatic adenomas in the high-dose mice compared with the controls, but an equal percentage of hepatocellular carcinomas occurred in both control and dosed groups.

Several nonneoplastic changes were observed and were considered to be either spontaneous or intercurrent disease processes. One change that occurred in the dosed mice but not in the controls consisted of deposits of dark brown, finely granular pigment in the lamina propria of the small intestine. The pigment change occurred in 46/49 low-dose and 43/47 high-dose males and in 43/48 low-dose and 42/47 high-dose females. It appeared similar to that described in the rats of this study with respect to its location, dose relationship, and staining characteristics.

The results of the histopathologic examination indicate that 4-amino-2-nitrophenol was not carcinogenic in mice but induced the deposition of pigment in the lamina propria of the small intestine under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F 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.

The results of the Cochran-Armitage test for dose-related trend and those of the Fisher exact test comparing the incidences of tumors in each of the dosed groups with that in the control group are not significant in the positive direction in either sex.

Significant results in the negative direction are observed in the incidence of alveolar/bronchiolar carcinoma of the lung in male mice; however, when combined incidences of animals with either adenoma or carcinoma of the lungs are analyzed, there is no significant difference between control and dosed groups. In female mice, a significant trend ($P = 0.035$) in the negative direction in the incidences of follicular-cell adenoma or papillary adenoma of the thyroid is observed, but the results of the Fisher exact tests are not significant.

In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one or less than one is included; this indicates the absence of significant positive results. It should also be noted that each of the intervals (except that for the incidence of alveolar/bronchiolar carcinoma of the lung in male mice) has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by 4-amino-2-nitrophenol, which could not be detected under the conditions of this test.

V. DISCUSSION

On the basis of rate of growth, mortality, or other clinical signs, there was little evidence of toxicity of 4-amino-2-nitrophenol in the dosed rats or mice. Mean body weights of the female mice were only slightly lower than those of the controls throughout much of the bioassay. The survival of either rats or mice was not affected by the test chemical, and at the end of the bioassay, the survival of the animals in dosed and control groups of both the rats and the mice was at least 60%. Sufficient numbers of animals were at risk for the development of late-appearing tumors. Since both male and female mice receiving 4-amino-2-nitrophenol had little or no depression in mean weights and their survival was comparable to controls, they may have been able to tolerate a higher dose.

In rats, transitional-cell carcinomas of the urinary bladder showed a dose-related trend in the males ($P < 0.001$) and occurred at a significantly higher incidence ($P = 0.018$) in the high-dose males than in the matched-control males (controls 0/15, low-dose 0/46, high-dose 11/39 [28%]). Carcinomas of the bladder also occurred in one low-dose female and two high-dose females, but in none of the control females. Transitional-cell papillomas of the bladder occurred in two additional high-dose males, and transitional-cell hyperplasia of the bladder occurred in four

additional high-dose males, but neither lesion occurred in control males. No tumors of the bladder were found among 220 male and 220 female historical-control Fischer 344 rats at this laboratory.

In mice, no tumors occurred in dosed groups of males or females at incidences that were significantly higher than those in the corresponding matched-control groups.

Deposition of pigment occurred in the lamina propria of the small intestine in at least 91% of the animals in the dosed groups of rats and in at least 89% of the animals in the dosed groups of mice, but in none of the control groups of either species.

The LD_{50} of 4-amino-2-nitrophenol in Charles River CD rats has been reported as 3,300 mg/kg when the chemical was administered orally and 302 mg/kg when it was given by intraperitoneal injection (Burnett et al., 1976; Burnett et al., 1977). A hair dye containing the chemical caused no embryotoxic or teratogenic effects in CD rats when it was applied to the skin at 2 ml/kg at intervals during pregnancy (Burnett et al., 1976) and also did not induce a dominant lethal effect when it was tested in mature male CD rats by intraperitoneal injection at a dose of 20 mg/kg (Burnett et al., 1977). When a hair dye containing 4-amino-2-nitrophenol as well as Cl Acid Black 107 was applied to the skin

of DBAf or A strain mice, tumors of lymphoid origin developed at a higher incidence in the DBAf strain and at earlier times in both strains than in corresponding untreated controls (Venitt and Searle, 1976). Two dosed DBAf females developed sarcomas of the genital tract at weeks 66 and 69 (Venitt and Searle, 1976). Positive results were also obtained when the same hair dye (Venitt and Searle, 1976) or the 4-amino-2-nitrophenol alone (Garner and Nutman, 1977) was tested in the Salmonella mutagenicity test (McCann et al., 1975).

It is concluded that under the conditions of the bioassay, 4-amino-2-nitrophenol was carcinogenic for male Fischer 344 rats, inducing transitional-cell carcinomas of the urinary bladder; the transitional-cell carcinomas of the urinary bladder observed in three dosed female rats may also have been associated with administration of the 4-amino-2-nitrophenol. The test chemical was not carcinogenic for male or female B6C3F1 mice at the doses tested.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN

RATS FED 4-AMINO-2-NITROPHENOL IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED 4–AMINO–2–NITROPHENOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	19	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (5%)	1 (2%)	1 (2%)
FIBROMA	1 (5%)	1 (2%)	1 (2%)
FIBROSARCOMA			1 (2%)
LIPOMA			1 (2%)
NEUROFIBROMA	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(19)	(50)	(48)
SQUAMOUS CELL CARCINOMA, METASTA	1 (5%)	1 (2%)	1 (2%)
TRANSITIONAL-CELL CARCINOMA, MET			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)		1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
LEUKEMIA,NOS		3 (6%)	2 (4%)
UNDIFFERENTIATED LEUKEMIA	3 (15%)	7 (14%)	7 (14%)
LYMPHOCYTIC LEUKEMIA			1 (2%)
GRANULOCYTIC LEUKEMIA		2 (4%)	1 (2%)
#SPLEEN	(17)	(45)	(47)
MALIG.LYMPHOMA, UNDIFFER-TYPE		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			
#SALIVARY GLAND	(16)	(49)	(47)
SQUAMOUS CELL CARCINOMA, INVASIV			1 (2%)
#LIVER	(19)	(48)	(48)
HEPATOCELLULAR ADENOMA		1 (2%)	
URINARY SYSTEM			
#URINARY BLADDER	(15)	(46)	(39)
TRANSITIONAL-CELL PAPILLOMA			2 (5%)
TRANSITIONAL-CELL CARCINOMA			11 (28%)
HEMANGIOMA			1 (3%)
ENDOCRINE SYSTEM			
#PITUITARY	(15)	(40)	(39)
CHROMOPHOBE ADENOMA	2 (13%)	4 (10%)	8 (21%)
#ADRENAL	(18)	(46)	(49)
CORTICAL CARCINOMA			1 (2%)
PHEOCHROMOCYTOMA	1 (6%)	1 (2%)	2 (4%)
#THYROID	(18)	(42)	(44)
FOLLICULAR-CELL ADENOMA	1 (6%)		1 (2%)
FOLLICULAR-CELL CARCINOMA			1 (2%)
C-CELL ADENOMA	2 (11%)	2 (5%)	1 (2%)
C-CELL CARCINOMA	1 (6%)		
#PANCREATIC ISLETS	(18)	(46)	(49)
ISLET-CELL ADENOMA	2 (11%)	1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
#TESTIS	(17)	(50)	(50)
INTERSTITIAL-CELL TUMOR	15 (88%)	50 (100%)	41 (82%)
NERVOUS 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
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	5	6	8
MORIBUND SACRIFICE	3	7	9
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	12	37	33
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	16	50	48
TOTAL PRIMARY TUMORS	31	75	86
TOTAL ANIMALS WITH BENIGN TUMORS	15	50	46
TOTAL BENIGN TUMORS	26	60	60
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	14	23
TOTAL MALIGNANT TUMORS	5	15	26
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	2
TOTAL SECONDARY TUMORS	1	1	3
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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED 4–AMINO–2–NITROPHENOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	@50	50
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	48
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(49)	(50)
FIBROMA			1 (2%)
*SUBCUT TISSUE	(20)	(49)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
FIBROMA		2 (4%)	1 (2%)
LIPOMA			1 (2%)
OSTEOSARCOMA			1 (2%)
CHONDROSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#TRACHEA	(18)	(45)	(48)
CARCINOMA-IN-SITU, NOS	1 (6%)		
#LUNG	(18)	(48)	(46)
CARCINOMA, NOS, METASTATIC	1 (6%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (6%)		
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (6%)		
CHONDROSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(50)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)
LEUKEMIA,NOS		2 (4%)	
UNDIFFERENTIATED LEUKEMIA	1 (5%)	4 (8%)	1 (2%)
LYMPHOCYTIC LEUKEMIA	1 (5%)		
GRANULOCYTIC LEUKEMIA			2 (4%)
#SPLEEN	(19)	(48)	(47)
HEMANGIOMA			1 (2%)

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

@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A MALE IN A FEMALE GROUP.

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#MANDIBULAR L. NODE	(19)	(49)	(47)
CARCINOMA, NOS, METASTATIC			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(48)	(48)
CARCINOMA, NOS, METASTATIC	1 (5%)		
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(48)
CARCINOMA, NOS, METASTATIC	1 (5%)		
#URINARY BLADDER	(15)	(43)	(44)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	2 (5%)
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(48)	(45)
CHROMOPHOBE ADENOMA	8 (44%)	26 (54%)	20 (44%)
#ADRENAL	(19)	(48)	(48)
PHEOCHROMOCYTOMA	1 (5%)		
#THYROID	(17)	(44)	(47)
ADENOMA, NOS			1 (2%)
FOLLICULAR-CELL ADENOMA			1 (2%)
C-CELL ADENOMA	1 (6%)	2 (5%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(50)
ADENOMA, NOS		1 (2%)	1 (2%)
FIBROADENOMA	1 (5%)	3 (6%)	5 (10%)
#UTERUS	(17)	(47)	(48)
LEIOMYOMA			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
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	5	6
MORIBUND SACRIFICE	2	8	7
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	14	36	37
ANIMAL MISSING			
ANIMAL DELETED(WRONG SEX)		1	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	34	28
TOTAL PRIMARY TUMORS	16	42	41
TOTAL ANIMALS WITH BENIGN TUMORS	11	30	23
TOTAL BENIGN TUMORS	12	34	33
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	8	8
TOTAL MALIGNANT TUMORS	4	8	8
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		2
TOTAL SECONDARY TUMORS	3		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 FED 4-ANIMO-2-NITROPHENOL IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED 4-AMINO-2-NITROPHENOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(20)	(49)	(48)
ADENOMATOUS POLYP, NOS		1 (2%)	
#LUNG	(20)	(49)	(48)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)	10 (20%)	7 (15%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (15%)		
PAPILLARY ADENOCARCINOMA, METAST	1 (5%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (4%)	1 (2%)
GRANULOCYTIC LEUKEMIA	1 (5%)		
#LYMPH NODE	(19)	(50)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#MANDIBULAR L. NODE	(19)	(50)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)		
#MESENTERIC L. NODE	(19)	(50)	(48)
NEOPLASM, NOS			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#LIVER	(20)	(50)	(49)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		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
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(49)
NEOPLASM, NOS			1 (2%)
HEPATOCELLULAR ADENOMA	3 (15%)	13 (26%)	12 (24%)
HEPATOCELLULAR CARCINOMA	3 (15%)	7 (14%)	7 (14%)
SARCOMA, NOS			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(18)	(44)	(45)
FOLLICULAR-CELL CARCINOMA			1 (2%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
LIPOMA			1 (2%)
ALL OTHER SYSTEMS			
THORAX			
LIPOSARCOMA			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	8	6
MORIBUND SACRIFICE	1		1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	17	41	43
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	32	28
TOTAL PRIMARY TUMORS	13	36	36
TOTAL ANIMALS WITH BENIGN TUMORS	5	23	18
TOTAL BENIGN TUMORS	5	24	20
TOTAL ANIMALS WITH MALIGNANT TUMORS	8	12	13
TOTAL MALIGNANT TUMORS	8	12	14
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	1
TOTAL SECONDARY TUMORS	1	1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			2
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED 4–AMINO–2–NITROPHENOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROSARCOMA		1 (2%)	
RHABDOMYOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)	3 (6%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)	1 (2%)	1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	2 (4%)	2 (4%)
LYMPHOCYTIC LEUKEMIA		1 (2%)	
*MEDIASTINUM	(20)	(50)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
#SPLEEN	(20)	(50)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
#LYMPH NODE	(20)	(49)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		2 (4%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#MESENTERIC L. NODE	(20)	(49)	(48)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (5%)		
#LIVER	(20)	(50)	(50)
MALIG.LYMPHOMA, UNDIFFER-TYPE		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
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
#PEYERS PATCH	(19)	(48)	(47)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
HEPATOCELLULAR ADENOMA		1 (2%)	2 (4%)
HEPATOCELLULAR CARCINOMA		1 (2%)	
#STOMACH	(19)	(49)	(47)
PAPILLOMA, NOS			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(19)	(47)	(46)
CORTICAL ADENOMA	1 (5%)	1 (2%)	1 (2%)
#THYROID	(17)	(40)	(42)
PAPILLARY ADENOMA		1 (3%)	
FOLLICULAR-CELL ADENOMA	2 (12%)		
#PANCREATIC ISLETS	(20)	(49)	(49)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOMA, NOS	1 (5%)		
#UTERUS	(19)	(48)	(49)
ENDOMETRIAL STROMAL POLYP			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
#OVARY	(18)	(41)	(45)
TERATOMA, BENIGN			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	4	5
MORIBUND SACRIFICE			1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	19	46	44
ANIMAL MISSING			

@ 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*	8	17	16
TOTAL PRIMARY TUMORS	9	19	17
TOTAL ANIMALS WITH BENIGN TUMORS	6	6	9
TOTAL BENIGN TUMORS	6	7	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	12	8
TOTAL MALIGNANT TUMORS	3	12	8
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN RATS FED 4-AMINO-2-NITROPHENOL IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED 4-AMINO-2-NITROPHENOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	19	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
CYST, NOS		1 (2%)	
EPIDERMAL INCLUSION CYST		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
ABSCESS, NOS			1 (2%)
RESPIRATORY SYSTEM			
#TRACHEA	(17)	(42)	(46)
INFLAMMATION, NOS			1 (2%)
#LUNG	(19)	(50)	(48)
MINERALIZATION			1 (2%)
CONGESTION, NOS	4 (21%)	8 (16%)	5 (10%)
INFLAMMATION, FOCAL			1 (2%)
BRONCHOPNEUMONIA, ACUTE			2 (4%)
PNEUMONIA, CHRONIC MURINE	11 (58%)	35 (70%)	35 (73%)
HYPERPLASIA, ADENOMATOUS	1 (5%)	2 (4%)	1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(17)	(45)	(47)
CONGESTION, NOS			1 (2%)
PIGMENTATION, NOS			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(18)	(50)	(50)
THROMBOSIS, 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
#HEART/ATRIUM	(18)	(50)	(50)
THROMBOSIS, NOS	1 (6%)	2 (4%)	1 (2%)
#MYOCARDIUM	(18)	(50)	(50)
FIBROSIS	15 (83%)	32 (64%)	32 (64%)
DEGENERATION, NOS	1 (6%)		2 (4%)
*GASTRODUODENAL ARTER	(20)	(50)	(50)
PERIVASCULITIS	1 (5%)		
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(16)	(49)	(47)
ATROPHY, NOS			1 (2%)
ATROPHY, DIFFUSE	1 (6%)		
#LIVER	(19)	(48)	(48)
CONGESTION, NOS			1 (2%)
GRANULOMA, NOS			1 (2%)
DEGENERATION, NOS	3 (16%)	5 (10%)	5 (10%)
NECROSIS, FOCAL		1 (2%)	
METAMORPHOSIS FATTY	1 (5%)	6 (13%)	7 (15%)
LIPOIDOSIS	1 (5%)		
FOCAL CELLULAR CHANGE			1 (2%)
INCLUSION, CYTOPLASMIC		1 (2%)	
HYPERPLASTIC NODULE			1 (2%)
HYPERPLASIA, FOCAL	11 (58%)	28 (58%)	17 (35%)
#LIVER/CENTRILOBULAR	(19)	(48)	(48)
CONGESTION, NOS	1 (5%)		1 (2%)
DEGENERATION, NOS			1 (2%)
NECROSIS, NOS			1 (2%)
METAMORPHOSIS FATTY		1 (2%)	
*BILE DUCT	(20)	(50)	(50)
HYPERPLASIA, NOS	9 (45%)	26 (52%)	19 (38%)
#PANCREAS	(18)	(46)	(49)
ATROPHY, FOCAL	6 (33%)	9 (20%)	10 (20%)
#PANCREATIC ACINUS	(18)	(46)	(49)
HYPERPLASIA, FOCAL			1 (2%)
#SMALL INTESTINE	(15)	(45)	(45)
PIGMENTATION, NOS		44 (98%)	43 (96%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#LARGE INTESTINE	(15)	(42)	(44)
INFLAMMATION, NOS		1 (2%)	
NEMATODIASIS	3 (20%)	10 (24%)	7 (16%)
URINARY SYSTEM			
#KIDNEY	(19)	(50)	(50)
INFLAMMATION, CHRONIC	17 (89%)	48 (96%)	49 (98%)
#KIDNEY/TUBULE	(19)	(50)	(50)
NECROSIS, NOS	1 (5%)		
PIGMENTATION, NOS			3 (6%)
#URINARY BLADDER	(15)	(46)	(39)
HYPERPLASIA, EPITHELIAL			4 (10%)
ENDOCRINE SYSTEM			
#PITUITARY	(15)	(40)	(39)
CYST, NOS		1 (3%)	3 (8%)
#ADRENAL	(18)	(46)	(49)
HEMORRHAGE	1 (6%)		
LIPOIDOSIS			2 (4%)
#ADRENAL CORTEX	(18)	(46)	(49)
HYPERPLASIA, FOCAL			2 (4%)
#ADRENAL MEDULLA	(18)	(46)	(49)
CYST, NOS		1 (2%)	
#THYROID	(18)	(42)	(44)
HYPERPLASIA, C-CELL		1 (2%)	1 (2%)
HYPERPLASIA, FOLLICULAR-CELL	1 (6%)	2 (5%)	
#THYROID FOLLICLE	(18)	(42)	(44)
HYPERTROPHY, FOCAL		1 (2%)	
#PANCREATIC ISLETS	(18)	(46)	(49)
HYPERTROPHY, NOS		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	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
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(20)	(50)	(50)
INFLAMMATION, ACUTE	1 (5%)		2 (4%)
INFLAMMATION, ACUTE FOCAL			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
GRANULOMA, NOS		1 (2%)	
#TESTIS	(17)	(50)	(50)
NECROSIS, NOS			2 (4%)
NECROSIS, FAT			1 (2%)
ATROPHY, NOS		1 (2%)	2 (4%)
NERVOUS SYSTEM			
#BRAIN	(18)	(46)	(48)
ABSCESS, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(50)	(50)
HEMORRHAGE			1 (2%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
NECROSIS, FAT		1 (2%)	
*PLEURA	(20)	(50)	(50)
FOAM-CELL		2 (4%)	
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			
AUTO/NECROPSY/NO HISTO	1		

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED 4-AMINO-2-NITROPHENOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	@50	50
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	48
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(49)	(50)
EPIDERMAL INCLUSION CYST	1 (5%)		
INFLAMMATION, NOS	1 (5%)		
NECROSIS, NOS	1 (5%)		
*SUBCUT TISSUE	(20)	(49)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
DERMAL INCLUSION CYST		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(18)	(48)	(46)
CONGESTION, NOS		2 (4%)	
BRONCHOPNEUMONIA, NOS			1 (2%)
PNEUMONIA, CHRONIC MURINE	12 (67%)	37 (77%)	34 (74%)
FOAM-CELL		2 (4%)	
HYPERPLASIA, ADENOMATOUS		4 (8%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(48)	(47)
HEMORRHAGIC CYST			1 (2%)
HEMOSIDEROSIS		1 (2%)	
#MESENTERIC L. NODE	(19)	(49)	(47)
CYTOLOGIC ALTERATION, NOS	1 (5%)		
CIRCULATORY SYSTEM			
#HEART/ATRIUM	(20)	(49)	(48)
THROMBOSIS, NOS			1 (2%)

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

@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A MALE IN A FEMALE GROUP.

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#MYOCARDIUM	(20)	(49)	(48)
INFLAMMATION, ACUTE FOCAL			1 (2%)
FIBROSIS	12 (60%)	28 (57%)	18 (38%)
*PULMONARY ARTERY	(20)	(49)	(50)
MINERALIZATION		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(18)	(48)	(45)
ATROPHY, NOS			2 (4%)
ATROPHY, DIFFUSE	1 (6%)		
#LIVER	(20)	(48)	(48)
ABSCESS, NOS		1 (2%)	
GRANULOMA, NOS			1 (2%)
DEGENERATION, NOS	3 (15%)	3 (6%)	4 (8%)
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY	3 (15%)	3 (6%)	3 (6%)
HEPATOCYTOMEGALY			1 (2%)
GLYCOGENIC CELL			1 (2%)
HYPERPLASIA, NODULAR		2 (4%)	1 (2%)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL	12 (60%)	31 (65%)	29 (60%)
ANGIECTASIS			1 (2%)
#LIVER/KUPFFER CELL	(20)	(48)	(48)
CYTOPLASMIC VACUOLIZATION	1 (5%)		
*BILE DUCT	(20)	(49)	(50)
HYPERPLASIA, NOS	7 (35%)	1 (2%)	3 (6%)
#PANCREAS	(18)	(48)	(46)
ATROPHY, NOS			1 (2%)
ATROPHY, FOCAL		6 (13%)	4 (9%)
ATROPHY, DIFFUSE			1 (2%)
HYPERPLASTIC NODULE		1 (2%)	
#SMALL INTESTINE	(17)	(44)	(47)
PIGMENTATION, NOS		43 (98%)	43 (91%)
#LARGE INTESTINE	(18)	(46)	(47)
NEMATODIASIS	6 (33%)	6 (13%)	8 (17%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(48)
INFLAMMATION, NOS		1 (2%)	
ABSCESS, NOS		1 (2%)	
INFLAMMATION, CHRONIC	18 (90%)	45 (92%)	46 (96%)
DEGENERATION, HYALINE			1 (2%)
#KIDNEY/TUBULE	(20)	(49)	(48)
NEPHROSIS, NOS			1 (2%)
NECROSIS, NOS	1 (5%)	1 (2%)	
PIGMENTATION, NOS			2 (4%)
#URINARY BLADDER	(15)	(43)	(44)
METAMORPHOSIS FATTY			1 (2%)
LIPOIDOSIS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(48)	(45)
CYST, NOS	2 (11%)	4 (8%)	9 (20%)
HEMORRHAGIC CYST		2 (4%)	
#ADRENAL	(19)	(48)	(48)
HEMORRHAGE		1 (2%)	
LIPOIDOSIS	1 (5%)	1 (2%)	1 (2%)
#ADRENAL CORTEX	(19)	(48)	(48)
LIPOIDOSIS		1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)
#THYROID	(17)	(44)	(47)
HYPERPLASIA, FOCAL			1 (2%)
HYPERPLASIA, C-CELL	1 (6%)	3 (7%)	1 (2%)
HYPERPLASIA, FOLLICULAR-CELL			2 (4%)
#PANCREATIC ISLETS	(18)	(48)	(46)
ATROPHY, FOCAL	1 (6%)		
HYPERPLASIA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(50)
DILATATION/DUCTS		4 (8%)	2 (4%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#UTERUS	(17)	(47)	(48)
THROMBUS, ORGANIZED		1 (2%)	
BLOOD CLOT, POSTMORTEM		1 (2%)	
PYOMETRA			1 (2%)
NECROSIS, NOS			1 (2%)
#CERVIX UTERI	(17)	(47)	(48)
INFLAMMATION, NOS		1 (2%)	
#UTERUS/ENDOMETRIUM	(17)	(47)	(48)
INFLAMMATION, NOS		1 (2%)	1 (2%)
INFLAMMATION, ACUTE			2 (4%)
HYPERPLASIA, NOS	1 (6%)	2 (4%)	2 (4%)
HYPERPLASIA, CYSTIC	1 (6%)	2 (4%)	1 (2%)
#OVARY	(17)	(47)	(48)
FOLLICULAR CYST, NOS		1 (2%)	
INFLAMMATION, ACUTE			1 (2%)
DEGENERATION, NOS			1 (2%)
NECROSIS, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(19)	(49)	(47)
INFLAMMATION, ACUTE FOCAL			1 (2%)
#BRAIN	(19)	(49)	(47)
DEMYELINIZATION	1 (5%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(20)	(49)	(50)
FOAM-CELL			2 (4%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*MESENTERY	(20)	(49)	(50)
ARTERIOSCLEROSIS, NOS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY/NO HISTO			2

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE FED 4-AMINO-2-NITROPHENOL IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED 4–AMINO–2–NITROPHENOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(48)
CONGESTION, CHRONIC PASSIVE		1 (2%)	
INFLAMMATION, INTERSTITIAL			1 (2%)
PNEUMONIA, ASPIRATION		1 (2%)	1 (2%)
PNEUMONIA, CHRONIC MURINE	2 (10%)	11 (22%)	2 (4%)
HYPERPLASIA, ADENOMATOUS		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(48)	(47)
DEGENERATION, HYALINE	1 (5%)		
NECROSIS, CASEOUS	1 (5%)		
ANGIECTASIS	1 (5%)		
HYPERPLASIA, LYMPHOID		1 (2%)	
HEMATOPOIESIS			1 (2%)
#MESENTERIC L. NODE	(19)	(50)	(48)
INFLAMMATION, HEMORRHAGIC		1 (2%)	
INFLAMMATION, GRANULOMATOUS	1 (5%)		
CIRCULATORY SYSTEM			
*PULMONARY ARTERY	(20)	(50)	(50)
FIBROSIS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(49)
NECROSIS, FOCAL	1 (5%)		3 (6%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, CASEOUS	1 (5%)		
INFARCT, NOS	1 (5%)		3 (6%)
METAMORPHOSIS FATTY	1 (5%)	4 (8%)	2 (4%)
NUCLEAR ENLARGEMENT		1 (2%)	
CYTOPLASMIC VACUOLIZATION	1 (5%)		
HEPATOCYTOMEGALY			1 (2%)
HEMATOPOIESIS			2 (4%)
#LIVER/PERIPORTAL	(20)	(50)	(49)
MONOCYTOSIS			1 (2%)
#SMALL INTESTINE	(20)	(49)	(47)
PIGMENTATION, NOS		46 (94%)	43 (91%)
#PEYERS PATCH	(20)	(49)	(47)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
#COLON	(20)	(50)	(48)
NEMATODIASIS	2 (10%)	9 (18%)	10 (21%)
PARASITISM	1 (5%)		
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(49)
INFLAMMATION, CHRONIC	1 (5%)	2 (4%)	3 (6%)
INFARCT, NOS			1 (2%)
#URINARY BLADDER	(18)	(46)	(48)
INFLAMMATION, CHRONIC		1 (2%)	
ENDOCRINE SYSTEM			
#PANCREATIC ISLETS	(19)	(49)	(47)
HYPERTROPHY, NOS		1 (2%)	
HYPERPLASIA, NOS	3 (16%)	8 (16%)	
REPRODUCTIVE SYSTEM			
#TESTIS	(18)	(50)	(49)
CALCIFICATION, NOS	1 (6%)		
NERVOUS SYSTEM			
#BRAIN	(19)	(50)	(50)
MINERALIZATION	1 (5%)	1 (2%)	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
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
NECROSIS, FAT		1 (2%)	2 (4%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3		2

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED 4-AMINO-2-NITROPHENOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
HEMORRHAGE	1 (5%)		
INFLAMMATION, INTERSTITIAL		2 (4%)	
PNEUMONIA, CHRONIC MURINE	5 (25%)	15 (31%)	10 (20%)
PERIVASCULAR CUFFING		2 (4%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(50)	(50)
INFARCT, NOS		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	
#LYMPH NODE	(20)	(49)	(48)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
#MANDIBULAR L. NODE	(20)	(49)	(48)
HYPERPLASIA, LYMPHOID		1 (2%)	
#MESENTERIC L. NODE	(20)	(49)	(48)
INFLAMMATION, GRANULOMATOUS	1 (5%)		
CIRCULATORY SYSTEM			
#CARDIAC VALVE	(19)	(48)	(50)
INFLAMMATION, NOS			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
*PULMONARY ARTERY	(20)	(50)	(50)
HYPERPLASIA, LYMPHOID		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(19)	(49)	(46)
PERIVASCULAR CUFFING		1 (2%)	
#LIVER	(20)	(50)	(50)
INFLAMMATION, ACUTE FOCAL	1 (5%)		
PERIVASCULAR CUFFING		1 (2%)	1 (2%)
NECROSIS, FOCAL		1 (2%)	
METAMORPHOSIS FATTY	1 (5%)		
#PANCREAS	(20)	(49)	(49)
CYST, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE	1 (5%)		
#SMALL INTESTINE	(19)	(48)	(47)
PIGMENTATION, NOS		43 (90%)	42 (89%)
#PEYERS PATCH	(19)	(48)	(47)
HYPERPLASIA, LYMPHOID			1 (2%)
#COLON	(19)	(48)	(49)
NEMATODIASIS	1 (5%)	2 (4%)	
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
PERIVASCULAR CUFFING		3 (6%)	3 (6%)
NEPHROSIS, HEMOGLOBINURIC			1 (2%)
METAPLASIA, OSSEOUS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(38)	(44)
ANGIECTASIS			1 (2%)
#ADRENAL	(19)	(47)	(46)
CYST, NOS		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
LIPOIDOSIS		1 (2%)	
#PANCREATIC ISLETS	(20)	(49)	(49)
HYPERTROPHY, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS	(19)	(48)	(49)
HYDROMETRA	4 (21%)	8 (17%)	13 (27%)
CYST, NOS			1 (2%)
THROMBUS, ORGANIZED			1 (2%)
INFLAMMATION, NOS			1 (2%)
PYOMETRA			1 (2%)
#UTERUS/ENDOMETRIUM	(19)	(48)	(49)
HYPERPLASIA, NOS		2 (4%)	
HYPERPLASIA, CYSTIC	1 (5%)	5 (10%)	1 (2%)
#OVARY	(18)	(41)	(45)
CYST, NOS	2 (11%)	2 (5%)	2 (4%)
FOLLICULAR CYST, NOS	1 (6%)	1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(19)	(49)	(49)
MINERALIZATION		3 (6%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
NECROSIS, FAT			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
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
POSTMORTEM CHANGE	1 (5%)		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	2	1

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED 4-AMINO-2-NITROPHENOL IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 4-Amino-2-Nitrophenol in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Malignant Lymphoma, Lymphocytic Leukemia, Undifferentiated Leukemia, or Leukemia, NOS[b]	3/20 (15)	11/50 (22)	10/50 (20)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.467	1.333
Lower Limit		0.450	0.398
Upper Limit		7.594	7.002
Weeks to First Observed Tumor	105	86	83
Hematopoietic System: All Lymphomas or Leukemias[b]	3/20 (15)	13/50 (26)	11/50 (22)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.733	1.467
Lower Limit		0.556	0.450
Upper Limit		8.773	7.594
Weeks to First Observed Tumor	105	86	73

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 4-Amino-2-Nitrophenol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Urinary Bladder: Transitional-cell Carcinoma[b]	0/15 (0)	0/46 (0)	11/39 (28)
P Values[c,d]	P < 0.001	N.S.	P = 0.018
Departure from Linear Trend[e]	P = 0.030		
Relative Risk[f]		--	Infinite
Lower Limit		--	1.367
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	90
Urinary Bladder: Transitional-cell Papilloma[b]	0/15 (0)	0/46 (0)	2/39 (5)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		--	Infinite
Lower Limit		--	0.120
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	90

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 4-Amino-2-Nitrophenol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	2/15 (13)	4/40 (10)	8/39 (21)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.750	1.538
Lower Limit		0.125	0.366
Upper Limit		7.797	13.883
Weeks to First Observed Tumor	96	105	70
Thyroid: C-cell Adenoma or Carcinoma[b]	3/18 (17)	2/42 (5)	1/44 (2)
P Values[c,d]	P = 0.043(N)	N.S.	N.S.
Relative Risk[f]		0.286	0.136
Lower Limit		0.026	0.003
Upper Limit		2.323	1.592
Weeks to First Observed Tumor	99	105	105

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 4-Amino-2-Nitrophenol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pancreatic Lslets: Islet-cell Adenoma[b]	2/18 (11)	1/46 (2)	1/49 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.196	0.184
Lower Limit		0.004	0.003
Upper Limit		3.586	3.372
Weeks to First Observed Tumor	105	105	105
Testis: Interstitial-cell Tumor[b]	15/17 (88)	50/50 (100)	41/50 (82)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.011		
Relative Risk[f]		1.133	0.929
Lower Limit		0.996	0.798
Upper Limit		Infinite	1.283
Weeks to First Observed Tumor	90	86	78

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 4-Amino-2-Nitrophenol in the Diet[a]

(continued)

[a]Dosed groups received 1,250 or 2,500 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 $P < 0.05$; 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 $P < 0.05$; 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 probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 4-Amino-2-Nitrophenol in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Malignant Lymphoma, Lymphocytic Leukemia, Undifferentiated Leukemia, or Leukemia, NOS[b]	2/20 (10)	6/49 (12)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.224	0.400
Lower Limit		0.248	0.032
Upper Limit		11.802	5.277
Weeks to First Observed Tumor	100	86	63
Hematopoietic System: All Lymphomas or Leukemias[b]	2/20 (10)	6/49 (12)	4/50 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.224	0.800
Lower Limit		0.248	0.128
Upper Limit		11.802	8.436
Weeks to First Observed Tumor	100	86	63

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 4-Amino-2-Nitrophenol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Urinary Bladder: Transitional-cell Carcinoma[b]	0/15 (0)	1/43 (2)	2/44 (5)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.020	0.107
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	79	61
Pituitary: Chromophobe Adenoma[b]	8/18 (44)	26/48 (54)	20/45 (44)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.219	1.000
Lower Limit		0.695	0.546
Upper Limit		2.571	2.192
Weeks to First Observed Tumor	72	76	92

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 4-Amino-2-Nitrophenol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma[b]	1/17 (6)	2/44 (5)	0/47 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.773	0.000
Lower Limit		0.044	0.000
Upper Limit		44.565	6.754
Weeks to First Observed Tumor	105	105	--
Mammary Gland: Fibroadenoma or Adenoma, NOS[b]	1/20 (5)	4/49 (8)	6/50 (12)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.633	2.400
Lower Limit		0.179	0.325
Upper Limit		78.704	108.021
Weeks to First Observed Tumor	105	105	92

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 4-Amino-2-Nitrophenol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	1/20 (5)	3/49 (6)	5/50 (10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.224	2.000
Lower Limit		0.108	0.249
Upper Limit		62.958	92.596
Weeks to First Observed Tumor	105	105	92

[a]Dosed groups received 1,250 or 2,500 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 $P < 0.05$; 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 $P < 0.05$; 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 probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN

MICE ADMINISTERED 4-AMINO-2-NITROPHENOL IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 4-Amino-2-Nitrophenol in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	2/20 (10)	10/49 (20)	7/48 (15)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		2.041	1.458
Lower Limit		0.498	0.316
Upper Limit		18.154	13.664
Weeks to First Observed Tumor	105	70	105
Lung: Alveolar/Bronchiolar Carcinoma[b]	3/20 (15)	0/49 (0)	0/48 (0)
P Values[c,d]	P = 0.005(N)	P = 0.022(N)	P = 0.023(N)
Departure from Linear Trend[e]	P = 0.015		
Relative Risk[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		0.673	0.686
Weeks to First Observed Tumor	105	--	--

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 4-Amino-2-Nitrophenol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	5/20 (25)	10/49 (20)	7/48 (15)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.816	0.583
Lower Limit		0.302	0.187
Upper Limit		2.740	2.109
Weeks to First Observed Tumor	105	70	105
Hematopoietic System: Lymphoma[b]	1/20 (5)	5/50 (10)	4/50 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		2.000	1.600
Lower Limit		0.249	0.175
Upper Limit		92.596	77.169
Weeks to First Observed Tumor	105	83	93

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 4-Amino-2-Nitrophenol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma or Leukemia[b]	2/20 (10)	5/50 (10)	4/50 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.000	0.800
Lower Limit		0.184	0.128
Upper Limit		10.007	8.436
Weeks to First Observed Tumor	69	83	93
Liver: Hepatocellular Carcinoma[b]	3/20 (15)	7/50 (14)	7/49 (14)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.933	0.952
Lower Limit		0.245	0.250
Upper Limit		5.215	5.317
Weeks to First Observed Tumor	95	87	93

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 4-Amino-2-Nitrophenol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	6/20 (30)	18/50 (36)	19/49 (39)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.200	1.293
Lower Limit		0.557	0.607
Upper Limit		3.238	3.452
Weeks to First Observed Tumor	95	78	93

[a]Dosed groups received 1,250 or 2,500 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 $P < 0.05$; 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 $P < 0.05$; 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 probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 4-Amino-2-Nitrophenol in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	2/20 (10)	3/49 (6)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.612	0.400
Lower Limit		0.078	0.032
Upper Limit		6.996	5.277
Weeks to First Observed Tumor	105	105	105
Hematopoietic System: Malignant Lymphoma or Lymphocytic Leukemia[b]	3/20 (15)	10/50 (20)	8/50 (16)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.333	1.067
Lower Limit		0.398	0.295
Upper Limit		7.002	5.813
Weeks to First Observed Tumor	85	76	64

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 4-Amino-2-Nitrophenol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Adenoma or Papillary Adenoma[b]	2/17 (12)	1/40 (3)	0/42 (0)
P Values[c,d]	P = 0.035(N)	N.S.	N.S.
Relative Risk[f]		0.213	0.000
Lower Limit		0.004	0.000
Upper Limit		3.873	1.353
Weeks to First Observed Tumor	105	105	--

[a]Dosed groups received 1,250 or 2,500 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 $P < 0.05$; 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 $P < 0.05$; 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 probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the control group.

Review of the Bioassay of 4-Amino-2-Nitrophenol*
for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup of the
Clearinghouse on Environmental Carcinogens

April 26, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1978, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/ Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 4-Amino-2-Nitrophenol for carcinogenicity.

The primary reviewer said that the compound induced bladder cancer in treated male rats and that the evidence was suggestive for a similar effect in females. No carcinogenic effect was observed among treated mice. After a brief description of the experimental design, he opined that the incidence of bladder cancer was not dose related, as indicated in the report. He noted that the compound was the only phenol he was aware of which was (systemically) carcinogenic. Based on the relatively low incidence of bladder cancer and long latent period, the primary reviewer concluded that the compound did not pose a carcinogenic risk to humans.

The secondary reviewer agreed with the conclusion that the compound was carcinogenic in rats. He said that a conclusion on the carcinogenicity of 4-Amino-2-Nitrophenol in mice could not be made since it appeared that a maximum tolerated dose was not achieved. Had higher doses been

administered, the elevated incidence of liver tumors in treated male mice may have increased to a statistically significant number. The secondary reviewer concluded that 4-Amino-2-Nitrophenol poses a carcinogenic risk to humans.

A Subgroup member disagreed with the primary reviewer's conclusion with respect to human risk. Unlike the results of this study, the primary reviewer argued that human carcinogens induce a high yield of cancer in a relatively short time in experimental animals.

In response to a question, a Program staff pathologist said that the presence of bladder calculi in the rats were not noted by the testing laboratories' pathologists. In his experience, he added, bladder parasites have not been found in rats used in bioassay studies. It was noted that only one of the bladder tumors metastasized. The Program staff pathologist continued that the finding was not unusual since bladder tumors normally do not metastasize even when induced by strong carcinogens. A Subgroup member suggested that the conclusion on the carcinogenicity of 4-Amino-2-Nitrophenol should be qualified, since the urine was not analyzed for crystals.

A motion was approved unanimously that the report on the bioassay of 4-Amino-2-Nitrophenol be accepted as written.

Members present were:

Michael Shimkin (Acting Chairman), University of California at San Diego
Joseph Highland, Environmental Defense Fund
George Roush, Jr., Monsanto Company
Louise Strong, University of Texas Health Sciences Center
John Weisburger, American Health Foundation
(Sidney Wolfe, Health Research Group, submitted a written review)

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

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