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

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REPORT ON THE BIOASSAY OF CUPFERRON
FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of cupferron 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 significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because 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 a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of cupferron was conducted by Mason Research Institute, Worcester, Massachusetts, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (1,2) and Dr. E. K. Weisburger (1). The principal investigators for the contract were Dr. E. Smith (3) and Dr. A. Handler (3). Animal treatment and observation were supervised by Mr. G. Wade (3) and Ms. E. Zepp (3). Chemical analysis was performed by Midwest Research Institute (4) and the analytical results were reviewed by Dr. N. Zimmerman (5).

Histopathologic examinations were performed by Dr. R. W. Fleischman (3), Dr. A. S. Krishna Murthy (3), Dr. A. Russfield (3), and Dr. D. S. Wyand (3) at the Mason Research Institute, the pathology narratives were written by Dr. D. S. Wyand (3), and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (6).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (7); the statistical analysis was performed by Mr. W. W. Belew (5,8) using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (9).

This report was prepared at METREK, a Division of The MITRE Corporation (5) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (5), task leader Dr. M. R. Kornreich (5,10), senior biologist Ms. P. Walker (5), biochemist Dr. B. Fuller (5), and technical editor Ms. P. A. Miller (5). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1,10), Dr. R. A. Griesemer (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,11), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A bioassay of cupferron for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F1 mice. Cupferron was administered in the feed, at either of two concentrations, to groups of 49 or 50 male and 50 female animals of each species. The time-weighted average high and low dietary concentrations of cupferron were, respectively, 0.30 and 0.15 percent for male and female rats, and 0.4 and 0.2 percent for male and female mice. After a 78-week period of compound administration, observation of the rats continued for an additional period of up to 28 weeks and observation of the mice continued for an additional period of up to 18 weeks.

For each species, 50 animals of each sex were placed on test as controls and fed only the basal diet.

Among both sexes of rats and mice there was a significant positive association between the dose of cupferron administered and mortality; however, in all groups of animals sufficient numbers survived long enough to establish the carcinogenicity of this compound.

There were significant positive associations between the concentrations of cupferron administered to male and female rats and the incidences of: squamous-cell carcinomas of the forestomach, hepatocellular carcinomas and neoplastic nodules, and hemangiosarcomas. When a binomial distribution and a spontaneous incidence rate corresponding to the appropriate historical control incidence were assumed, the incidences of auditory sebaceous gland neoplasms in female rats and female mice were significant. There were significant positive associations between the concentrations administered and: the incidences of hepatocellular carcinomas in female mice, the incidences of hemangiosarcomas in both sexes of mice, and the incidence of Harderian gland adenomas in both sexes of mice.

Under the conditions of this bioassay cupferron was carcinogenic in Fischer 344 rats, causing hemangiosarcomas, hepatocellular carcinomas, and squamous-cell carcinomas of the forestomach in males and females as well as carcinomas of the auditory sebaceous gland in females. The chemical was also carcinogenic in B6C3F1 mice, causing hemangiosarcomas in males and hepatocellular carcinomas, carcinomas of the auditory sebaceous gland, a combination of hemangiosarcomas and hemangiomas, and adenomas of the Harderian gland in females.

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I. INTRODUCTION

Cupferron (Figure 1) (NCI No. C03258), an N-nitroso hydroxylamine derivative used primarily as a reagent in analytical chemistry, was selected for bioassay by the National Cancer Institute because of the suspected carcinogenicity of nitrosamines.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is N-hydroxy-N-nitroso-benzenamine, ammonium salt.* It is also known as N-nitroso-N-phenyl-hydroxylamine, ammonium salt; and as ammonium nitrosophenylhydroxylamine.

As an analytical reagent, cupferron is used to separate copper and iron from other metals and to separate tin from zinc (Windholz, 1976; Schlecten and Thompson, 1970). It also finds application as a quantitative reagent for vanadates and titanium and for the colorimetric estimation of aluminum (Windholz, 1976).

The chelating properties of cupferron as well as its ability to inhibit respiration (and therefore oxygen consumption) in certain plants have been employed in numerous experimental studies dealing with elucidation of the mechanism of radiation-induced chromosomal breakage in plants and subsequent repair (Kihlman, 1958; Kihlman, 1959; Davis, 1969; Gilot-Delhalle et al., 1973). In addition, cupferron has been found to increase the fungicidal activity of zinc dimethyldithiocarbamate (Ziram), a widely used agricultural fungicide (Matolcsy et al., 1971).

*The CAS registry number is 135-20-6.

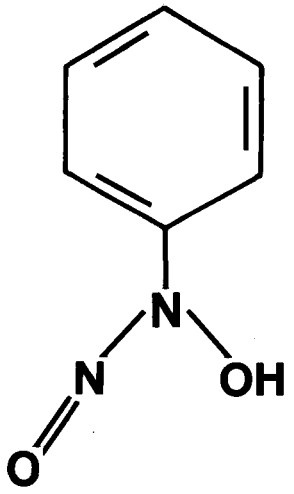


FIGURE 1
CHEMICAL STRUCTURE OF CUPFERRON

Production statistics for cupferron are not available. However, the compound is produced in commercial quantities (greater than 1000 pounds or $1000 in value annually) by three U.S. companies (Stanford Research Institute, 1977).

The potential for exposure to cupferron appears to be greatest for those engaged in analytical or research studies involving its use. Workers at manufacturing plants may also experience significant contact with the chemical.

II. MATERIALS AND METHODS

A. Chemicals

Cupferron (N-nitroso-N-phenyl-hydroxylamine) was purchased by the NCI for Mason Research Institute from Eastman Kodak Company, Rochester, New York and chemical analysis was performed by Midwest Research Institute, Kansas City, Missouri. The experimentally determined melting point of 154° to 156°C, although narrow in its range, suggested the presence of minor impurities due to its difference from the literature value of 163° to 164°C (Bamberger and Baudisch, 1909). The results of elemental analysis, although close to theoretical, confirmed this suggestion. Thin-layer chromatography was performed utilizing two solvent systems (methanol:chloroform and butanol:benzene), visualized with ultraviolet light and ferricyanide-ferrichloride. The first procedure indicated the presence of two impurities, while the second revealed no indication of impurities. The higher polarity of the first system may have contributed to this difference. High-pressure liquid chromatography indicated the presence of one less motile impurity. The result of titration of the NH_4^+ group with ceric sulfate was approximately 98 percent of the theoretical. Infrared analysis was consistent with the structure of the compound. Ultraviolet analysis revealed λ_{max} at 219 and 282 with molar extinction coefficients (ϵ) of 107.5×10^2 and 100.4×10^2, while the published literature values are λ_{max} at 221 and 293 and respective ϵ values of 91.45×10^2 and 107.5×10^2. If the shifting value of the upper λ_{max} were ignored,

the purity approximation would be 93 percent; however, as a result of the upper λ_{max} difference, the ϵ cannot be utilized to suggest purity.

Throughout this report the term cupferron is utilized to represent this chemical.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® (Allied Mills, Inc., Chicago, Illinois). Cupferron was administered to the dosed animals as a component of the diet. Proper amounts of the chemical were weighed out of a working stock bottle of presifted chemical under a fume hood. Ammonium carbonate in a gauze bag was suspended in the stock container to retard decomposition. The compound was hand-blended in an aluminum bowl with an aliquot of the ground feed. Once visual uniformity was attained, the mixture was placed into a 6 kg capacity Patterson-Kelley twin-shell stainless steel V-blender along with the remainder of the meal and blended for 20 minutes. Prepared diets were placed in double plastic bags and stored in the dark at 4°C. The mixture was used for 1 week only.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. Fischer 344 rats and B6C3F1 mice were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Animals were supplied by Charles River Breeding Laboratories,

Inc., Wilmington, Massachusetts. Dosed and control animals for both species were received in separate shipments.

Upon arrival, a sample of animals from each shipment was examined for parasites and other signs of disease. The remaining animals were quarantined by species for 2 weeks prior to initiation of test. Animals were assigned to groups and distributed among cages so that average body weight per cage was approximately equal for a given sex and species.

D. Animal Maintenance

All animals were housed by species in rooms having a temperature range of 23° to 34°C. Incoming air was filtered through Tri-Dek® 15/40 denier Dacron® filters (Tri-Dim Filter Corp., Hawthorne, New Jersey) providing six changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle.

Rats were housed five per cage by sex. During quarantine and for the first 14 months of study, they were kept in galvanized-steel wire-mesh cages suspended above newspapers. Newspapers were replaced daily and cages and racks washed weekly. For the remainder of the study, rats were kept in suspended polycarbonate cages equipped with disposable nonwoven fiber filter sheets. Clean bedding and cages were provided twice weekly. SAN-I-CEL® (Paxton Processing Company, Paxton, Illinois) corncob bedding was used for the first 6 months that rats were housed in polycarbonate cages. Aspen hardwood chip

bedding (American Excelsior Company, Baltimore, Maryland) was used for the remainder of the study. Stainless steel cage racks were cleaned once every 2 weeks, and disposable filters were replaced at that time.

Mice were housed by sex in polycarbonate shoe box type cages. During quarantine and periods of chemical administration, cages were fitted with perforated stainless steel lids. During the observation period following chemical administration, stainless steel wire bar lids were used. Both types of lids were from Lab Products, Inc., Garfield, New Jersey. Nonwoven fiber filter bonnets were used over cage lids. Dosed and control mice were housed 10 per cage for the first 12 or 11 months of study, respectively, and five per cage thereafter. Cages, lids, filters, and bedding were provided three times per week when cage populations were ten and twice per week when cage populations were reduced to five. For the first year of the study, corncob bedding (SAN-I-CEL®) was used. A second corncob bedding (Bed-o-Cobs®, The Andersons Cob Division, Maumee, Ohio) was used for the next 8 months, followed by Aspen bedding for the remainder of the study. Reusable filter bonnets and pipe racks were sanitized every 2 weeks throughout the study.

Water was available from 250 ml polycarbonate water bottles equipped with rubber stoppers and stainless steel sipper tubes. Bottles were replaced twice weekly and, for rats only, water was

supplied as needed between changes. Food and water were available ad libitum.

Pelleted Wayne Lab-Blox® was supplied during the quarantine and final observation periods. During chemical administration, all dosed animals were supplied with Wayne Lab-Blox® meal containing the appropriate concentration of cupferron. Control animals had untreated meal available. For the first 13 months of study, feed was dispensed to all rats and to dosed mice in Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc., Woburn, Massachusetts) equipped with stainless steel baffles. This same feed distribution system was utilized for control mice for the first 14 months of study. Thereafter, feed was dispensed in stainless steel gangstyle feed hoppers (Scientific Cages, Inc., Bryan, Texas). Pellets were fed to rats on the cage floor during quarantine and final observation periods. Mice were fed pellets from feeders incorporated into the cage lids during the final observation period. Feed hoppers were changed twice weekly. Food was replenished daily in Alpine® feed cups.

Low dose rats were housed in rooms with other rats receiving diets containing* p-cresidine (120-71-8); 2,3,5,6-tetrachloro-4-nitroanisole (2438-88-2); 4-chloro-o-phenylenediamine (95-83-0); 4-chloro-m-phenylenediamine (5131-60-2); and 1H-benzotriazole (95-14-7). High dose and control rats were housed in a room with rats intubated with m-cresidine (102-50-1); and with other rats receiving

*CAS registry numbers are given in parentheses.

diets containing fenaminosulf (140-56-7) and 2,5-dithiobiurea (142-46-1).

Dosed and control mice were in a room in which other mice were receiving diets containing 2,5-dithiobiurea (142-46-1); 4-chloro-o-phenylenediamine (95-83-0); o-anisidine hydrochloride (134-29-0); p-anisidine hydrochloride (20265-97-8); and fenaminosulf (140-56-7).

E. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of cupferron for administration to dosed animals in the chronic studies, subchronic toxicity studies were conducted with both rats and mice. Animals of each species were distributed among six groups, each consisting of five males and five females. Cupferron was incorporated into the basal laboratory diet and supplied ad libitum to five of the six rat groups in concentrations of 0.02, 0.04, 0.08, 0.15, and 0.30 percent and five of the six mouse groups in concentrations of 0.04, 0.08, 0.15, 0.30, and 0.60 percent. The sixth group of each species served as controls, receiving only the basal laboratory diet. The dosed dietary preparations were administered for 8 weeks. Survivors were sacrificed at the end of the test, and gross necropsies were performed.

The highest concentration causing no deaths, no compound-related gross abnormalities, and no mean group body weight depression in excess of 20 percent relative to controls was selected as the high dose utilized for the chronic bioassay.

There were no deaths among male rats during the 8-week subchronic study; however, one female in the group receiving 0.04 percent and one in the group receiving 0.30 percent died. At the highest concentration mean body weight depression was approximately 38 percent among male rats and approximately 20 percent among female rats. At gross necropsy it was observed that rats of both sexes had black spleens with dose-related, moderate to advanced enlargement of the organ. The high concentration chosen for the chronic bioassay was 0.30 percent for male and female rats.

There were no deaths among male or female mice during the 8-week subchronic study. At a concentration of 0.60 percent mean body weight depression was approximately 23 percent among male and female mice. At gross necropsy it was observed that mice receiving cupferron had black spleens and those mice which received dosages of more than 0.15 percent had spleens that were also moderately enlarged. The high concentration chosen for the chronic bioassay was 0.60 percent for male and female mice.

F. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, concentrations administered, duration of treated and untreated observation periods, and time-weighted average concentrations) are summarized in Tables 1 and 2.

Rats were all approximately 6 weeks old at the time they were placed on test; however, control rats were approximately 10 weeks

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
CUPFERRON FEEDING EXPERIMENT

	INITIAL GROUP SIZE	CUPFERRON CONCENTRATION (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	50	0	0	110
LOW DOSE	50	0.15 0	78	26
HIGH DOSE	49	0.30 0	78	19
FEMALE				
CONTROL	50	0	0	110
LOW DOSE	50	0.15 0	78	28
HIGH DOSE	50	0.30 0	78	28

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
CUPFERRON FEEDING EXPERIMENT

	INITIAL GROUP SIZE	CUPFERRON CONCENTRATION (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE CONCENTRATION[a]
MALE					
CONTROL	50	0	0	98	0
LOW DOSE	50	0.3 0.1 0	35 43	 18	0.2
HIGH DOSE	50	0.6 0.2 0	35 43	 17	0.4
FEMALE					
CONTROL	50	0	0	98	0
LOW DOSE	50	0.3 0.1 0	35 43	 18	0.2
HIGH DOSE	50	0.6 0.2 0	35 43	 17	0.4

[a] Time-weighted average concentration = $\frac{\Sigma(\text{concentration X weeks received})}{\Sigma(\text{weeks receiving chemical})}$

older than the dosed rats. The dietary concentrations of cupferron utilized were 0.30 and 0.15 percent. Throughout this report those rats receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups. Dosed rats were supplied with feed containing cupferron for a total of 78 weeks, followed by an observation period of up to 28 weeks.

All mice were approximately 6 weeks old at the time they were placed on test; however, control mice were approximately 5 weeks older than the dosed mice. The dietary concentrations of cupferron initiallly utilized were 0.60 and 0.30 percent for the first 35 weeks of compound administration. Throughout this report, those mice initially receiving the former concentration are referred to as the high dose groups and those initially receiving the latter concentration are referred to as the low dose groups. In week 36 the concentrations for high and low dose mice were lowered to 0.2 and 0.1 percent, respectively, for the remainder of the period of compound administration, due to high mortality and excess mean body weight depression. Dosed mice were supplied with feed containing cupferron for a total of 78 weeks, followed by an observation period of up to 18 weeks.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. Body weights were recorded twice weekly for the first

12 weeks of the study and at monthly intervals thereafter. From the first day, all animals were inspected twice daily for mortality. Food consumption, for two cages from each group, was monitored for seven consecutive days once a month for the first nine months of the bioassay and for three consecutive days each month thereafter. The presence of tissue masses and lesions was determined by monthly observation and palpation of each animal.

A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized by carbon dioxide inhalation, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in 10 percent buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination. An occasional section was subjected to special staining techniques for more definitive diagnosis.

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, nasal cavity, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, mammary gland, uterus, ovary and brain.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were placed on experiment 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) 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

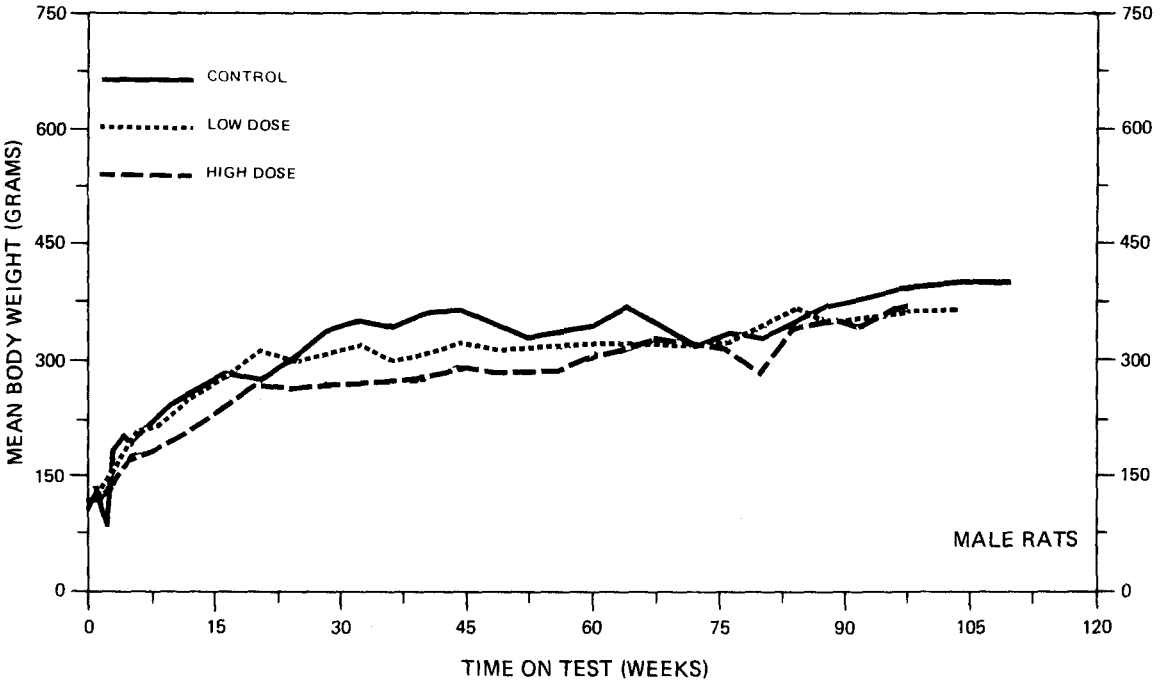
There were no generally consistent relationships in mean body weight patterns among male rats; however, there was slight mean body weight depression among dosed female rats when compared to controls (Figure 2). Fluctuations in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variations.

The only clinical abnormality observed among dosed or control rats was a hard, crusted subcutaneous lesion on the anterior surface of a control male that subsequently died.

B. Survival

The estimated probabilities of survival for male and female rats in the control and cupferron-dosed groups are shown in Figure 3. For male and female rats the Tarone test indicated a significant ($P < 0.001$) positive association between dosage and mortality. The departure from linear trend for males was also significant ($P = 0.016$).

For males five rats were sacrificed from the control group in week 78. In the high dose group the median survival on test was 63 weeks with the last rat dead by week 98. For the low dose group the median survival on test was 84 weeks with the last rat dead by week 105. In the control group 64 percent (32/50) survived on test until the termination of the study. The early mortality may have been associated with tumor incidence since 78 percent (38/49) of the low



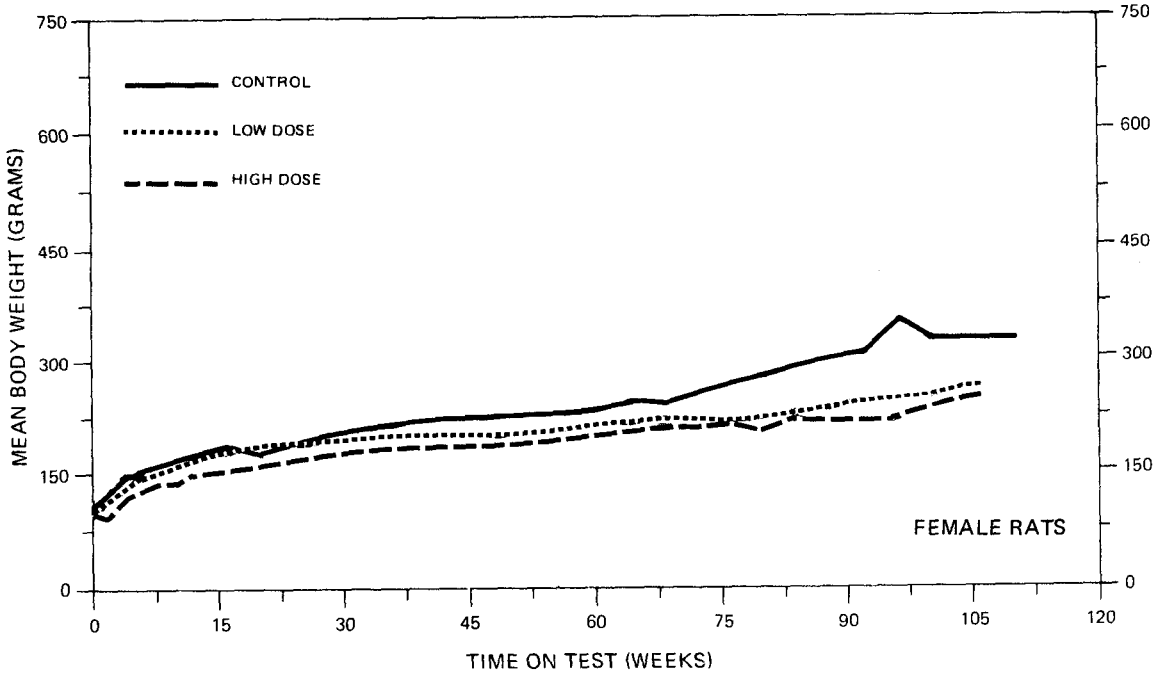


FIGURE 2
GROWTH CURVES FOR CUPFERRON CHRONIC STUDY RATS

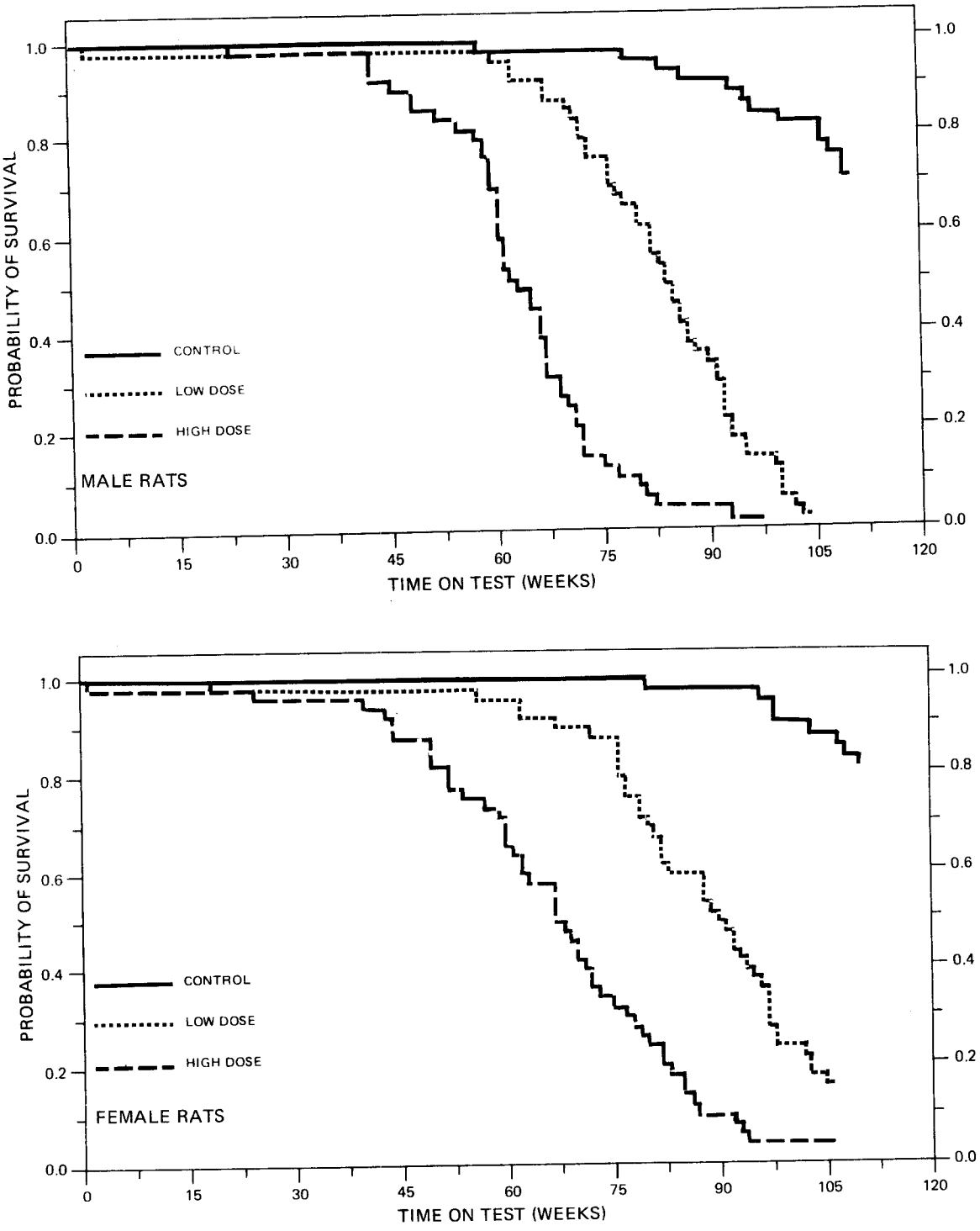


FIGURE 3
SURVIVAL COMPARISONS OF CUPFERRON CHRONIC STUDY RATS

dose males and 80 percent (35/44) of the high dose males examined developed a hemangiosarcoma as early as week 42, while no cases of this tumor were observed in the control male rats.

For females five rats were sacrificed from the control group in week 78. In the high dose group the median survival was 68 weeks, with only two rats remaining alive on test until the end of the study. The median survival was 91 weeks for the low dose group, with eight animals surviving on test until the end of the study. In the control group 72 percent (36/50) of the female rats survived on test until the end of the study. The early mortality may have resulted from tumor incidence since 62 percent (28/45) of the low dose females and 79 percent (37/47) of the high dose females examined developed a hemangiosarcoma, while no cases of this tumor were observed in the control female rats.

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).

Dosed rats had increased incidences of hepatocellular carcinoma; carcinoma and papilloma of the forestomach; hemangiosarcoma; fibromas; and tumors of the adrenal medulla and auditory sebaceous glands.

The incidence of hepatocellular carcinoma in dosed rats was significantly elevated in both males (4/43 [9 percent] high dose;

8/48 [17 percent] low dose) and females (10/44 [23 percent] high dose; 24/44 [55 percent] low dose). Neoplastic nodules, basophilic foci, and clear-cell foci, while occurring in lesser numbers than carcinoma, were only found in dosed rats. The histopathology of hepatocellular carcinoma and altered cellular foci was similar to that described by Squire and Levitt (1975). Hepatocellular carcinoma was generally characterized as an expanding mass of hepatocytes that produced compression of the adjacent parenchyma and lacked portal radicles. Variation in cytomorphology was a common feature of the tumors. Hepatocytes were basophilic, either small or large cell type, and had vacuolated or granular cytoplasm and normal-sized or enlarged nuclei. Mitotic activity was minimal to moderate. Cytoarchitectural changes included a trabecular growth pattern, one-cell-thick or multiple-cell-thick liver plates, and acinar formations. The latter two arrangements of the neoplastic liver cells were often associated with moderate degrees of anaplasia (e.g., cytoplasmic basophilia, large cell type, nuclear enlargement, and hyperchromatism). Occasional areas of necrosis were noted. Metastases of hepatocellular carcinoma were not found.

The squamous epithelium of the forestomach was altered in a number of ways. Squamous-cell papilloma (10/38 [26 percent] high dose, 16/48 [33 percent] low dose males; 6/43 [14 percent] high dose, 6/43 [14 percent] low dose females) and focal and diffuse basal-cell hyperplasia (16/38 [42 percent] high dose, 26/48 [54 percent] low

dose males; 14/43 [33 percent] high dose, 17/43 [40 percent] low dose females) was significantly elevated in males and females. Carcinoma in situ was noted in a few instances, but this was still considered to be compound-related. These diagnoses overlap, with a single animal often having two or three of the above changes. The squamous-cell carcinomas of the forestomach (17/38 [45 percent] high dose, 19/48 [40 percent] low dose males; 22/43 [51 percent] high dose, 14/43 [33 percent] low dose females) originated from the prickle-cell layer and were invasive. Most had locally invaded the lamina propria, but some had extensively invaded through the stomach wall and had invaded other visceral organs by direct extension. A few tumors showed distant metastases in lymph nodes, lungs, and liver. Some tumors had extensive pearl formation, while other tumors were composed of invading nests and cords of more undifferentiated squamous cells. Both focal and diffuse basal-cell hyperplasia were commonly seen. This was characterized by downgrowths of multiple layers of basal cells but without invasion of the lamina propria or overlying stratified squamous epithelium. Carcinoma in situ was judged to be present when loss of polarity and cellular atypia occurred throughout the squamous epithelium, but there was no invasion through the basement membrane. Squamous papilloma resembled a warty or villous nodule with extensive keratinization and lack of invasion.

Hemangiosarcomas, mostly originating in the spleen, were found in very high frequency in both males (35/44 [80 percent] high dose,

38/49 [78 percent] low dose) and females (37/47 [79 percent] high dose, 28/45 [62 percent] low dose). These were composed of variable-sized blood spaces from capillary size to massive hematomas. There were solid sarcomatous areas and villus-like structures projecting into large blood spaces. The cells were pleomorphic, and mitotic figures were common.

Subcutaneous fibromas, circumscribed tumors composed of well-differentiated fibroblasts and mature collagen, were of moderately elevated frequency in the low dose males. The incidence in the high dose group was comparable to that in the controls.

Carcinomas of the auditory sebaceous glands occurred in 4/47 (9 percent) high dose and 5/45 (11 percent) low dose females and only 1/49 (2 percent) control females. A few were seen in male rats. A rare tumor, ganglioneuroma of the adrenal medulla, was found in two high dose male rats and one high dose female rat. This incidence, although low, was judged to be compound-related. Histologically, these tumors were composed of nerve cells in an abundant glial fibrillary stroma.

There were numerous inflammatory and degenerative lesions noted which are common in aging rats, none of which were judged to be related to the administration of cupferron.

On the basis of the data presented by this histopathologic examination, it is evident that cupferron is a carcinogenic compound in Fischer 344 rats. The increased incidences of hepatocellular

carcinomas, squamous-cell carcinomas of the stomach, hemangiosarcomas, gliomas, ganglioneuroma and tumors of auditory sebaceous glands were all believed to be compound-related.

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

In both male and female rats increased incidences of hemangiosarcomas were observed. For both sexes the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dosage and tumor incidence. A significant departure from linearity was also observed for both males ($P < 0.001$) and females ($P = 0.009$), principally due to the elevated incidence of this tumor in the dosed rats compared to the controls. For both sexes the Fisher exact tests supported these findings with significant ($P <$ [illegible]) comparisons of both high and low dose to controls. Based on these results, the administration of cupferron was associated with an increased incidence of hemangiosarcomas in both male and female rats.

In both male and female rats numerous stomach squamous-cell carcinomas were observed. For both sexes the Cochran-Armitage test was significant ($P < 0.001$). In both sexes these results were supported by significant ($P < 0.001$) Fisher exact test comparisons of

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH CUPFERRON[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	1/50(0.02)	15/49(0.31)	5/44(0.11)
P Values[c]	N.S.	P < 0.001	N.S.
Departure from Linear Trend[e]	P < 0.001	---	---
Relative Risk (Control)[d]	---	15.310	5.682
Lower Limit	---	2.518	0.670
Upper Limit	---	625.800	262.000
Weeks to First Observed Tumor	110	62	60
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	10/50(0.20)	2/49(0.04)	2/44(0.05)
P Values[c]	P = 0.008(N)	P = 0.015(N)	P = 0.024(N)
Relative Risk (Control)[d]	---	0.204	0.227
Lower Limit	---	0.003	0.025
Upper Limit	---	0.894	0.991
Weeks to First Observed Tumor	78	91	42
Circulatory System: Hemangiosarcoma[b]	0/50(0.00)	38/49(0.78)	35/44(0.80)
P Values[c]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	13.280	13.665
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	62	45

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	0/49(0.00)	8/48(0.17)	4/43(0.09)
P Values[c]	N.S.	P = 0.003	P = 0.044
Departure from Linear Trend[e]	P = 0.016	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	2.334	1.058
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	84	72
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	0/49(0.00)	12/48(0.25)	5/43(0.12)
P Values[c]	P = 0.048	P < 0.001	P = 0.020
Departure from Linear Trend[e]	P = 0.001	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	3.747	1.440
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	84	67
Stomach: Squamous-Cell Carcinoma[b]	0/49(0.00)	19/48(0.40)	17/38(0.45)
P Values[c]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P = 0.028	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	6.245	7.024
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	73	58

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Stomach: Squamous-Cell Carcinoma or Squamous-Cell Papilloma[b]	0/49(0.00)	32/48(0.67)	24/38(0.63)
P Values[c]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	10.995	10.279
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	62	45
Stomach: Carcinoma In Situ NOS[b]	0/49(0.00)	4/48(0.08)	1/38(0.03)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.039	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.947	0.069
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	71	50
Pituitary: Adenoma NOS or Chromophobe Adenoma[b]	7/45(0.16)	3/38(0.08)	0/31(0.00)
P Values[c]	P = 0.016(N)	N.S.	P = 0.021(N)
Relative Risk (Control)[d]	---	0.508	0.000
Lower Limit	---	0.090	0.000
Upper Limit	---	2.048	0.735
Weeks to First Observed Tumor	78	73	---

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant[b]	3/50(0.06)	6/48(0.13)	6/36(0.17)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.083	2.778
Lower Limit	---	0.474	0.636
Upper Limit	---	12.229	16.051
Weeks to First Observed Tumor	78	80	65
Thyroid: C-Cell Carcinoma[b]	2/37(0.05)	0/34(0.00)	1/31(0.03)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	0.597
Lower Limit	---	0.000	0.010
Upper Limit	---	3.634	10.873
Weeks to First Observed Tumor	109	---	67
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	3/37(0.08)	0/34(0.00)	1/31(0.03)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	0.398
Lower Limit	---	0.000	0.008
Upper Limit	---	1.784	4.643
Weeks to First Observed Tumor	109	---	67

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Testis: Interstitial-Cell Tumor[b]	42/50(0.84)	27/47(0.57)	8/36(0.22)
P Values[c]	P < 0.001(N)	P = 0.004(N)	P < 0.001(N)
Relative Risk (Control)[d]	---	0.684	0.265
Lower Limit	---	0.535	0.145
Upper Limit	---	0.911	0.467
Weeks to First Observed Tumor	78	67	59
Cerebrum: Astrocytoma[b]	0/50(0.00)	2/44(0.05)	1/38(0.03)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.336	0.070
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	71	58
Body Cavities: Malignant Mesothelioma or Mesothelioma NOS[b]	0/50(0.00)	5/49(0.10)	1/44(0.02)
P Values[c]	P = 0.350	P = 0.027	P = 0.468
Departure from Linear Trend[e]	P = 0.011	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	1.287	0.061
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	84	72

TABLE 3 (CONCLUDED)

[a]Treated groups received time-weighted average doses of 0.15 or 0.30 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 $P < 0.05$.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE RATS TREATED WITH CUPFERRON[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	2/49(0.04)	6/45(0.13)	2/47(0.04)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.048	---	---
Relative Risk (Control)[d]	---	3.267	1.043
Lower Limit	---	0.621	0.078
Upper Limit	---	31.720	13.860
Weeks to First Observed Tumor	108	76	75
Subcutaneous Tissue: Sarcoma NOS or Fibrosarcoma[b]	0/49(0.00)	3/45(0.07)	1/47(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.656	0.056
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	76	67
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	7/49(0.14)	3/45(0.07)	0/47(0.00)
P Values[c]	P = 0.006(N)	N.S.	P = 0.008(N)
Relative Risk (Control)[d]	---	0.467	0.000
Lower Limit	---	0.082	0.000
Upper Limit	---	1.904	0.536
Weeks to First Observed Tumor	96	77	---

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Circulatory System: Hemangiosarcoma[b]	0/49(0.00)	28/45(0.62)	37/47(0.79)
P Values[c]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P = 0.009	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	10.180	13.240
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	62	43
Liver: Hepatocellular Carcinoma[b]	1/48(0.02)	24/44(0.55)	10/44(0.23)
P Values[c]	P = 0.012	P < 0.001	P = 0.002
Departure from Linear Trend[e]	P < 0.001	---	---
Relative Risk (Control)[d]	---	26.180	10.910
Lower Limit	---	4.657	1.657
Upper Limit	---	1024.000	459.900
Weeks to First Observed Tumor	98	79	70
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	1/48(0.02)	26/44(0.59)	12/44(0.23)
P Values[c]	P = 0.004	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001	---	---
Relative Risk (Control)[d]	---	28.360	13.090
Lower Limit	---	5.104	2.074
Upper Limit	---	1100.000	542.600
Weeks to First Observed Tumor	98	79	70

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Stomach: Carcinoma In Situ NOS[b]	0/49(0.00)	3/43(0.07)	0/43(0.00)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.011	---	---
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.686	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	81	---
Stomach: Squamous-Cell Carcinoma[b]	0/49(0.00)	14/43(0.33)	22/43(0.51)
P Values[c]	P < 0.001	P < 0.001	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	4.986	8.205
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	76	60
Stomach: Squamous-Cell Carcinoma or Squamous-Cell Papilloma[b]	0/49(0.00)	19/43(0.44)	24/43(0.56)
P Values[c]	P < 0.001	P < 0.001	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	6.990	9.022
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	76	60

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma NOS or Chromophobe Adenoma[b]	17/39(0.44)	4/35(0.11)	0/34(0.00)
P Values[c]	P < 0.001(N)	P = 0.002(N)	P < 0.001(N)
Relative Risk (Control)[d]	---	0.262	0.000
Lower Limit	---	0.072	0.000
Upper Limit	---	0.710	0.208
Weeks to First Observed Tumor	78	76	---
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant[b]	3/49(0.06)	7/41(0.17)	6/42(0.14)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.789	2.333
Lower Limit	---	0.684	0.533
Upper Limit	---	15.720	13.603
Weeks to First Observed Tumor	110	97	79
Mammary Gland: Fibroadenoma[b]	12/49(0.24)	10/45(0.22)	1/47(0.02)
P Values[c]	P = 0.003(N)	N.S.	P = 0.002(N)
Relative Risk (Control)[d]	---	0.907	0.087
Lower Limit	---	0.390	0.002
Upper Limit	---	2.056	0.550
Weeks to First Observed Tumor	103	93	106

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Nervous System/Brain: Astrocytoma, Oligodendroglioma, or Glioma NOS[b]	1/49(0.02)	5/43(0.12)	3/42(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	5.698	3.500
Lower Limit	---	0.673	0.294
Upper Limit	---	262.570	179.151
Weeks to First Observed Tumor	98	88	52
Zymbal's Gland: Ceruminous Carcinoma[b]	1/49(0.02)	4/45(0.09)	2/47(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	4.356	2.085
Lower Limit	---	0.453	0.112
Upper Limit	---	209.400	120.400
Weeks to First Observed Tumor	80	67	54
Zymbal's Gland: Squamous-Cell Carcinoma or Ceruminous Carcinoma[b]	1/49(0.02)	5/45(0.11)	4/47(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	5.444	4.170
Lower Limit	---	0.642	0.433
Upper Limit	---	251.270	200.705
Weeks to First Observed Tumor	80	76	54

TABLE 4 (CONCLUDED)

[a]Treated groups received time-weighted average doses of 0.15 or 0.30 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 $P < 0.05$.

both high and low dose to control group. Based on these results, the administration of cupferron was associated with an increased incidence of squamous-cell carcinomas of the stomach in both male and female rats.

In both male and female rats numerous stomach squamous-cell carcinomas were observed. For both sexes the Cochran-Armitage test was significant ($P < 0.001$). In both sexes these results were supported by significant ($P < 0.001$) Fisher exact test comparisons of both high and low dose to control group. Based on these results, the administration of cupferron was associated with an increased incidence of squamous-cell carcinomas of the stomach in both male and female rats.

In both male and female rats increased incidences of liver neoplasms were observed. When incidences were combined so that the numerator represented rats with either a hepatocellular carcinoma or a neoplastic nodule, for male rats the Cochran-Armitage test indicated a significant ($P = 0.048$) positive association between dose and incidence. The Fisher exact test supported these findings with significant comparisons of both high dose ($P = 0.020$) and low dose ($P < 0.001$) to control. Similarly, for females the Cochran-Armitage test on the combined incidence of hepatocellular carcinomas and neoplastic nodules of the liver was significant ($P = 0.012$) and both the high dose ($P = 0.002$) and low dose ($P < 0.001$) Fisher exact test comparisons were significant. Based on these results, the administration of

cupferron was associated with the increased incidence of liver neoplasms in both male and female rats.

For male rats the Fisher exact test indicated a significantly ($P < 0.001$) higher incidence of fibromas of the subcutaneous tissue in the low dose than in the control. The Cochran-Armitage test and the comparison of high dose to control, however, were not significant. In historical control data compiled by this laboratory for the NCI Carcinogenesis Testing Program, 11/250 (4 percent) of the untreated male Fischer 344 rats had this tumor.

The combined incidence of squamous-cell carcinomas or ceruminous carcinomas of the Zymbal's gland in female rats, although increased in the dosed groups compared to the controls, did not yield significant results using the Fisher exact or Cochran-Armitage tests. They were, however, rare tumors occurring in only 0.8 percent (2/249) of the untreated historical control female Fischer 344 rats. The incidence rates observed under the conditions of this experiment were 11 percent (5/45) for the low dose group and 9 percent (4/47) for the high dose group--compared to 2 percent (1/49) in the control group. When a binomial distribution with a spontaneous incidence rate of 2/249 was assumed, the probability of observing 5 or more animals with such a tumor out of 45 animals (as in the low dose female group) was $P < 0.0001$ and the probability of observing 4 animals with such a tumor out of 47 animals (as in the high dose female group) was $P < 0.0006$, both significant results.

For male rats the low dose to control Fisher exact comparison of the combined incidences of mesothelioma NOS and malignant mesothelioma of the body cavities had a probability level of $P = 0.027$, a marginal result which was not significant under the Bonferroni criterion.

A number of possible negative associations between dose and incidence were observed: leukemia/malignant lymphomas, interstitial-cell tumors of the testis, and pituitary adenomas in the males, plus leukemia/malignant lymphomas, mammary fibroadenomas, and pituitary adenomas in the females. Since all of these tumors are relatively late-developing tumors it is possible that these results merely reflected the higher early morality noted in dosed rats of both sexes.

A rare tumor--a ganglioneuroma of the adrenal gland or adrenal medulla--was observed in 2/36 high dose males. In historical data, none of the 250 untreated males had this tumor. When a binomial distribution with a spontaneous incidence rate of 1/251 was assumed (the most conservative approach), the probability of observing 2 or more rats with such a tumor out of 36 males was $P < 0.009$, a significant result. In other historical data of interest 32/250 (13 percent) of the males and 16/249 (6 percent) of the females had an adrenal pheochromocytoma or a malignant pheochromocytoma; none of the 249 historical control females had a glioma of the brain or an adrenal ganglioneuroma.

Thus, the statistical results indicated that the administration of cupferron was associated with the increased incidence of

hemangiosarcomas, squamous-cell carcinomas of the stomach, and liver neoplasms in both male and female rats--and possibly was associated with the increased incidence of ganglioneuromas of the adrenal gland or adrenal medulla in males and of carcinomas of the Zymbal's gland in females.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

Distinct compound-related mean body weight depression was evident in both male and female dosed mice when compared to controls (Figure 4). Fluctuations in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variations.

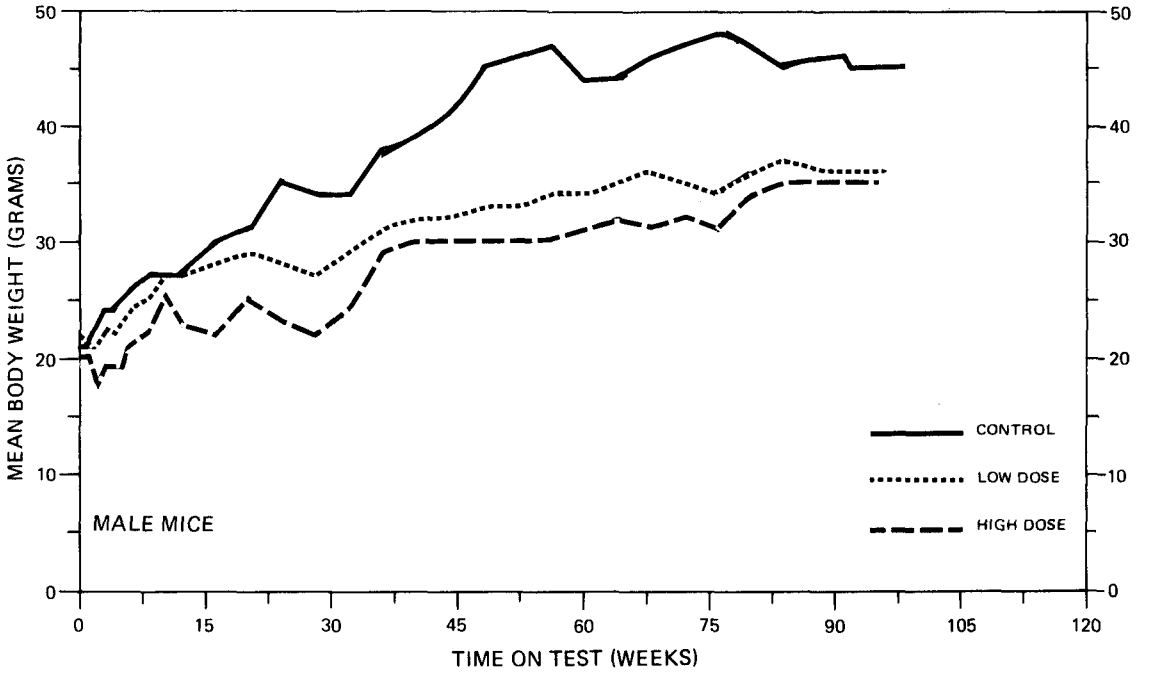
No clinical abnormalities were reported for dosed or control mice of either sex.

B. Survival

The estimated probabilities of survival for male and female mice in the control and cupferron-dosed groups are shown in Figure 5. The Tarone test indicated a significant positive association between dosage and mortality for both male ($P < 0.001$) and female ($P = 0.009$) mice.

For males, five mice were sacrificed from the high dose group in week 78 and five from the control group in week 80. Adequate numbers of males were at risk from late-developing tumors as 62 percent (31/50) of the high dose, 88 percent (44/50) of the low dose, and 98 percent (49/50) of the control mice survived on test at least 75 weeks.

For females five mice were sacrificed from the high dose group in week 78 and five from the control group in week 80. With 58 percent (29/50) of the high dose, 68 percent (34/50) of the low dose



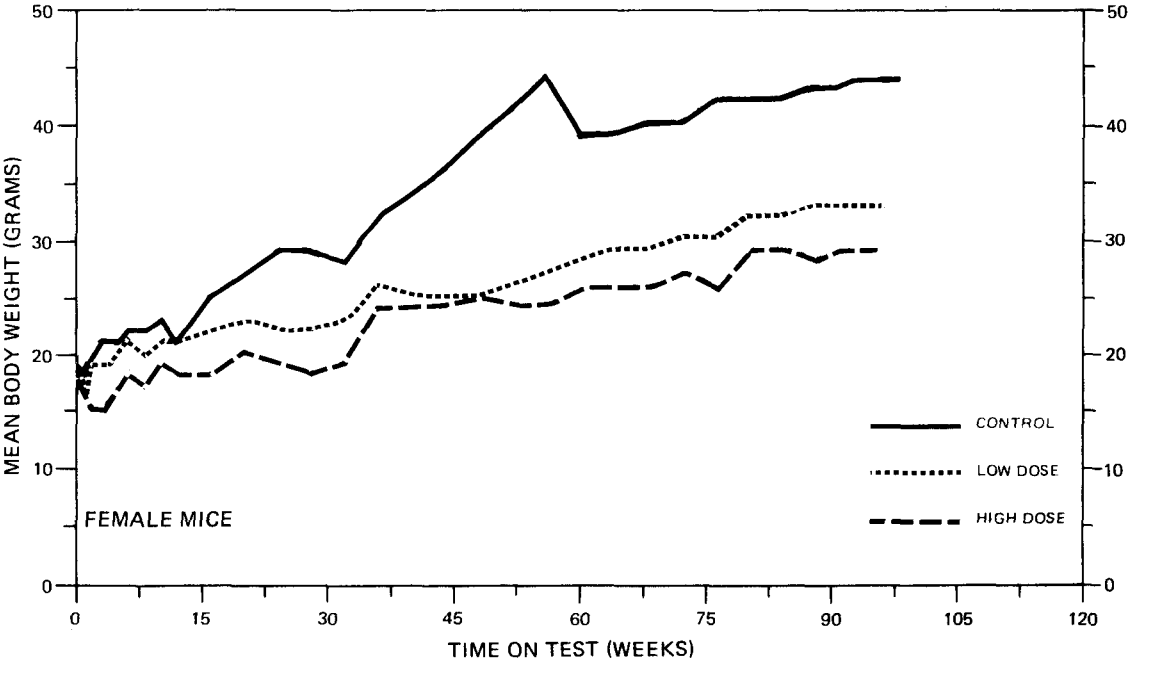
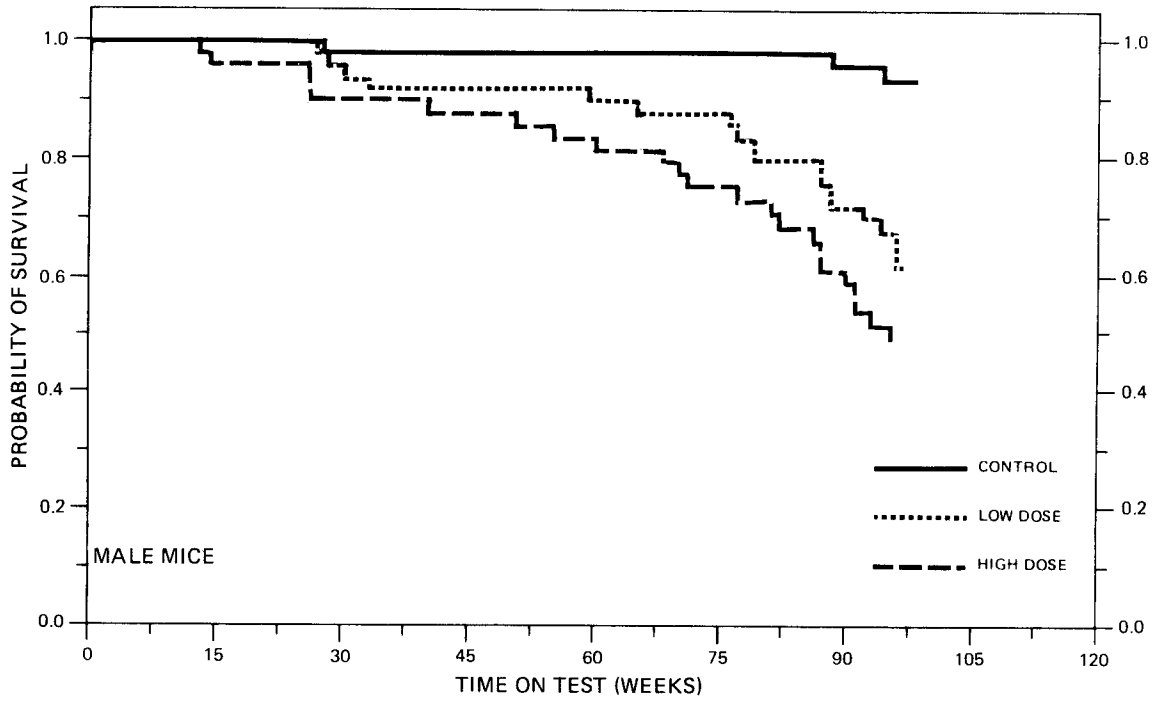


FIGURE 4
GROWTH CURVES FOR CUPFERRON CHRONIC STUDY MICE



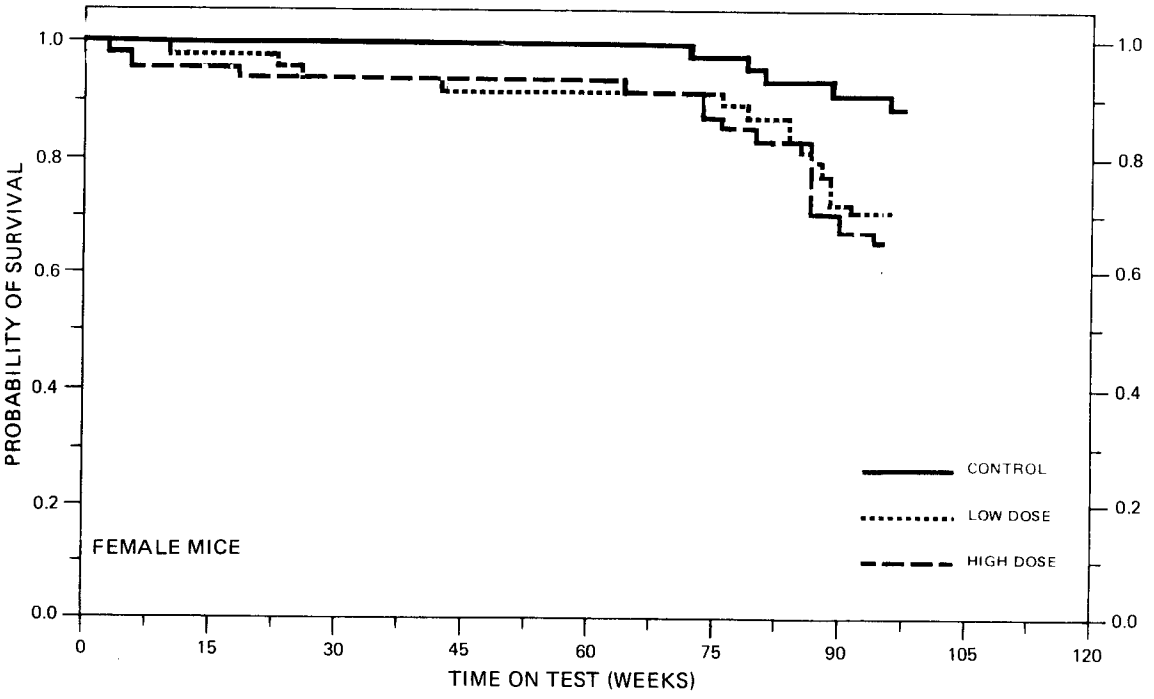


FIGURE 5
SURVIVAL COMPARISONS OF CUPFERRON CHRONIC STUDY MICE

and 80 percent (40/50) of the controls surviving on test until the termination of the study, adequate numbers of female mice 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).

Dosed mice had increased but relatively low incidences of hepatocellular neoplasms, hemangiomas and hemangiosarcomas, Harderian gland adenomas, and tumors of the Zymbal's gland.

The incidence of hepatocellular carcinoma was considerably higher in dosed female mice (13/45 [29 percent] high dose, 9/46 [20 percent] low dose) than in the controls (2/49 [4 percent]). This increase was not seen in dosed male mice. The hepatocellular carcinomas were cellular, pleomorphic masses of hepatocytes devoid of any architectural arrangement and infiltrating the adjoining parenchyma. In each sex, a few hepatocellular carcinomas had metastasized to the lungs. Hepatocellular adenomas were generally spherical noninfiltrating expanding nodules of well-differentiated hepatocytes which moderately compressed adjacent hepatic cords. In some the cells were smaller than normal and hyperbasophilic while others had cells larger than normal with eosinophilic cytoplasmic vacuolation. Hepatocytes were arranged in thickened trabecular and sheet-like growth patterns. Mitotic figures and small bile ducts were rare.

The incidence of hemangiosarcomas was elevated in the high dose male mice (7/40 [18 percent]), low dose female mice (5/47 [11 percent]), and high dose female mice (6/46 [13 percent]) with 1/50 (2 percent) in control males and 1/50 (2 percent) in control females. The number of hemangiomas was elevated in low dose females (5/47 [11 percent]; controls 0/50). The majority of hemangiosarcomas were found in the spleen, and although histologically malignant, there was only one instance of pulmonary metastasis. The hemangiosarcomas were characterized by irregular blood spaces with ingrowths of pleomorphic lining cells. Some tumors had large blood-filled spaces, while others had extensive areas of solid spindle-shaped sarcomatous cells. Mitotic figures were numerous. Hemangiomas tended to have blood spaces bordered by several layers of cells with uniform size and shape of the lining epithelium. Mitotic figures were rare in hemangiomas. These tumors were also more circumscribed than their malignant counterparts.

Neoplasms of the Zymbal's gland were found in 3/47 (6 percent) high dose females and in 1/45 (2 percent) high dose males; none were detected in controls.

The number of adenomas of the Harderian gland was elevated in the low dose (3/45 [7 percent]) and high dose (4/40 [10 percent]) males and the low dose (2/47 [4 percent]) and high dose (6/46 [13 percent]) females as compared with controls (female 0/50; male 0/50). These were well-differentiated, circumscribed expanding tumors with

a papillary glandular structure composed of epithelial cells closely resembling the normal glandular epithelium. No mitoses were seen.

There were numerous inflammatory and degenerative lesions noted, as expected, in aging mice. None were judged to be related to the administration of cupferron.

The results of this histopathologic examination indicate that cupferron induced hemangiomas, hepatocellular carcinomas, and Zymbal's gland carcinomas in female mice, and adenomas of the Harderian gland and hemangiosarcomas in both males and females. Therefore, the compound is considered to be carcinogenic in B6C3F1 mice.

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 cupferron-dosed groups and where such tumors were observed in at least 5 percent of the group.

In both male and female mice numerous hemangiosarcomas were observed. The Cochran-Armitage test indicated a significant positive association between dose and tumor incidence for both males ($P = 0.008$) and females ($P = 0.038$). For males the Fisher exact test supported these findings with a significant ($P = 0.013$) comparison of the high dose group to the control. For females when incidences were combined so that the numerator represented mice with either a hemangiosarcoma or a hemangioma, the Fisher exact test comparison of low

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH CUPFERRON[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Skin and Subcutaneous Tissue: Squamous-Cell Carcinoma[b]	0/50(0.00)	3/45(0.07)	2/40(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.669	0.370
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	95	78
Subcutaneous Tissue: Sarcoma NOS[b]	0/50(0.00)	1/45(0.02)	0/40(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.060	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	96	---
Lung: Alveolar/Bronchiolar Carcinoma[b]	3/47(0.06)	0/44(0.00)	0/40(0.00)
P Values[c]	P = 0.047(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	1.769	1.941
Weeks to First Observed Tumor	98	---	---

TABLE 5 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	7/47(0.15)	8/44(0.18)	5/40(0.13)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.221	0.839
Lower Limit	---	0.422	0.226
Upper Limit	---	3.620	2.819
Weeks to First Observed Tumor	98	79	87
Circulatory System: Hemangiosarcoma[b]	1/50(0.02)	3/45(0.07)	7/40(0.18)
P Values[c]	P = 0.008	N.S.	P = 0.013
Relative Risk (Control)[d]	---	3.333	8.750
Lower Limit	---	0.279	1.194
Upper Limit	--	171.000	382.700
Weeks to First Observed Tumor	98	87	70
Liver: Hepatocellular Carcinoma[b]	15/49(0.31)	9/44(0.20)	8/40(0.20)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.668	0.653
Lower Limit	---	0.287	0.267
Upper Limit	---	1.454	1.459
Weeks to First Observed Tumor	94	79	86

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Harderian Gland: Adenoma NOS[b]	0/50(0.00)	3/45(0.07)	4/40(0.10)
P Values[c]	P = 0.028	N.S.	P = 0.036
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.669	1.161
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	95	95

[a]Treated groups received time-weighted average doses of 0.2 or 0.4 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 fo the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE MICE TREATED WITH CUPFERRON[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	3/50(0.06)	3/45(0.07)	1/46(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.111	0.362
Lower Limit	---	0.156	0.007
Upper Limit	---	7.893	4.318
Weeks to First Observed Tumor	80	84	95
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	4/50(0.08)	11/45(0.24)	9/46(0.20)
P Values[c]	N.S.	P = 0.027	N.S.
Relative Risk (Control)[d]	---	3.056	2.446
Lower Limit	---	0.983	0.737
Upper Limit	---	12.240	10.160
Weeks to First Observed Tumor	80	84	79
Hematopoietic System: Malignant Lymphoma[b]	6/50(0.12)	7/47(0.15)	4/46(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.241	0.725
Lower Limit	---	0.385	0.160
Upper Limit	---	4.146	2.852
Weeks to First Observed Tumor	98	87	94

TABLE 6 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Circulatory System: Hemangiosarcoma[b]	1/50(0.02)	5/47(0.11)	6/46(0.13)
P Values[c]	P = 0.038	N.S.	P = 0.044
Relative Risk (Control)[d]	---	5.319	6.522
Lower Limit	---	0.627	0.836
Upper Limit	---	245.700	292.800
Weeks to First Observed Tumor	98	84	64
Circulatory System: Hemangiosarcoma or Hemangioma[b]	1/50(0.02)	10/47(0.21)	6/46(0.13)
P Values[c]	N.S.	P = 0.003	P = 0.044
Departure from Linear Trend[e]	P = 0.017	---	---
Relative Risk (Control)[d]	---	10.638	6.522
Lower Limit	---	1.610	0.836
Upper Limit	---	449.503	292.687
Weeks to First Observed Tumor	98	84	64
Liver: Hepatocellular Carcinoma[b]	2/49(0.04)	9/46(0.20)	13/45(0.29)
P Values[c]	P = 0.001	P = 0.019	P = 0.001
Relative Risk (Control)[d]	---	4.794	7.078
Lower Limit	---	1.063	1.730
Upper Limit	---	43.590	61.400
Weeks to First Observed Tumor	98	79	74

TABLE 6 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	2/49(0.04)	12/46(0.26)	16/45(0.36)
P Values[c]	P < 0.001	P = 0.002	P < 0.001
Relative Risk (Control)[d]	---	6.391	8.711
Lower Limit	---	1.533	2.222
Upper Limit	---	56.028	73.866
Weeks to First Observed Tumor	98	79	74
Ovary: Tubular-Cell Adenoma[b]	0/48(0.00)	1/37(0.03)	3/41(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.070	0.706
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	96	95
Harderian Gland: Adenoma NOS[b]	0/50(0.00)	2/47(0.04)	6/46(0.13)
P Values[c]	P = 0.006	N.S.	P = 0.010
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.315	1.740
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	96	87

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Ovary: Granulosa-Cell Tumor[b]	0/48(0.00)	2/37(0.05)	1/41(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.385	0.063
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	96	95
Zymbal's Gland: Squamous-Cell Carcinoma or Sebaceous Adenocarcinoma[b]	0/50(0.00)	0/47(0.00)	3/46(0.07)
P Values[c]	P = 0.033	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.654
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	87

[a]Treated groups received time-weighted average doses of 0.2 or 0.4 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 $P < 0.05$.

dose to control was significant (P = 0.003). The comparison of high dose to control had a probability level of P = 0.044, a marginal result which was not significant under the Bonferroni criterion. Based on these results, administration of cupferron was associated with an increased incidence of hemangiosarcomas in male and either hemangiosarcomas or hemangiomas in female mice.

For female mice the incidence of hepatocellular carcinomas was increased in the dosed groups compared to the control group. The Cochran-Armitage test showed a significant (P = 0.001) positive association between dose and tumor incidence compared to the control. This was supported by significant Fisher exact test results comparing the incidences in the high dose (P = 0.001) and the low dose (P = 0.019) to that in the control. Based upon these results the administration of cupferron was associated with an increased incidence of hepatocellular carcinomas in female mice.

In both male and female mice increased incidences of Harderian gland adenomas NOS were also observed in both dosed groups. For females the Cochran-Armitage test showed a significant (P = 0.006) positive association between chemical administration and tumor incidence. This was supported by a significant (P = 0.010) Fisher exact test comparison of the high dose group to the control. For male mice the Cochran-Armitage test was significant (P = 0.028), but the Fisher exact tests were not significant under the Bonferroni criterion. Based on these results the administration of cupferron was associated

with an increased incidence of Harderian gland adenomas in female mice.

In female mice an increase in the combined incidence of squamous-cell carcinomas or sebaceous adenocarcinomas of the Zymbal's gland was observed in the high dose group. The Cochran-Armitage test showed a significant ($P = 0.033$) positive association between dosage and tumor incidence, but Fisher exact tests were not significant. These rare tumors did not occur in any of the untreated 275 historical control B6C3F1 female mice observed by Mason Research Institute for the NCI Carcinogenesis Testing Program. When a binominal distribution with a 1/276 probability of spontaneous incidence was assumed (the most conservative approach), the probability of observing 3 mice with such tumors out of 46 female mice was $P < 0.0007$, a significant result.

For male mice the Cochran-Armitage test showed a significant ($P = 0.047$) negative trend for alveolar/bronchiolar carcinoma, but the Fisher exact tests were not significant. For alveolar/bronchiolar neoplasms in the females the comparison of low dose to control was $P = 0.027$, a marginal result which was not significant under the Bonferroni criterion.

Thus, the statistical results indicated that the administration of cupferron was associated with an increased incidence of hemangiosarcomas in male mice and of hepatocellular carcinomas, of Harderian

gland adenomas, of hemangiosarcomas or hemangiomas, and possibly of carcinomas of the Zymbal's gland in female mice.

V. DISCUSSION

Among both sexes of rats and mice there was a significant positive association between the dosage of cupferron administered and mortality which could be attributed to tumor formation.

For male and female rats there was a significant positive association between dosage and the incidences of circulatory hemangiosarcomas (i.e., 0/50, 38/49 [78 percent], and 35/44 [80 percent] in the control, low dose, and high dose males, respectively, and 0/49, 28/45 [62 percent], and 37/47 [79 percent] in the control, low dose, and high dose females). For both sexes the high dose to control and low dose to control Fisher exact comparisons were also significant.

Squamous-cell carcinomas of the forestomach were observed at increased incidences in the dosed rats (i.e., 0/49, 19/48 [40 percent], and 17/38 [45 percent] in the control, low dose, and high dose males, respectively, and 0/49, 14/43 [33 percent], and 22/43 [51 percent] in the control, low dose, and high dose females). The Cochran-Armitage tests for both sexes were significant and all the Fisher exact comparisons supported these findings. In addition to the squamous-cell carcinomas of the forestomach, squamous-cell papillomas and basal-cell hyperplasias of the forestomach were observed at increased incidences in the dosed rats when compared to controls, further strengthening the association between abnormalities of the squamous epithelium of the forestomach and administration of cupferron.

Hepatocellular carcinomas, relatively uncommon tumors in Fischer 344 rats (i.e, 0/250 and 1/249 observed in the Mason Research Institute historical control male and female rats, respectively), were detected in 0/49, 8/48 (17 percent), and 4/43 (9 percent) of the control, low dose, and high dose males, respectively, and in 1/48 (2 percent), 24/44 (55 percent), and 10/44 (23 percent) of the control, low dose, and high dose females. When the rats having hepatocellular carcinomas were combined with rats having neoplastic nodules and the resulting incidences (i.e., 0/49, 12/48 [25 percent], and 5/43 [12 percent] control, low dose, and high dose males, respectively, and 1/48 [2 percent], 26/44 [59 percent], and 12/44 [23 percent] control, low dose, and high dose females) analyzed, the Cochran-Armitage tests for both sexes provided significant positive associations between dosage and incidence. High dose to control and low dose to control Fisher exact comparisons for each sex supported the relationship.

When female rats with squamous-cell carcinoma of the auditory sebaceous gland (i.e., 0/49, 1/45 [2 percent], and 2/47 [4 percent] for the control, low dose, and high dose, respectively) were combined with those having ceruminous carcinoma (i.e., 1/49 [2 percent], 4/45 [9 percent], and 2/47 [4 percent] for the control, low dose, and high dose, respectively), no statistically significant results were provided using either the Cochran-Armitage test or the Fisher exact test. However, when a binominal distribution and a spontaneous incidence rate corresponding to the appropriate historical control incidence

were assumed, the incidences of neoplasms of the auditory sebaceous gland observed in this bioassay were significant.

In addition, several uncommon tumors were observed in male and female rats, further strengthening the evidence for carcinogenic action of cupferron in rats.

For female mice hepatocellular carcinomas were observed in 2/49 (4 percent), 9/46 (20 percent), and 13/45 (29 percent) of the control, low dose, and high dose groups, respectively. Statistical analysis indicated a significant positive association between dosage and tumor incidence and both the high dose to control and low dose to control Fisher exact comparisons reinforced the finding.

Hemangiosarcomas occurred in mice of both sexes. For males and females there was a statistically significant positive association between the cupferron dosages administered and tumor incidence. For males these results were supported by the high dose to control Fisher exact test. For females only the low dose to control Fisher exact comparison was significant and that occurred only when the female rats with circulatory hemangiosarcomas and/or hemangiomas were combined and the resulting tumor incidences analyzed.

Adenomas of the Harderian gland were observed in dosed male and female mice. For both sexes the Cochran-Armitage test was significantly positive. The high dose to control Fisher exact comparison supported this finding for the females. The same was not true, however, for males.

When female mice with squamous-cell carcinoma of the auditory sebaceous gland (i.e., 0/50, 0/47, 2/46 [4 percent] for the control, low dose, and high dose groups, respectively) were combined with those having sebaceous adenocarcinoma (i.e., 0/50, 0/47, and 1/46 [2 percent] for the control, low dose, and high dose groups, respectively), no statistically significant results were provided using either the Cochran-Armitage test or the Fisher exact test. However, when a binominal distribution and a spontaneous incidence rate corresponding to the appropriate historical control incidence were assumed, the incidences of auditory neoplasms observed in this bioassay were significant.

Due to the extensive carcinogenic effect on multiple tissues, it must be considered that dose-related trends and incidences of tumors at a particular site may have been modulated by competing or enhancing effects from several types and sites of tumors in both rats and mice.

Under the conditions of this bioassay cupferron was carcinogenic in Fischer 344 rats, causing hemangiosarcomas, hepatocellular carcinomas, and squamous-cell carcinomas of the forestomach in males and females as well as carcinomas of the auditory sebaceous gland in females. The chemical was also carcinogenic in B6C3F1 mice, causing hemangiosarcomas in males and hepatocellular carcinomas, carcinomas of the auditory sebaceous gland, a combination of hemangiosarcomas and hemangiomas, and adenomas of the Harderian gland in females.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH CUPFERRON

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH CUPFERRON

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0180	HIGH DOSE 01-0185
ANIMALS INITIALLY IN STUDY	50	50	@50
ANIMALS NECROPSIED	50	49	44
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	49	44
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(44)
SEBACEOUS ADENOCARCINOMA		1 (2%)	1 (2%)
FIBROMA	1 (2%)		1 (2%)
FIBROSARCOMA	1 (2%)		
HEMANGIOSARCOMA		1 (2%)	
*SUBCUT TISSUE	(50)	(49)	(44)
SARCOMA, NOS	1 (2%)		1 (2%)
FIBROMA	1 (2%)	15 (31%)	5 (11%)
FIBROSARCOMA	1 (2%)	1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(49)	(47)	(43)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)		1 (2%)
HEMANGIOSARCOMA, METASTATIC		2 (4%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(44)
MALIGNANT LYMPHOMA, NOS			1 (2%)
LEUKEMIA,NOS	1 (2%)		
MYELOMONOCYTIC LEUKEMIA	9 (18%)	2 (4%)	1 (2%)
#SPLEEN	(50)	(46)	(43)
HEMANGIOSARCOMA		38 (83%)	35 (81%)
#TRACHEAL LYMPH NODE	(49)	(43)	(39)
C-CELL CARCINOMA, METASTATIC			1 (3%)
#MEDIASTINAL L.NODE	(49)	(43)	(39)
FIBROSARCOMA, METASTATIC		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS
@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A FEMALE IN A MALE GROUP.

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0180	HIGH DOSE 01-0185
CIRCULATORY SYSTEM			
#HEART	(48)	(47)	(43)
SARCOMA, NOS, METASTATIC	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(49)	(48)	(43)
NEOPLASTIC NODULE		4 (8%)	1 (2%)
HEPATOCELLULAR CARCINOMA		8 (17%)	4 (9%)
SARCOMA, NOS		1 (2%)	
FIBROSARCOMA, METASTATIC		1 (2%)	
#STOMACH	(49)	(48)	(38)
CARCINOMA-IN-SITU, NOS		4 (8%)	1 (3%)
SQUAMOUS CELL PAPILLOMA		16 (33%)	10 (26%)
SQUAMOUS CELL CARCINOMA		19 (40%)	17 (45%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(38)	(31)
ADENOMA, NOS	5 (11%)	3 (8%)	
CHROMOPHOBE ADENOMA	2 (4%)		
#ADRENAL	(50)	(48)	(36)
PHEOCHROMOCYTOMA	3 (6%)	4 (8%)	5 (14%)
PHEOCHROMOCYTOMA, MALIGNANT		2 (4%)	1 (3%)
HEMANGIOSARCOMA		1 (2%)	
GANGLIONEUROMA			1 (3%)
#ADRENAL MEDULLA	(50)	(48)	(36)
GANGLIONEUROMA			1 (3%)
#THYROID	(37)	(34)	(31)
FOLLICULAR-CELL CARCINOMA	1 (3%)		
C-CELL ADENOMA	1 (3%)		
C-CELL CARCINOMA	2 (5%)		1 (3%)
#PANCREATIC ISLETS	(47)	(41)	(31)
ISLET-CELL ADENOMA	1 (2%)		

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0180	HIGH DOSE 01-0185
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(49)	(44)
CARCINOMA,NOS	2 (4%)		
#TESTIS	(50)	(47)	(36)
INTERSTITIAL-CELL TUMOR	42 (84%)	27 (57%)	8 (22%)
NERVOUS SYSTEM			
#CEREBRUM	(50)	(44)	(38)
ASTROCYTOMA		2 (5%)	1 (3%)
#CEREBRAL CORTEX	(50)	(44)	(38)
GLIOMA, NOS	1 (2%)		
#CEREBELLUM	(50)	(44)	(38)
FIBROSARCOMA, INVASIVE			1 (3%)
SPECIAL SENSE ORGANS			
*EAR CANAL	(50)	(49)	(44)
FIBROSARCOMA			1 (2%)
*ZYMBAL'S GLAND	(50)	(49)	(44)
CERUMINOUS CARCINOMA		2 (4%)	1 (2%)
MUSCULOSKEIETAL SYSTEM			
*SKELETAL MUSCLE	(50)	(49)	(44)
HEMANGIOSARCOMA, INVASIVE			1 (2%)
BODY CAVITIES			
*BODY CAVITIES	(50)	(49)	(44)
MESOTHELIOMA, NOS		2 (4%)	1 (2%)
MESOTHELIOMA, MALIGNANT		3 (6%)	
*ABDOMINAL CAVITY	(50)	(49)	(44)
SARCOMA, NOS		1 (2%)	
FIBROSARCOMA		1 (2%)	
ALL OTHER SYSTEMS			
NONE			

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

TABLE A1 (CONCLUDED)

	CONTROL(UNTR) 01-0160	LOW DOSE 01-0180	HIGH DOSE 01-0185
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	5	32	31
MORIBUND SACRIFICE	8	18	18
SCHEDULED SACRIFICE	5		
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	32		
ANIMAL MISSING			
ANIMAL DELETED(WRONG SEX)			1
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	49	45	41
TOTAL PRIMARY TUMORS	76	158	101
TOTAL ANIMALS WITH BENIGN TUMORS	46	37	21
TOTAL BENIGN TUMORS	56	65	31
TOTAL ANIMALS WITH MALIGNANT TUMORS	17	44	39
TOTAL MALIGNANT TUMORS	20	87	68
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	3	3
TOTAL SECONDARY TUMORS	1	4	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		6	2
TOTAL UNCERTAIN TUMORS		6	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

TABLE A2
SUMMARY OF THE INCIDENT OF NEOPLASMS IN FEMALE RATS TREATED WITH CUPFERRON

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0180	HIGH DOSE 02-0185
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	45	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	44	44
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(45)	(47)
SQUAMOUS CELL CARCINOMA			1 (2%)
SEBACEOUS ADENOCARCINOMA			1 (2%)
*SUBCUT TISSUE	(49)	(45)	(47)
SARCOMA, NOS		2 (4%)	1 (2%)
FIBROMA	2 (4%)	6 (13%)	2 (4%)
FIBROSARCOMA		1 (2%)	
HEMANGIOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(49)	(44)	(41)
SQUAMOUS CELL CARCINOMA, METASTA			2 (5%)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(45)	(47)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
LEUKEMIA,NOS	1 (2%)		
MYELOMONOCYTIC LEUKEMIA	6 (12%)	2 (4%)	
#SPLEEN	(47)	(44)	(44)
SQUAMOUS CELL CARCINOMA, INVASIV			2 (5%)
HEMANGIOSARCOMA		28 (64%)	37 (84%)
#LYMPH NODE	(44)	(38)	(40)
SQUAMOUS CELL CARCINOMA, METASTA			1 (3%)
#PANCREATIC L.NODE	(44)	(38)	(40)
SQUAMOUS CELL CARCINOMA, METASTA			1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0180	HIGH DOSE 02-0185
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(48)	(44)	(44)
SQUAMOUS CELL CARCINOMA, INVASIV			3 (7%)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
NEOPLASTIC NODULE		2 (5%)	2 (5%)
HEPATOCELLULAR CARCINOMA	1 (2%)	24 (55%)	10 (23%)
HEMANGIOSARCOMA, METASTATIC			1 (2%)
#PANCREAS	(48)	(37)	(38)
HEMANGIOSARCOMA, INVASIVE		1 (3%)	
#PERIPANCREATIC TISSU	(48)	(37)	(38)
SQUAMOUS CELL CARCINOMA, METASTA		1 (3%)	
#STOMACH	(49)	(43)	(43)
CARCINOMA-IN-SITU, NOS		3 (7%)	
SQUAMOUS CELL PAPILLOMA		6 (14%)	6 (14%)
SQUAMOUS CELL CARCINOMA		14 (33%)	22 (51%)
#COLON	(49)	(41)	(40)
ADENOMATOUS POLYP, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(48)	(44)	(44)
HEMANGIOSARCOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(39)	(35)	(34)
ADENOMA, NOS	15 (38%)	4 (11%)	
CHROMOPHOBE ADENOMA	2 (5%)		
#ADRENAL	(49)	(41)	(42)
SQUAMOUS CELL CARCINOMA, INVASIV			1 (2%)
CORTICAL ADENOMA		2 (5%)	
PHEOCHROMOCYTOMA	3 (6%)	4 (10%)	1 (2%)

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0180	HIGH DOSE 02-0185
PHECCHROMOCYTOMA, MALIGNANT		3 (7%)	5 (12%)
GANGLIONEUROMA			1 (2%)
#THYROID	(45)	(31)	(35)
FOLLICULAR-CELL CARCINOMA		1 (3%)	
C-CELL CARCINOMA	2 (4%)	1 (3%)	
PAPILLARY CYSTADENOMA, NOS		1 (3%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(45)	(47)
ADENOCARCINOMA, NOS			1 (2%)
FIBROADENOMA	12 (24%)	10 (22%)	1 (2%)
*CLITORAL GLAND	(49)	(45)	(47)
CARCINOMA,NOS		1 (2%)	
ADENOMA, NOS	1 (2%)		
#UTERUS	(46)	(42)	(40)
ENDOMETRIAL STROMAL POLYP	5 (11%)	3 (7%)	
#OVARY	(47)	(42)	(40)
HEMANGIOSARCOMA		1 (2%)	
NERVOUS SYSTEM			
*CEREBRUM	(49)	(43)	(42)
ASTROCYTOMA		2 (5%)	2 (5%)
OLIGODENDROGLIOMA		1 (2%)	
#BRAIN	(49)	(43)	(42)
GLIOMA, NOS		1 (2%)	
ASTROCYTOMA	1 (2%)	1 (2%)	1 (2%)
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(49)	(45)	(47)
ADENOCARCINOMA, NOS		1 (2%)	
*ZYMBAL'S GLAND	(49)	(45)	(47)
SQUAMOUS CELL CARCINOMA		1 (2%)	2 (4%)
CERUMINOUS CARCINOMA	1 (2%)	4 (9%)	2 (4%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0180	HIGH DOSE 02-0185
BODY CAVITIES			
*ABDOMINAL CAVITY	(49)	(45)	(47)
SARCOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
DIAPHRAGM			
SQUAMOUS CELL CARCINOMA, INVASIV			1
OMENTUM			
SQUAMOUS CELL CARCINOMA			1
HEMANGIOSARCOMA		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	2	27	29
MORIBUND SACRIFICE	6	15	19
SCHEDULED SACRIFICE	5		
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	8	2
ANIMAL MISSING	1		

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0180	HIGH DOSE 02-0185
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	31	42	42
TOTAL PRIMARY TUMORS	52	136	101
TOTAL ANIMALS WITH BENIGN TUMORS	27	24	12
TOTAL BENIGN TUMORS	40	37	12
TOTAL ANIMALS WITH MALIGNANT TUMORS	10	41	42
TOTAL MALIGNANT TUMORS	12	97	87
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	4
TOTAL SECONDARY TUMORS		3	12
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		2	2
TOTAL UNCERTAIN TUMORS		2	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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH CUPFERRON

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH CUPFERRON

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0185	HIGH DOSE 05-0190
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	2
ANIMALS NECROPSIED	50	45	40
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	44	40
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(45)	(40)
SQUAMOUS CELL CARCINOMA		1 (2%)	1 (3%)
*SUBCUT TISSUE	(50)	(45)	(40)
SQUAMOUS CELL CARCINOMA		2 (4%)	1 (3%)
SARCOMA, NOS		1 (2%)	
HEMANGIOSARCOMA		2 (4%)	2 (5%)
RESPIRATORY SYSTEM			
#LUNG	(47)	(44)	(40)
HEPATOCELLULAR CARCINOMA, METAST	2 (4%)		2 (5%)
ALVEOLAR/BRONCHIOLAR ADENOMA	4 (9%)	8 (18%)	5 (13%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (6%)		
HEMANGIOMA			1 (3%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(45)	(40)
MALIGNANT LYMPHOMA, NOS	1 (2%)	3 (7%)	2 (5%)
GRANULOCYTIC LEUKEMIA		1 (2%)	
#SPLEEN	(49)	(44)	(38)
HEMANGIOSARCOMA	1 (2%)	1 (2%)	4 (11%)
MALIGNANT LYMPHOMA, NOS		1 (2%)	1 (3%)
#MESENTERIC L. NODE	(40)	(38)	(33)
MALIGNANT LYMPHOMA, NOS		1 (3%)	
#PEYERS PATCH	(49)	(41)	(37)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0185	HIGH DOSE 05-0190
DIGESTIVE SYSTEM			
#LIVER	(49)	(44)	(40)
HEPATOCELLULAR ADENOMA			1 (3%)
HEPATOCELLULAR CARCINOMA	15 (31%)	9 (20%)	8 (20%)
SARCOMA, NOS		1 (2%)	
#STOMACH	(49)	(42)	(39)
SQUAMOUS CELL PAPILLOMA		2 (5%)	
ADENOMATOUS POLYP, NOS	1 (2%)		
#JEJUNUM	(49)	(41)	(37)
CYSTADENOCARCINOMA, NOS			1 (3%)
URINARY SYSTEM			
#KIDNEY	(49)	(44)	(40)
PAPILLARY CYSTADENOMA, NOS			1 (3%)
ENDOCRINE SYSTEM			
#THYROID	(42)	(39)	(33)
FOLLICULAR-CELL CARCINOMA			1 (3%)
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(45)	(40)
CARCINOMA,NOS		1 (2%)	
SQUAMOUS CELL CARCINOMA		1 (2%)	
#TESTIS	(49)	(44)	(40)
INTERSTITIAL-CELL TUMOR	1 (2%)		
NERVOUS SYSTEM			
#BRAIN/MENINGES	(48)	(42)	(39)
FIBROSARCOMA		1 (2%)	
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(45)	(40)
ADENOMA, NOS		3 (7%)	4 (10%)

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

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0185	HIGH DOSE 05-0190
*ZYMBAL'S GLAND	(50)	(45)	(40)
SQUAMOUS CELL CARCINOMA			1 (3%)
MUSCULOSKELETAL SYSTEM			
*BONE/UPPER EXTREMITY	(50)	(45)	(40)
OSTEOSARCOMA		1 (2%)	
BODY CAVITIES			
*BODY CAVITIES	(50)	(45)	(40)
HEMANGIOSARCOMA			1 (3%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	3	11	19
MORIBUND SACRIFICE		8	4
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	42	29	20
ANIMAL MISSING		1	2

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0185	HIGH DOSE 05-0190
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	25	26
TOTAL PRIMARY TUMORS	26	41	35
TOTAL ANIMALS WITH BENIGN TUMORS	6	12	10
TOTAL BENIGN TUMORS	6	13	12
TOTAL ANIMALS WITH MALIGNANT TUMORS	18	21	23
TOTAL MALIGNANT TUMORS	20	28	23
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		2
TOTAL SECONDARY TUMORS	2		2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH CUPFERRON

	CONTROL(UNTR) 06-0160	LOW DOSE 06-0185	HIGH DOSE 06-0190
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	50	47	46
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	46	46
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(47)	(46)
SQUAMOUS CELL CARCINOMA		1 (2%)	
HEMANGIOSARCOMA			1 (2%)
*SUBCUT TISSUE	(50)	(47)	(46)
FIBROSARCOMA	1 (2%)		1 (2%)
HEMANGIOMA		2 (4%)	
HEMANGIOSARCOMA	1 (2%)	2 (4%)	
RESPIRATORY SYSTEM			
*EXTERNAL NOSE	(50)	(47)	(46)
SARCOMA, NOS		1 (2%)	
#LUNG	(50)	(45)	(46)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)	8 (18%)	8 (17%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (6%)	3 (7%)	1 (2%)
HEMANGIOSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(47)	(46)
MALIGNANT LYMPHOMA, NOS	3 (6%)	3 (6%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		3 (6%)	2 (4%)
#SPLEEN	(49)	(44)	(42)
TRANSITIONAL-CELL CARCINOMA, MET		1 (2%)	
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA	1 (2%)	1 (2%)	4 (10%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		1 (2%)
#MANDIBULAR L. NODE	(40)	(42)	(40)
MALIGNANT LYMPHOMA, NOS	1 (3%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0185	HIGH DOSE 06-0190
#PULMONARY LYMPH NODE	(40)	(42)	(40)
TRANSITIONAL-CELL CARCINOMA, MET		1 (2%)	
#PEYERS PATCH	(49)	(44)	(44)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
#KIDNEY	(49)	(45)	(43)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(49)	(46)	(45)
TRANSITIONAL-CELL CARCINOMA, MET		1 (2%)	
HEPATOCELLULAR ADENOMA		3 (7%)	3 (7%)
HEPATOCELLULAR CARCINOMA	2 (4%)	9 (20%)	13 (29%)
SARCOMA, NOS, METASTATIC		1 (2%)	
#STOMACH	(49)	(43)	(42)
SQUAMOUS CELL PAPILLOMA			2 (5%)
URINARY SYSTEM			
#KIDNEY/CORTEX	(49)	(45)	(43)
TUBULAR-CELL ADENOMA		1 (2%)	
#URINARY BLADDER	(50)	(44)	(45)
TRANSITIONAL-CELL CARCINOMA		2 (5%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#UTERUS	(49)	(41)	(43)
HEMANGIOMA		1 (2%)	

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0185	HIGH DOSE 06-0190
#UTERINE SEROSA	(49)	(41)	(43)
HEMANGIOMA		1 (2%)	
#OVARY	(48)	(37)	(41)
GRANULOSA-CELL TUMOR		2 (5%)	1 (2%)
TUBULAR ADENOMA		1 (3%)	3 (7%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(47)	(46)
SQUAMOUS CELL CARCINOMA			1 (2%)
ADENOMA, NOS		2 (4%)	6 (13%)
*ZYMBAL'S GLAND	(50)	(47)	(46)
SQUAMOUS CELL CARCINOMA			2 (4%)
SEBACEOUS ADENOCARCINOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(47)	(46)
HEMANGIOSARCOMA		1 (2%)	1 (2%)
ALL OTHER SYSTEMS			
THIGH			
HEMANGIOSARCOMA		1	

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

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0185	HIGH DOSE 06-0190
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	3	8	12
MORIBUND SACRIFICE	2	6	4
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	40	34	29
ANIMAL MISSING		1	
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	33	31
TOTAL PRIMARY TUMORS	15	50	52
TOTAL ANIMALS WITH BENIGN TUMORS	1	17	16
TOTAL BENIGN TUMORS	1	20	22
TOTAL ANIMALS WITH MALIGNANT TUMORS	11	22	21
TOTAL MALIGNANT TUMORS	14	28	29
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	2	2
TOTAL SECONDARY TUMORS	1	4	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		2	1
TOTAL UNCERTAIN TUMORS		2	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH CUPFERRON

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH CUPFERRON

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0180	HIGH DOSE 01-0185
ANIMALS INITIALLY IN STUDY	50	50	@50
ANIMALS NECROPSIED	50	49	44
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	49	44
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(44)
INFLAMMATION, SUPPURATIVE	1 (2%)		
ULCER, ACUTE			1 (2%)
*SUBCUT TISSUE	(50)	(49)	(44)
EPIDERMAL INCLUSION CYST		1 (2%)	
ABSCESS, NOS		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(49)	(47)	(43)
CONGESTION, CHRONIC PASSIVE	1 (2%)		
INFLAMMATION, INTERSTITIAL	4 (8%)		
FIBROSIS, DIFFUSE	1 (2%)		
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, ADENOMATOUS			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)		
#LUNG/ALVEOLI	(49)	(47)	(43)
HEMORRHAGE	1 (2%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(46)	(43)
CONGESTION, NOS		1 (2%)	
CONGESTION, ACUTE		1 (2%)	
CONGESTION, PASSIVE		4 (9%)	3 (7%)
CONGESTION, ACUTE PASSIVE			1 (2%)
HEMATOMA, NOS		1 (2%)	
FIBROSIS	1 (2%)	2 (4%)	
HEMOSIDEROSIS	2 (4%)	3 (7%)	4 (9%)
METAPLASIA, OSSEOUS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS
@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A FEMALE IN A MALE GROUP.

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0180	HIGH DOSE 01-0185
ERYTHROPOIESIS		1 (2%)	2 (5%)
#MANDIBULAR L. NODE	(49)	(43)	(39)
HYPERPLASIA, PLASMA CELL	1 (2%)	2 (5%)	
#MEDIASTINAL L.NODE	(49)	(43)	(39)
CONGESTION, NOS		2 (5%)	1 (3%)
HEMORRHAGE		2 (5%)	
HEMOSIDEROSIS		12 (28%)	4 (10%)
#PANCREATIC L.NODE	(49)	(43)	(39)
CONGESTION, NOS		1 (2%)	2 (5%)
HEMOSIDEROSIS		4 (9%)	9 (23%)
#MESENTERIC L. NODE	(49)	(43)	(39)
HEMOSIDEROSIS		4 (9%)	5 (13%)
HYPERPLASIA, PLASMA CELL	1 (2%)		
#RENAL LYMPH NODE	(49)	(43)	(39)
HEMOSIDEROSIS			1 (3%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(48)	(47)	(43)
INFLAMMATION, INTERSTITIAL	1 (2%)		
FIBROSIS	2 (4%)		
DEGENERATION, NOS	1 (2%)		
*BLOOD VESSEL	(50)	(49)	(44)
THROMBOSIS, NOS			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(50)	(40)	(40)
HYPERPLASIA, INTRADUCTAL	1 (2%)		
DYSPLASIA, NOS			1 (3%)
#LIVER	(49)	(48)	(43)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
INFLAMMATION, CHRONIC DIFFUSE			1 (2%)
NECROSIS, FOCAL	3 (6%)	1 (2%)	
BASOPHILIC CYTO CHANGE			1 (2%)
CLEAR-CELL CHANGE			1 (2%)
HYPERPLASIA, FOCAL	1 (2%)		

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0180	HIGH DOSE 01-0185
ANGIECTASIS		3 (6%)	
#LIVER/CENTRILOBULAR	(49)	(48)	(43)
CONGESTION, PASSIVE	1 (2%)		
NECROSIS, NOS		2 (4%)	
NECROSIS, DIFFUSE		5 (10%)	10 (23%)
#LIVER/KUPFFER CELL	(49)	(48)	(43)
HEMOSIDEROSIS		15 (31%)	28 (65%)
*BILE DUCT	(50)	(49)	(44)
HYPERPLASIA, NOS	2 (4%)		
#PANCREAS	(47)	(41)	(31)
INFLAMMATION, NOS	2 (4%)		
INFLAMMATION, CHRONIC			2 (6%)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
#STOMACH	(49)	(48)	(38)
HYPERPLASIA, BASAL CELL		26 (54%)	16 (42%)
HYPERKERATOSIS	1 (2%)		
ACANTHOSIS	1 (2%)		
#GASTRIC MUCOSA	(49)	(48)	(38)
EROSION		2 (4%)	
NECROSIS, FOCAL		2 (4%)	
#PEYERS PATCH	(49)	(45)	(37)
HYPERPLASIA, NOS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(50)	(48)	(39)
HYDRONEPHROSIS		1 (2%)	
CONGESTION, NOS	1 (2%)		
GLOMERULONEPHRITIS, NOS	4 (8%)		
PYELONEPHRITIS, ACUTE		1 (2%)	
NEPHROSIS, NOS	35 (70%)	5 (10%)	2 (5%)
HEMOSIDEROSIS		1 (2%)	
#KIDNEY/TUBULE	(50)	(48)	(39)
CALCIFICATION, NOS			1 (3%)
HEMOSIDEROSIS		35 (73%)	38 (97%)
#URINARY BLADDER	(50)	(44)	(38)
INFLAMMATION, ACUTE NECROTIZING		1 (2%)	

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0180	HIGH DOSE 01-0185
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(38)	(31)
CONGESTION, NOS	1 (2%)		
#ADRENAL MEDULLA	(50)	(48)	(36)
HYPERPLASIA, FOCAL			1 (3%)
#THYROID	(37)	(34)	(31)
CYSTIC FOLLICLES	1 (3%)		
HYPERPLASIA, C-CELL	2 (5%)		
REPRODUCTIVE SYSTEM			
#PROSTATE	(48)	(46)	(36)
INFLAMMATION, NOS	3 (6%)		
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, ACUTE NECROTIZING		1 (2%)	
#TESTIS	(50)	(47)	(36)
PERIVASCULITIS	1 (2%)		
CALCIFICATION, NOS	3 (6%)		
CALCIFICATION, FOCAL	1 (2%)		
ATROPHY, NOS	11 (22%)	12 (26%)	3 (8%)
HYPERPLASIA, INTERSTITIAL CELL	4 (8%)		1 (3%)
NERVOUS SYSTEM			
#CEREBRUM	(50)	(44)	(38)
ABSCESS, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(50)	(49)	(44)
FIBROSIS, DIFFUSE	1 (2%)		

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

TABLE C1 (CONCLUDED)

	CONTROL(UNTR) 01-0160	LOW DOSE 01-0180	HIGH DOSE 01-0185
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
NECROSIS, FAT		1	
OMENTUM			
HEMOSIDEROSIS			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			1
AUTO/NECROPSY/HISTO PERF		1	
AUTOLYSIS/NO NECROPSY		1	5

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

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH CUPFERRON

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0180	HIGH DOSE 02-0185
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	45	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	44	44
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(45)	(47)
SEBACEOUS CYST		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(49)	(44)	(41)
THROMBOSIS, NOS		1 (2%)	
CONGESTION, NOS		1 (2%)	
CONGESTION, ACUTE PASSIVE	1 (2%)		
HEMORRHAGE		1 (2%)	
CALCIFICATION, NOS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(47)	(44)	(44)
CONTRACTURE		1 (2%)	
CONGESTION, NOS		1 (2%)	
CONGESTION, PASSIVE		1 (2%)	3 (7%)
HEMOSIDEROSIS	3 (6%)	10 (23%)	3 (7%)
ATROPHY, NOS		3 (7%)	
HEMATOPOIESIS		2 (5%)	1 (2%)
ERYTHROPOIESIS		2 (5%)	
#SPLENIC CAPSULE	(47)	(44)	(44)
HYPERPLASIA, NOS			1 (2%)
#LYMPH NODE	(44)	(38)	(40)
HEMOSIDEROSIS			1 (3%)
#MANDIBULAR L. NODE	(44)	(38)	(40)
HYPERPLASIA, PLASMA CELL			1 (3%)
#MEDIASTINAL L.NODE	(44)	(38)	(40)
HEMORRHAGE		6 (16%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0180	HIGH DOSE 02-0185
HEMOSIDEROSIS		5 (13%)	2 (5%)
HYPERPLASIA, PLASMA CELL		1 (3%)	
#PANCREATIC L.NODE	(44)	(38)	(40)
HEMORRHAGE		1 (3%)	
HEMOSIDEROSIS		6 (16%)	7 (18%)
#MESENTERIC L. NODE	(44)	(38)	(40)
CONGESTION, NOS			1 (3%)
HEMOSIDEROSIS		2 (5%)	7 (18%)
#RENAL LYMPH NODE	(44)	(38)	(40)
HEMOSIDEROSIS		1 (3%)	1 (3%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(48)	(44)	(41)
FIBROSIS	1 (2%)		
CALCIFICATION, NOS		1 (2%)	
CALCIFICATION, FOCAL		1 (2%)	1 (2%)
*AORTA	(49)	(45)	(47)
MEDIAL CALCIFICATION		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(48)	(44)	(44)
HEMORRHAGE		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL	1 (2%)		
METAMORPHOSIS FATTY	1 (2%)	2 (5%)	
BASOPHILIC CYTO CHANGE	2 (4%)		
CLEAR-CELL CHANGE		1 (2%)	2 (5%)
HYPERPLASIA, FOCAL	1 (2%)	1 (2%)	
ANGIECTASIS			2 (5%)
#LIVER/CENTRILOBULAR	(48)	(44)	(44)
NECROSIS, DIFFUSE		3 (7%)	2 (5%)
#LIVER/KUPFFER CELL	(48)	(44)	(44)
HEMOSIDEROSIS		9 (20%)	33 (75%)
*BILE DUCT	(49)	(45)	(47)
HYPERPLASIA, NOS	2 (4%)		

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0180	HIGH DOSE 02-0185
HYPERPLASIA, FOCAL	1 (2%)		
#PANCREAS	(48)	(37)	(38)
HEMORRHAGIC CYST			1 (3%)
INFLAMMATION, CHRONIC		1 (3%)	
#STOMACH	(49)	(43)	(43)
EPIDERMAL INCLUSION CYST		1 (2%)	
INFLAMMATION, NOS	1 (2%)		
HYPERPLASIA, BASAL CELL		17 (40%)	14 (33%)
#GASTRIC MUCOSA	(49)	(43)	(43)
VESICLE			1 (2%)
EROSION			2 (5%)
CALCIFICATION, NOS		2 (5%)	
#GASTRIC SUBMUCOSA	(49)	(43)	(43)
EDEMA, NOS	1 (2%)		
#PEYERS PATCH	(49)	(43)	(40)
HYPERPLASIA, NOS	2 (4%)		
#COLON	(49)	(41)	(40)
PARASITISM	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(48)	(44)	(44)
EMBRYONAL DUCT CYST		1 (2%)	
GLOMERULONEPHRITIS, NOS	4 (8%)		
NEPHROSIS, NOS	29 (60%)	2 (5%)	2 (5%)
NECROSIS, MEDULLARY		1 (2%)	
#KIDNEY/CORTEX	(48)	(44)	(44)
CYST, NOS			2 (5%)
METAMORPHOSIS FATTY	1 (2%)		
#KIDNEY/TUBULE	(48)	(44)	(44)
NEPHROSIS, NOS		1 (2%)	
CALCIFICATION, NOS		1 (2%)	2 (5%)
HEMOSIDEROSIS		42 (95%)	40 (91%)
#KIDNEY/PELVIS	(48)	(44)	(44)
CALCIFICATION, NOS			1 (2%)
#U.BLADDER/SUBMUCOSA	(49)	(40)	(37)
HEMORRHAGE			1 (3%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0180	HIGH DOSE 02-0185
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(49)	(41)	(42)
HEMOSIDEROSIS		1 (2%)	
#THYROID	(45)	(31)	(35)
HYPERPLASIA, C-CELL	2 (4%)	1 (3%)	
#PARATHYROID	(27)	(17)	(23)
HYPERPLASIA, NOS		1 (6%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(45)	(47)
DILATATION/DUCTS	1 (2%)		
*MAMMARY DUCT	(49)	(45)	(47)
HYPERPLASIA, CYSTIC	1 (2%)		
#UTERUS	(46)	(42)	(40)
HYDROMETRA	1 (2%)		
HEMATOMA, NOS	1 (2%)		
POLYP, INFLAMMATORY	1 (2%)		
#UTERUS/ENDOMETRIUM	(46)	(42)	(40)
HYPERPLASIA, CYSTIC		1 (2%)	
#OVARY	(47)	(42)	(40)
INFLAMMATION, CHRONIC	1 (2%)		
NERVOUS SYSTEM			
#BRAIN	(49)	(43)	(42)
PERIVASCULITIS		1 (2%)	
#PONS	(49)	(43)	(42)
GLIOSIS		1 (2%)	
SPECIAL SENSE ORGANS			
*LENS CAPSULE	(49)	(45)	(47)
CALCIFICATION, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE C2 (CONCLUDED)

	CONTROL(UNTR) 02-0160	LOW DOSE 02-0180	HIGH DOSE 02-0185
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2		
ANIMAL MISSING/NO NECROPSY	1		
NECROPSY PERF/NO HISTO PERFORMED			2
AUTO/NECROPSY/NO HISTO		1	1
AUTOLYSIS/NO NECROPSY		5	3

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH CUPFERRON

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH CUPFERRON

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0185	HIGH DOSE 05-0190
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	2
ANIMALS NECROPSIED	50	45	40
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	44	40
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(45)	(40)
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC DIFFUSE			1 (3%)
*SUBCUT TISSUE	(50)	(45)	(40)
HEMORRHAGE		1 (2%)	
HEMATOMA, NOS	1 (2%)		
INFLAMMATION, ACUTE FOCAL	1 (2%)		
ABSCESS, NOS	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(47)	(44)	(40)
ABSCESS, NOS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(49)	(38)	(35)
HYPERPLASIA, HEMATOPOIETIC		2 (5%)	
#SPLEEN	(49)	(44)	(38)
RUPTURE			1 (3%)
HEMORRHAGE			1 (3%)
NECROSIS, FOCAL			1 (3%)
HYPERPLASIA, NOS			1 (3%)
HEMATOPOIESIS		3 (7%)	1 (3%)
ERYTHROPOIESIS	2 (4%)	2 (5%)	
GRANULOPOIESIS		1 (2%)	
#MEDIASTINAL L.NODE	(40)	(38)	(33)
HEMORRHAGE			1 (3%)
#MESENTERIC L. NODE	(40)	(38)	(33)
LYMPHANGIECTASIS			1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0185	HIGH DOSE 05-0190
HYPERPLASIA, LYMPHOID		2 (5%)	
#RENAL LYMPH NODE	(40)	(38)	(33)
HYPERPLASIA, NOS	2 (5%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(47)	(44)	(40)
INFLAMMATION, CHRONIC			1 (3%)
FIBROSIS, FOCAL			1 (3%)
#LIVER	(49)	(44)	(40)
CYST, NOS			2 (5%)
HEMORRHAGE			1 (3%)
HEMATOMA, NOS			1 (3%)
HEMORRHAGIC CYST			1 (3%)
SCAR			1 (3%)
NECROSIS, FOCAL			1 (3%)
NECROSIS, DIFFUSE			1 (3%)
METAMORPHOSIS FATTY	1 (2%)		
ANGIECTASIS	1 (2%)		
#LIVER/KUPFFER CELL	(49)	(44)	(40)
HYPERPLASIA, NOS	1 (2%)		
*BILE DUCT	(50)	(45)	(40)
INFLAMMATION, CHRONIC		1 (2%)	
#PANCREAS	(46)	(38)	(36)
CYSTIC DUCTS	1 (2%)		2 (6%)
INFLAMMATION, ACUTE/CHRONIC			1 (3%)
PERIVASCULITIS	1 (2%)		
NECROSIS, FAT	1 (2%)		
#PANCREATIC ACINUS	(46)	(38)	(36)
ATROPHY, NOS			2 (6%)
#STOMACH	(49)	(42)	(39)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
HYPERPLASIA, BASAL CELL		1 (2%)	

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0185	HIGH DOSE 05-0190
#PEYERS PATCH	(49)	(41)	(37)
INFLAMMATION, ACUTE	1 (2%)		
HYPERPLASIA, LYMPHOID	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(49)	(44)	(40)
HYDRONEPHROSIS	2 (4%)		
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC FOCAL			1 (3%)
INFARCT, NOS			1 (3%)
#KIDNEY/CORTEX	(49)	(44)	(40)
SCAR		1 (2%)	
#KIDNEY/GLOMERULUS	(49)	(44)	(40)
AMYLOIDOSIS		1 (2%)	
#U.BLADDER/SUBMUCOSA	(49)	(43)	(39)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
ENDOCRINE SYSTEM			
#THYROID	(42)	(39)	(33)
HYPERPLASIA, FOCAL	1 (2%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(45)	(40)
DILATATION, NOS	1 (2%)		
#TESTIS	(49)	(44)	(40)
HEMORRHAGE		1 (2%)	
NECROSIS, NOS		1 (2%)	1 (3%)
#TESTIS/TUBULE	(49)	(44)	(40)
NECROSIS, FOCAL	1 (2%)		
NERVOUS SYSTEM			
NONE			

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

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0185	HIGH DOSE 05-0190
SPECIAL SENSE ORGANS			
*EYE	(50)	(45)	(40)
PUS			1 (3%)
*EYE/CORNEA	(50)	(45)	(40)
ULCER, NOS			1 (3%)
MUSCULOSKELETAL SYSTEM			
*POPLITEAL MUSCLE	(50)	(45)	(40)
HEMORRHAGE		1 (2%)	
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(45)	(40)
ADHESION, NOS	1 (2%)		
*MESENTERY	(50)	(45)	(40)
STEATITIS	1 (2%)		
ABSCESS, NOS	1 (2%)		
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
STEATITIS	1		
NECROSIS, FAT	2		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	17	12	8
ANIMAL MISSING/NO NECROPSY		1	2
ACCIDENTAL DEATH		1	
AUTO/NECROPSY/HISTO PERF	1		
AUTO/NECROPSY/NO HISTO	1	1	
AUTOLYSIS/NO NECROPSY		3	8

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

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH CUPFERRON

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0185	HIGH DOSE 06-0190
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	50	47	46
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	46	46
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*BONE MARROW	(49)	(34)	(37)
HYPERPLASIA, HEMATOPOIETIC			1 (3%)
*SPLEEN	(49)	(44)	(42)
THROMBOSIS, NOS			1 (2%)
HEMATOMA, NOS			2 (5%)
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	
HEMATOPOIESIS			1 (2%)
ERYTHROPOIESIS	1 (2%)		1 (2%)
*MANDIBULAR L. NODE	(40)	(42)	(40)
HYPERPLASIA, PLASMA CELL	1 (3%)	1 (2%)	
*MEDIASTINAL L.NODE	(40)	(42)	(40)
HYPERPLASIA, NOS	1 (3%)		
*LUMBAR LYMPH NODE	(40)	(42)	(40)
HYPERPLASIA, NOS	1 (3%)		
*MESENTERIC L. NODE	(40)	(42)	(40)
HYPERPLASIA, NOS			1 (3%)
HYPERPLASIA, LYMPHOID		1 (2%)	
*RENAL LYMPH NODE	(40)	(42)	(40)
HYPERPLASIA, NOS	1 (3%)		

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0185	HIGH DOSE 06-0190
HYPERPLASIA, PLASMA CELL	1 (3%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(50)	(45)	(46)
INFLAMMATION, ACUTE DIFFUSE	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(49)	(46)	(45)
NECROSIS, NOS	1 (2%)	1 (2%)	1 (2%)
NECROSIS, FOCAL	1 (2%)		2 (4%)
INFARCT, NOS	1 (2%)		
MEGALOCYTOSIS		1 (2%)	
HYPERPLASTIC NODULE			1 (2%)
ANGIECTASIS		2 (4%)	
HEMATOPOIESIS			1 (2%)
#LIVER/CAUDATE LOBE	(49)	(46)	(45)
NECROSIS, NOS		1 (2%)	
#LIVER/KUPFFER CELL	(49)	(46)	(45)
HYPERPLASIA, NOS			1 (2%)
*BILE DUCT	(50)	(47)	(46)
INFLAMMATION, CHRONIC FOCAL	2 (4%)		
#STOMACH	(49)	(43)	(42)
INFLAMMATION, ACUTE FOCAL	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
#PEYERS PATCH	(49)	(44)	(44)
HYPERPLASIA, LYMPHOID	1 (2%)		
#COLON	(50)	(43)	(44)
NEMATODIASIS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(49)	(45)	(43)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		
INFLAMMATION, CHRONIC	2 (4%)		
INFLAMMATION, CHRONIC FOCAL	1 (2%)		

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0185	HIGH DOSE 06-0190
GLOMERULOSCLEROSIS, NOS	1 (2%)		1 (2%)
HYPERPLASIA, TUBULAR CELL			1 (2%)
#KIDNEY/TUBULE	(49)	(45)	(43)
CALCIFICATION, NOS		1 (2%)	
#URINARY BLADDER	(50)	(44)	(45)
INFLAMMATION, ACUTE FOCAL			1 (2%)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
#U.BLADDER/SUBMUCOSA	(50)	(44)	(45)
INFLAMMATION, CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC FOCAL	16 (32%)		
PERIVASCULITIS	1 (2%)		
#U.BLADDER/MUSCULARIS	(50)	(44)	(45)
CALCIUM DEPOSIT	1 (2%)		
ENDOCRINE SYSTEM			
#THYROID	(41)	(37)	(31)
HYPERPLASIA, C-CELL	2 (5%)		
HYPERPLASIA, FOLLICULAR-CELL	1 (2%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(49)	(41)	(43)
HYDROMETRA	5 (10%)	1 (2%)	
HEMATOMA, NOS		1 (2%)	
HEMATOMA, ORGANIZED		1 (2%)	
HEMORRHAGIC CYST		1 (2%)	
NECROSIS, FAT	1 (2%)		
CALCIFICATION, NOS	1 (2%)		
#UTERUS/ENDOMETRIUM	(49)	(41)	(43)
INFLAMMATION, SUPPURATIVE	2 (4%)		
HYPERPLASIA, CYSTIC	32 (65%)	10 (24%)	2 (5%)
#OVARY	(48)	(37)	(41)
CYST, NOS	6 (13%)	5 (14%)	5 (12%)
THROMBOSIS, NOS			1 (2%)
HEMATOMA, NOS			1 (2%)
HEMORRHAGIC CYST			1 (2%)
INFLAMMATION, SUPPURATIVE	1 (2%)		

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

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0185	HIGH DOSE 06-0190
INFLAMMATION, CHRONIC	1 (2%)		
NECROSIS, NOS			1 (2%)
HYPERPLASIA, ADENOMATOUS		2 (5%)	4 (10%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(49)	(41)	(45)
INFLAMMATION, NOS		1 (2%)	
#BRAIN	(49)	(41)	(45)
HEMORRHAGE		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(47)	(46)
AMYLOIDOSIS	1 (2%)		
OMENTUM			
PERIVASCULITIS	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	5	10
ANIMAL MISSING/NO NECROPSY		1	
AUTO/NECROPSY/HISTO PERF	2		
AUTO/NECROPSY/NO HISTO		1	
AUTOLYSIS/NO NECROPSY		2	4

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

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

June 29, 1978

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

The reviewer agreed with the conclusion in the report that Cupferron was carcinogenic in rats and mice, under the conditions of test. After a brief description of the experimental design, he said that the hemosiderosis detected in several organs should be further evaluated. He added that the hemosiderosis was not reported in the same target organs for the chronic phase as in the subchronic study. He also commented on the wide temperature variation in the animal rooms, on the fact that mice were housed in a room in which other chemicals were under study, and on the lack of data on the stability and concentration of Cupferron in the diet. The reviewer questioned the basis for selecting the chronic dose levels since it did not correspond with the criteria described in the protocol. He opined that the studies' shortcomings cast doubt on its validity. The reviewer said that the data do not allow an assessment of human risk.

A Program staff member said that the bioassay was too limited to allow an interpretation of the hemosiderosis problem. He noted that it did not appear to interfer with the survival of the treated animals. It was moved that the report on the bioassay of Cupferron be accepted as written. The motion was approved without objection.

Clearinghouse Members present:

Arnold L. Brown (Chairman), Mayo Clinic
Paul Nettesheim, National Institute of Environmental Health Sciences
Verne Ray, Pfizer Medical Research Laboratory
Verald K. Rowe, Dow Chemical U.S.A.
Michael B. Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center

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

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