National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 204
NTP No. 80-9
1980

BIOASSAY OF BENZOIN FOR POSSIBLE CARCINOGENICITY

CAS No. 119-53-9

NCI-CG-TR-204

NTP-80-9

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

BIOASSAY OF

BENZOIN

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 10105
and
National Toxicology Program
Research Triangle Park
Box 12233
North Carolina 27709

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

NIH Publication No. 80-1760
August 1980

BIOASSAY OF
BENZOIN
FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
National Cancer Institute/National Toxicology Program

FOREWORD

This report presents the results of the bioassay of benzoin conducted for the Carcinogenesis Testing Program, National Cancer Institute (NCI) National Toxicology Program (NTP). This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. A negative result, in which the test animals do not have a greater incidence of cancer than control animals, does not necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. A positive result demonstrates that the test chemical is carcinogenic for animals under the conditions of the test and indicates that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from chemicals found to be carcinogenic in animals requires a wider analysis.

CONTRIBUTORS

This bioassay of benzoin was conducted by Hazleton Laboratories America, Inc., Vienna, Virginia, initially under direct contract to NCI and later under a subcontract to Tracor Jitco, Inc., Rockville, Maryland, prime contractor for the NCI Carcinogenesis Testing Program.

The persons responsible for selecting the protocols used in this bioassay were Drs. O. G. Fitzhugh (1,2), J. F. Robens (1,3), M. B. Powers (4,5), and C. Cueto (6,7). The principal investigators were Drs. M. B. Powers (4,5) and R. W. Voelker (4). Ms. K. J. Petrovics (4) was responsible for data management, and Mr. G. Najarian (4,1) was the supervisor of animal care. Histopathologic examinations were performed by Drs. D. A. Banas (4) and R. W. Voelker (4). The pathology report and selected slides were evaluated by the NCI Pathology Working Group as described in Ward et al. (1978).

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (8). Statistical analyses were performed by Dr. J. R. Joiner (1) and Ms. S. Vatsan (1), using methods selected for the bioassay program by Dr. J. J. Gart (9).

Chemicals used in this bioassay were analyzed at Midwest Research Institute (10), and dose solutions containing the test chemical were analyzed at Hazleton Laboratories by Dr. R. P. Stanovick (4) and Mr. E. Missaghi (4). The results of these analyses were reviewed by Dr. S. S. Olin (1).

This report was prepared at Tracor Jitco in collaboration with Hazleton Laboratories and NCI. Those responsible for the report at Tracor Jitco (1) were Dr. L. A. Campbell, Acting Director of the Bioassay Program; Dr. S. S. Olin, Associate Director; Dr. R. L. Schueler, pathologist; Dr. D. J. Beach, reports manager; Dr. A. C. Jacobs, bioscience writer; and Dr. W. D. Theriault and Ms. M. Glasser, technical editors.

The following scientists at NCI (6) were responsible for evaluating the bioassay, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Michael P. Dieter, Dr. J. Fielding Douglas, Dr. Richard A. Griesemer, Dr. Charles K. Grieshaber, Dr. Thomas E. Hamm, Dr. William V. Hartwell, Dr. Y. Jack Lee, Dr. Morton H. Levitt (11), Dr. Harry Mahar, Dr. James McCoy, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Marcelina B. Powers, Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

---

(1) Now with Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland 20852.
(2) 4208 Dresden Street, Kensington, Maryland 20795.
(3) Now with Bureau of Veterinary Medicine, Food and Drug Administration, 5600 Fishers Lane, Rockville, Maryland 20851.
(4) Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike, Vienna, Virginia 22180.
(5) Now with Carcinogenesis Testing Program, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205.
(6) Carcinogenesis Testing Program, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205.
(7) Now with Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland 20795.
(8) EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland 20852.
(9) Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205.
(10) Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110.
(11) Now with Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, 20205.

SUMMARY

A bioassay of benzoin for possible carcinogenicity was conducted by incorporating the test chemical in diets of F344 rats and B6C3F1 mice. Benzoin is used as a photopolymerization catalyst, chemical intermediate, and flavor ingredient.

Groups of 50 male rats were fed diets containing 125 or 250 ppm benzoin for 104 weeks, and similar groups of female rats received feed containing 250 or 500 ppm. Groups of 50 mice of each sex were fed diets containing 2,500 or 5,000 ppm benzoin for 104 weeks. Groups of 50 untreated rats and mice of each sex were used as matched controls. Rats and mice of either sex probably could have tolerated higher doses. An increased incidence of lymphomas or leukemia occurred in dosed male rats, but the observed dose-related trend was not statistically significant.

Mean body weights and clinical signs of low-dose, high-dose, and control male and female rats and male mice were comparable throughout the study. After week 44, mean body weights of dosed female mice were slightly lower (10% or less) than those of the controls.

The incidences of lymphomas that occurred in male mice varied with each dose but were not statistically significant when compared with those of the matched controls.

Lymphomas or leukemias occurred in low-dose female mice at an incidence that was significant when compared with the matched controls. However, because the incidence of lymphomas or leukemias in the high-dose female mice was not significant, the occurrence of these tumors was not clearly related to administration of the test compounds.

Under the conditions of this bioassay, benzoin was not carcinogenic for F344 rats or B6C3F1 mice.

TABLE OF CONTENTS

		Page
I.	Introduction	1
II.	Materials and Methods	3
	A. Chemical	3
	B. Dietary Preparation	3
	C. Animals	4
	D. Animal Maintenance	4
	E. Range-Finding Studies	6
	F. 14-Day Repeated Dose Study	6
	G. Subchronic Studies	9
	H. Chronic Studies	12
	I. Clinical Examinations and Pathology	12
	J. Data Recording and Statistical Analyses	15
III.	Results - Rats	19
	A. Body Weights and Clinical Signs (Rats)	19
	B. Survival (Rats)	19
	C. Pathology (Rats)	22
	D. Statistical Analyses of Tumor Incidences (Rats)	23
IV.	Results - Mice	29
	A. Body Weights and Clinical Signs (Mice)	29
	B. Survival (Mice)	29
	C. Pathology (Mice)	29
	D. Statistical Analyses of Tumor Incidences (Mice)	32
V.	Discussion	39
VI.	Conclusions	41
VII.	Bibliography	43

APPENDIXES

Appendix A	Summary of the Incidence of Neoplasms in Rats Administered Benzoin in the Diet	45
Table A1	Summary of the Incidence of Neoplasms in Male Rats Administered Benzoin in the Diet	47
Table A2	Summary of the Incidence of Neoplasms in Female Rats Administered Benzoin in the Diet	51
Appendix B	Summary of the Incidence of Neoplasms in Mice Administered Benzoin in the Diet	55

		Page
Table B1	Summary of the Incidence of Neoplasms in Male Mice Administered Benzoin in the Diet	57
Table B2	Summary of the Incidence of Neoplasms in Female Mice Administered Benzoin in the Diet ..	61
Appendix C	Summary of the Incidence of Nonneoplastic Lesions in Rats Administered Benzoin in the Diet	65
Table C1	Summary of the Incidence of Nonneoplastic Lesions in Male Rats Administered Benzoin in the Diet	67
Table C2	Summary of the Incidence of Nonneoplastic Lesions in Female Rats Administered Benzoin in the Diet	75
Appendix D	Summary of the Incidence of Nonneoplastic Lesions in Mice Administered Benzoin in the Diet	83
Table D1	Summary of the Incidence of Nonneoplastic Lesions in Male Mice Administered Benzoin in the Diet	85
Table D2	Summary of the Incidence of Nonneoplastic Lesions in Female Mice Administered Benzoin in the Diet	90
Appendix E	Analysis of Benzoin (Lot No. 034) - Midwest Research Institute	95
Appendix F	Analysis of Benzoin (Lot No. 135) - Midwest Research Institute	99
Appendix G	Analysis of Formulated Diets for Stability of Benzoin	105
Appendix H	Analysis of Formulated Diets for Concentrations of Benzoin	109

TABLES

Table 1	Doses, Survival, and Mean Body Weights of Rats Administered Benzoin by Gavage for 14 days	7

Page

Table 2 Doses, Survival, and Mean Body Weights of Mice Administered Benzoin by Gavage for 14 Days 8

Table 3 Doses, Survival, and Mean Body Weights of Rats Fed Benzoin in the Diet for the First 90-Day Study 10

Table 4 Doses, Survival, and Mean Body Weights of Mice Fed Benzoin in the Diet for the 90-Day Study ... 11

Table 5 Doses, Survival, and Mean Body Weights of Rats Fed Benzoin in the Diet in the Second 90-Day Study 13

Table 6 Experimental Design of Chronic Feeding Studies with Benzoin in Rats and Mice 14

Table 7 Analyses of the Incidence of Primary Tumors in Male Rats Administered Benzoin in the Diet 24

Table 8 Analyses of the Incidence of Primary Tumors in Female Rats Administered Benzoin in the Diet ... 27

Table 9 Analyses of the Incidence of Primary Tumors in Male Mice Administered Benzoin in the Diet 33

Table 10 Analyses of the Incidence of Primary Tumors in Female Mice Administered Benzoin in the Diet ... 36

FIGURES

Figure 1 Growth Curves for Rats Administered Benzoin in the Diet 20

Figure 2 Survival Curves for Rats Administered Benzoin in the Diet 21

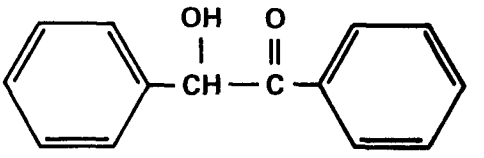
Figure 3 Growth Curves for Mice Administered Benzoin in the Diet 30

Figure 4 Survival Curves for Mice Administered Benzoin in the Diet 31

Figure 5 Infrared Absorption Spectrum of Benzoin 103

Figure 6 Nuclear Magnetic Resonance Spectrum of Benzoin 104

I. INTRODUCTION



BENZOIN

Benzoin (2-hydroxy-1,2-diphenylethanone) (CAS 119-53-9; NCI C50011) is used primarily as a photopolymerization catalyst in polyester resin manufacture. It is also used as a raw material for the production of wetting and emulsifying agents and stilbesterol products (Deinet and DiBella, 1969). Benzoin is approved by the U. S. Food and Drug Administration for use as a synthetic flavor (CFR, 1976). The following products may contain added benzoin: nonalcoholic beverages, 4.5 ppm; ice cream, 0.54 ppm; candy, 2.0 ppm; and baked goods, 0.10 ppm (Furia, 1975). Benzoin is also approved as a diluent in ink for marking fruits and vegetables (CFR, 1974). In 1958, production of benzoin was 27,000 pounds. The amount currently produced is proprietary information, but it exceeds 1,000 pounds (United States International Trade Commission, 1979). No toxicologic data for benzoin have been previously reported (U.S. Food and Drug Administration, 1979).

Benzoin was assigned for testing by the Chemical Carcinogenesis Testing Program because of its use as a flavor ingredient and as a chemical intermediate.

II. MATERIALS AND METHODS

A. Chemical

Benzoin was obtained in two batches from Stauffer Chemical Company (Westport, Connecticut). Lot No. 034 was used for the subchronic studies and Lot No. 135 was used for the chronic studies.

Elemental analysis; melting point; thin-layer chromatography; high-pressure liquid chromatography; and spectral analyses including infrared, ultraviolet, and nuclear magnetic resonance were performed at Midwest Research Institute (Kansas City, Missouri) (Appendixes E and F).

Results of elemental analysis of both batches were in agreement with the theoretical values. The infrared, ultraviolet, visible, and nuclear magnetic resonance spectra were consistent with the structure of benzoin and were identical to the literature spectra (Sadtler Standard Spectra).

The melting point was comparable with literature values (Okuzumi, 1961; Sharfstein, 1954; Kaji and Nagashima, 1956; and Sugihara and Newman, 1954). Two impurities (minor and slight traces) in Lot No. 135 were detected by thin-layer chromatography, and two impurities (equivalent to 1.1% and 0.4% of the benzoin peak area) were also detected by high-pressure liquid chromatography. A trace impurity with a higher r_f than benzoin, a slight trace at the origin, as well as a slight trace impurity with a lower r_f than benzoin were detected by thin-layer chromatography. An approximate 1% impurity in Lot No. 034 was detected by high-pressure chromatography. None of the impurities were identified.

B. Dietary Preparation

Corn oil (Duke's® Corn Oil, C. B. Sauer Co., Richmond, Virginia) was added to the animal stock feed, such that control and test diets contained 2% corn oil by weight. Test diets were prepared by first mixing the chemical with an aliquot of powdered Wayne® Lab Blox animal feed (Allied Mills, Chicago, Illinois) using a mortar and pestle. This pre-blend was

placed in a Patterson-Kelly®V-blender with the remainder of the feed and mixed for 15 minutes. The diets were sealed in labelled air-tight polyethylene buckets and stored at 4°C for no longer than 1 week.

Stability of benzoin in feed was determined at Midwest Research Institute by assaying sample diet mixtures containing 100,000 ppm benzoin that had been stored at -20°, 5°, 25°, and 35°C for 2 weeks.

Amounts of the test chemical present were determined by high-pressure liquid chromatography (Appendix G). The compound was stable in feed for 2 weeks at temperatures as high as 35°C.

Selected batches of the formulated diets administered during the chronic study were analyzed for benzoin. Results are summarized in Appendix H.

C. Animals

F344 rats and B6C3F1 mice were obtained from the NCI Frederick Cancer Research Center (Frederick, Maryland). Upon receipt, the animals were isolated for 2 weeks and examined for the presence of parasites or other diseases. They were then assigned to various groups so that the mean animal weights for each group of the same sex and species were approximately the same. At the beginning of the chronic studies, the rats were approximately 6 weeks old, and the mice were approximately 5 weeks old.

D. Animal Maintenance

The rats and mice were housed in solid-bottom polycarbonate cages (19.0" x 10.5" x 8.0" for rats and 11.5" x 7.25" x 5" for mice) (Lab Products, Inc., Garfield, N. J.) covered with nonwoven, fiber filter bonnets (Lab Products). Initially, the rats were housed five per cage; however, after 52 weeks male rats were housed two to three per cage. Mice were housed five per cage.

All cages, furnished with hardwood chip bedding (Sani-chips®, Shurfire Products Corporation, Beltsville, Md.), were changed twice per week. Wayne®Lab Blox Meal (Allied Mills, Inc., Chicago, Illinois) and untreated well water were provided ad libitum.

Feed hoppers were changed and washed once weekly. Cages, water bottles, and sipper tubes were washed at 81°C twice per week, and cage racks once per month using detergent Acclaim®(Economics Laboratory, St. Paul, Minn.). An industrial dishwasher was used for water bottles and sipper tubes; a cage and rack washer was used for the feed hoppers, cages, and racks.

Animal rooms were maintained at 20° to 24°C, and the relative humidity was 45% to 55%. In a single pass system, incoming air was filtered through 2-inch thick disposable fiberglass filters at a rate that allowed 12 changes of room air per hour. Fluorescent lighting was provided 12 hours per day.

Rats and mice were housed in separate rooms; control animals were housed in the same room as the respective dosed animals. The rats were housed in the same room as other rats on studies of the following chemicals:

Drinking Water Studies

(CAS 108-95-2) phenol

Feed Studies

(CAS 120-61-6) dimethyl terephthalate
(CAS 1346-67-7) titanium oxide

Gavage Studies

(CAS 108-60-1) bis(2-chloro-1-methylethyl)ether (BCPE)
(CAS 7446-34-6) selenium sulfide

Mice were housed in the same room as mice on studies of the following chemicals:

Drinking Water Studies
(CAS 108-95-2) phenol
Feed Studies

(CAS 120-61-6) dimethyl terephthalate
(CAS 1346-67-7) titanium oxide

Gavage Studies

(CAS 108-60-1) bis(2-chloro-1-methylethyl)ether (BCPE)
(CAS 7446-34-6) selenium sulfide

E. Range-Finding Studies

A range-finding study was conducted to determine the doses for the 14-day repeated dose study. The test chemical was diluted in corn oil and administered by gavage at three-fold increments between 31.6 and 10,000 mg/kg to two males and two females of each species. The animals were observed for 7 days and then killed and necropsied.

There was no mortality among the rats. Chemical-related effects consisted of depression, dyspnea, urine stains, ataxia, and unkempt fur in rats receiving the highest dose (10,000 mg/kg of body weight). Greenish-colored kidney cortices were observed at necropsy in rats receiving 10,000 mg/kg. No chemical-related effects were observed in rats receiving 3,160 mg/kg or less.

Mortality was 2/2 males and 1/2 females in mice receiving 10,000 mg/kg. The three mice that died had dark red areas on the liver. Depressed and labored respiration was observed in mice receiving the 10,000 mg/kg dose. No clinical signs were observed in mice receiving lower doses. The LD_{50} estimated for male mice was 5,620 mg/kg and 10,000 mg/kg for females.

F. 14-Day Repeated Dose Study

Fourteen-day repeated dose studies were conducted to determine the doses to be used in the 90-day subchronic studies. Benzoin suspended in 0.5% aqueous sodium carboxymethylcellulose was administered by gavage to groups of five males and five females of each species. Animals were observed daily and individual body weights were recorded at 0, 7, and 14 days. After 14 days, all survivors were killed and necropsied. Doses administered, survival, and mean body weights of the dosed groups are shown in Tables 1 and 2.

Deaths of rats receiving the test chemical were limited to three females receiving the 10,000 mg/kg dose. A dose-associated decrease in weight gain occurred among male rats, with no weight gain in those receiving 10,000 mg/kg. Significant weight gain was not detected in any of the dosed female rats, and those receiving 10,000 mg/kg lost weight. Hunched appearance and labored respiration were observed in both male and female rats receiving the 10,000 mg/kg dose. At necropsy, a solid silvery-white material was present

Table 1. Doses, Survival, and Mean Body Weights of Rats Administered Benzoin by Gavage for 14 Days

Dose (a) (mg/kg)	Survival (b)	Mean Body Weights (grams) Initial	Final	Gain
Male				
100	5/5	173	208	+35
316	5/5	173	202	+29
1,000	5/5	172	200	+28
3,160	5/5	175	200	+25
10,000	5/5	175	175	0
Female				
100	5/5	133	133	0
316	5/5	133	135	+2
1,000	5/5	133	135	+2
3,160	5/5	133	133	0
10,000	2/5	132	123	-9

(a) The benzoin was administered as a suspension in 0.5% aqueous sodium carboxymethylcellulose.
(b) Number surviving/number per group.

Table 2. Doses, Survival, and Mean Body Weights of Mice Administered Benzoin by Gavage for 14 Days

Dose (a) (mg/kg)	Survival (b)	Mean Body Weights (grams) Initial	Final	Gain
215	5/5	22	24	+2
464	5/5	22	23	+1
1,000	5/5	22	24	+2
2,150	5/5	22	25	+3
4,640	5/5	22	24	+2
Female				
215	5/5	20	22	+2
464	5/5	20	22	+2
1,000	5/5	20	22	+2
2,150	5/5	20	22	+2
4,640	5/5	20	22	+2

(a) The benzoin was administered as a suspension in 0.5% aqueous sodium carboxymethylcellulose.
(b) Number surviving/number per group.

in the stomach of rats that had received 3,160 and 10,000 mg/kg. The 14-day repeated-dose LD_{50} for female rats was estimated to be 8,268 mg/kg.

None of the mice died. Both male and female mice receiving the 4,640 mg/kg dose had enlarged lymph nodes. Enlarged spleens were seen in male mice receiving the highest dose.

G. Subchronic Studies

A 90-day subchronic study was conducted to determine the concentrations of benzoin to be used in the 2-year chronic study. Diets containing 0, 500, 1,500, 5,000, 15,000, or 50,000 ppm benzoin were fed to groups of 10 male and 10 female rats for 90 days. Similar groups of male and female mice were fed diets containing 0, 620, 1,250, 2,500, 5,000, or 10,000 ppm benzoin. Animals were observed daily for mortality. Individual animal weights, food consumption, appearance, and behavior were recorded weekly. After 13 weeks, test feed was removed and replaced with control feed for 1 week. After 14 weeks, all surviving animals were killed (following anesthetization by intraperitoneal injections of sodium pentobarbital-Diabutal®, Diamond Laboratories, Inc., Des Moines, Iowa) and necropsied. Representative tissues were examined microscopically as described in the section on chronic studies.

Doses administered, survival, and mean body weights of the dosed and control groups are shown in Tables 3 and 4.

Rats

No deaths occurred in rats at any of the doses tested. A depression in the mean body weight gain of more than 10% was observed in rats receiving the 5,000, 15,000, or 50,000 ppm.

Four male and two female rats that received 50,000 ppm, one male rat that received 15,000 ppm, one female rat that received 5,000 ppm, and one female rat that received 500 ppm benzoin had green-tinged cortices in the kidney. Discoloration of the liver was observed among one to four female rats at each dose level. A dose-related increase in the incidence and

Table 3. Doses, Survival, and Mean Body Weights of Rats Fed Benzoin in the Diet(a) for the First 90-Day Study

Dose (ppm)	Survival(b)	Mean Body Weights (grams) Initial	Final	Gain	Percent Weight Change Relative to Controls(c)
Male					
0	10/10	218	327	109	
500	10/10	218	336	118	+ 8.2
1,500	10/10	219	332	113	+ 3.6
5,000	10/10	219	313	94	-13
15,000	10/10	218	301	83	-23
50,000	10/10	218	310	92	-16
Female					
0	10/10	145	199	54	
500	10/10	145	199	54	0
1,500	10/10	145	197	52	- 4.0
5,000	10/10	144	192	48	-11.0
15,000	10/10	145	190	45	-17
50,000	10/10	145	186	41	-24

(a) Food consumption among all groups of male or female rats was comparable with that of the corresponding controls.
(b) Number surviving/number per group.
(c) Percent weight Change Relative to Controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

Table 4. Doses, Survival, and Mean Body Weights of Mice Fed Benzoin in the Diet(a) for the 90 Day Study

Dose (ppm)	Survival(b)	Mean Body Weights (grams) Initial	Final	Gain	Percent Weight Change Relative to Controls(c)
Male					
0	10/10	20	28	8	
620	10/10	19	27	8	0
1,250	10/10	20	26	6	-25.0
2,500	10/10	20	27	7	-12.5
5,000	10/10	19	28	9	+12.5
10,000	10/10	20	26	6	-25.0
Female					
0	10/10	17	25	8	
620	10/10	17	25	8	0
1,250	10/10	17	26	9	+11.0
2,500	10/10	17	25	8	0
5,000	10/10	17	25	8	0
10,000	10/10	17	25	8	0

(a) Food consumption among all groups of male and female mice was comparable with that of corresponding controls.
(b) Number surviving/number per group.
(c) Percent weight Change Relative to Controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

severity of interstitial nephritis was observed in all treated rats. Scattered vacuolated hepatocytes were present in the liver of all the females that received the 15,000 or 50,000 ppm doses.

A second 90-day subchronic study was conducted in rats at lower doses to determine the dose level at which there would be no compound-related interstitial nephritis. Doses, survival, and mean body weights of the dosed and control groups are shown in Table 5.

Survival was 100% for all groups. An increased incidence of interstitial nephritis characterized by focal areas of regenerative tubule epithelium and lymphocytes was observed in the kidneys of male rats receiving 250 or 500 ppm. The incidence of nephritis in all other dosed groups was comparable with that observed in the controls.

Mice

No evidence of any compound-related effect was detected during the 90-day subchronic study for mice.

As a result of the histopathologic findings of the subchronic studies, doses for the chronic studies were set at 125 and 250 ppm for male rats and at 250 and 500 ppm for female rats. For male and female mice, doses for the chronic studies were set at 2,500 and 5,000 ppm.

H. Chronic Studies

The number of animals in test groups, doses administered, and durations of the chronic studies are shown in Table 6.

I. Clinical Examinations and Pathology

Observations made of the rats and mice were recorded twice daily. Animals were examined for clinical signs and the presence of palpable masses, and findings were recorded weekly. Mean body weights were recorded every 2 weeks for the first 12 weeks, then monthly for the remainder of the study.

Table 5. Doses, Survival, and Mean Body Weights of Rats Fed Benzoin in the Diet(a) in the Second 90-Day Study

Dose (ppm)	Survival(b)	Mean Body Weights (grams) Initial	Final	Gain	Percent Weight Change Relative to Controls(c)
Male					
0	10/10	194	339	145	
30	10/10	193	328	135	- 6.8
60	10/10	193	341	148	+ 2.0
125	10/10	194	333	139	- 4.1
250	10/10	193	333	140	- 3.4
500	10/10	193	329	136	- 6.2
Female					
0	10/10	130	187	57	
30	10/10	129	188	59	+ 3.5
60	10/10	128	192	64	+12.0
125	10/10	128	186	56	- 1.8
250	10/10	130	189	59	+ 3.5
500	10/10	130	185	55	- 3.5

(a) Food consumption among all groups of male and female rats was comparable with that of the corresponding controls.

(b) Number surviving/number per group.

(c) Percent weight Change Relative to Controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

Table 6. Experimental Design of Chronic Feeding Studies with Benzoin in Rats and Mice

Sex, Species, and Test Group	Initial No. of Animals	Benzoin in Diet(a) (ppm)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male Rats				
Matched-Control	50	0	0	104
Low-Dose	50	125	104	0
High-Dose	50	250	104	0
Female Rats				
Matched-Control	50	0	0	104
Low-Dose	50	250	104	0
High-Dose	50	500	104	0
Male Mice				
Matched-Control	50	0	0	104-105
Low-Dose	50	2,500	104	0-1
High-Dose	50	5,000	104	0-1
Female Mice				
Matched-Control	50	0	0	104-105
Low-Dose	50	2,500	104	0-1
High-Dose	50	5,000	104	0-1

(a) Test and control diets were provided *ad libitum*.

Moribund animals and those that survived to the end of the study were killed with intraperitoneal injections of 0.3 to 0.5 ml containing 60 mg/ml of sodium pentobarbital (Diabutal®, Diamond Laboratories, Inc., Des Moines, Iowa) and necropsied.

Gross and microscopic examinations were performed on major tissues, major organs, and all gross lesions from animals killed at the study termination and from animals found dead. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, pancreas, stomach, small intestine, large intestine, gall bladder (mice), kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, and brain. Occasionally, additional tissues were also examined microscopically. Special staining techniques were used as necessary.

Necropsies were also performed on all animals found dead, unless precluded in whole or in part by autolysis or cannibalization. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group.

J. Data Recording and Statistical Analyses

Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's

methods for testing for a dose-related trend. One-tailed P values are reported for all tests except for the departure from linearity test, which is reported only when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When results for two dosed groups are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 is made. The Bonferroni test for inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.025. When 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.

Life table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died or was killed was entered as the time point of tumor observation. The methods of Cox and of Tarone were used for the statistical tests of the groups. The statistical tests were one-tailed.

The Cochran-Armitage test for linear trend in proportions with continuity correction (Armitage, 1971) was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance.

The approximate 95% confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971). The lower and upper limits of the confidence interval of the relative risk have been included in the tables of

statistical analyses. The interpretation of the limits is that, in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result has occurred (P less than 0.025 one-tailed test when the control incidence is not zero, P less than 0.050 when the control incidence is zero). When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

A. Body Weights and Clinical Signs (Rats)

Mean body weights of dosed male and female rats were similar to those of the corresponding control groups (see Figure 1). No benzoin-related clinical signs were observed. Food consumption among all groups of male or female rats was comparable with that of the corresponding controls.

B. Survival (Rats)

Estimates of the probabilities of survival for male and female rats administered benzoin in the diet at the doses of this bioassay, together with those of the matched controls, are shown by the Kaplan and Meier curves in Figure 2. The result of the Tarone test for dose-related trend in the proportions surviving is not significant in either sex.

In male rats, the results of the Cox test comparing the survival between the control group and the high-dose group indicates comparable survival among these groups; however, the results of the Cox test comparing the survival in the low-dose group with the controls and with the high-dose group are significant ($P=0.047$ and $P=0.005$, repectively) due to shortened survival in the low-dose group.

In male rats, 40/50 (80%) of the high-dose group, 25/50 (50%) of the low-dose group, and 36/50 (72%) of the control group lived to the end of the bioassay. In females, 42/50 (84%) of the high-dose group, 37/50 (74%) of the low-dose group, and 40/50 (80%) of the control group lived to the end of the study.

A sufficient number of rats of each sex was at risk for the development of late-appearing tumors.

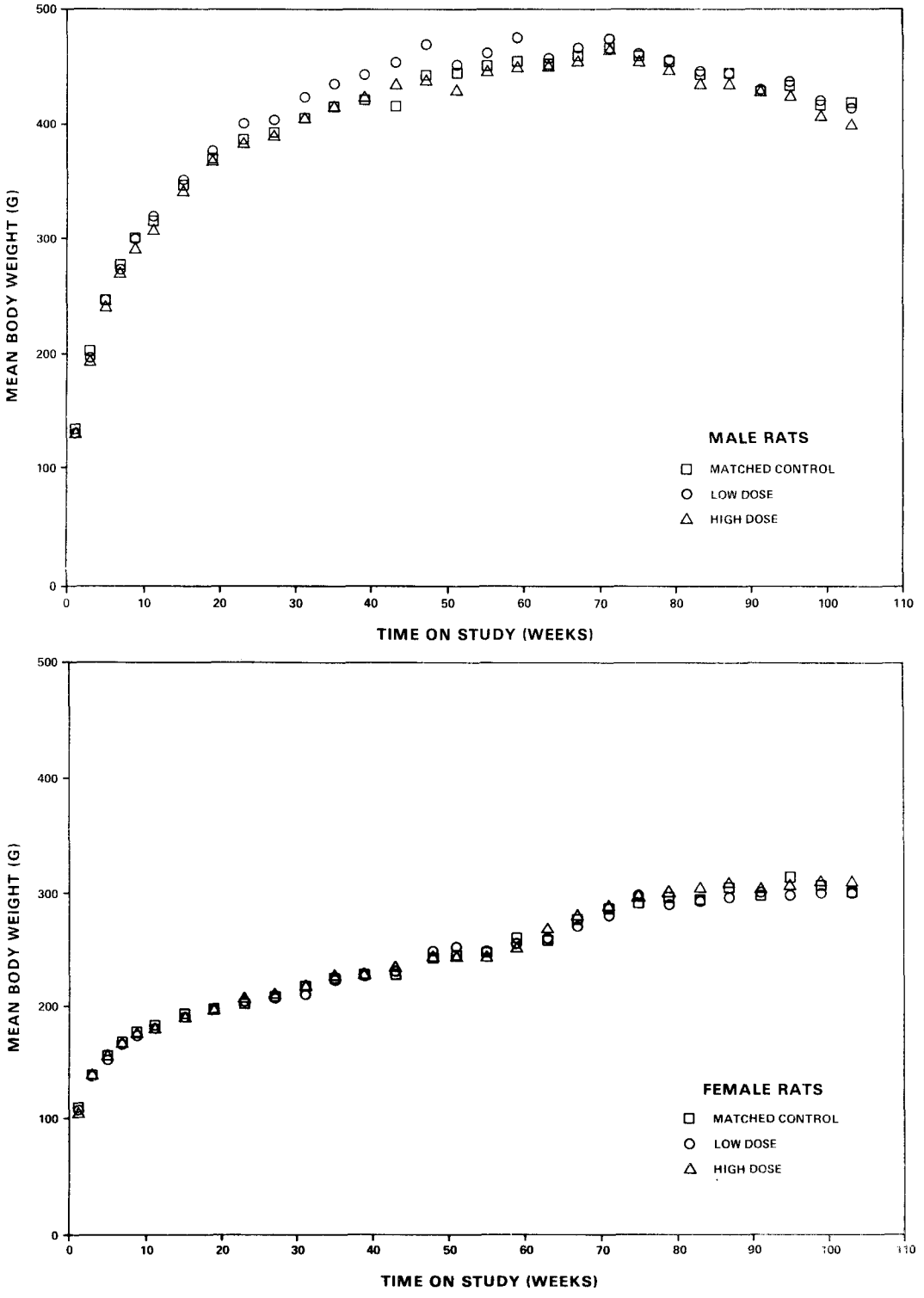


Figure 1. Growth Curves for Rats Administered Benzoin in the Diet

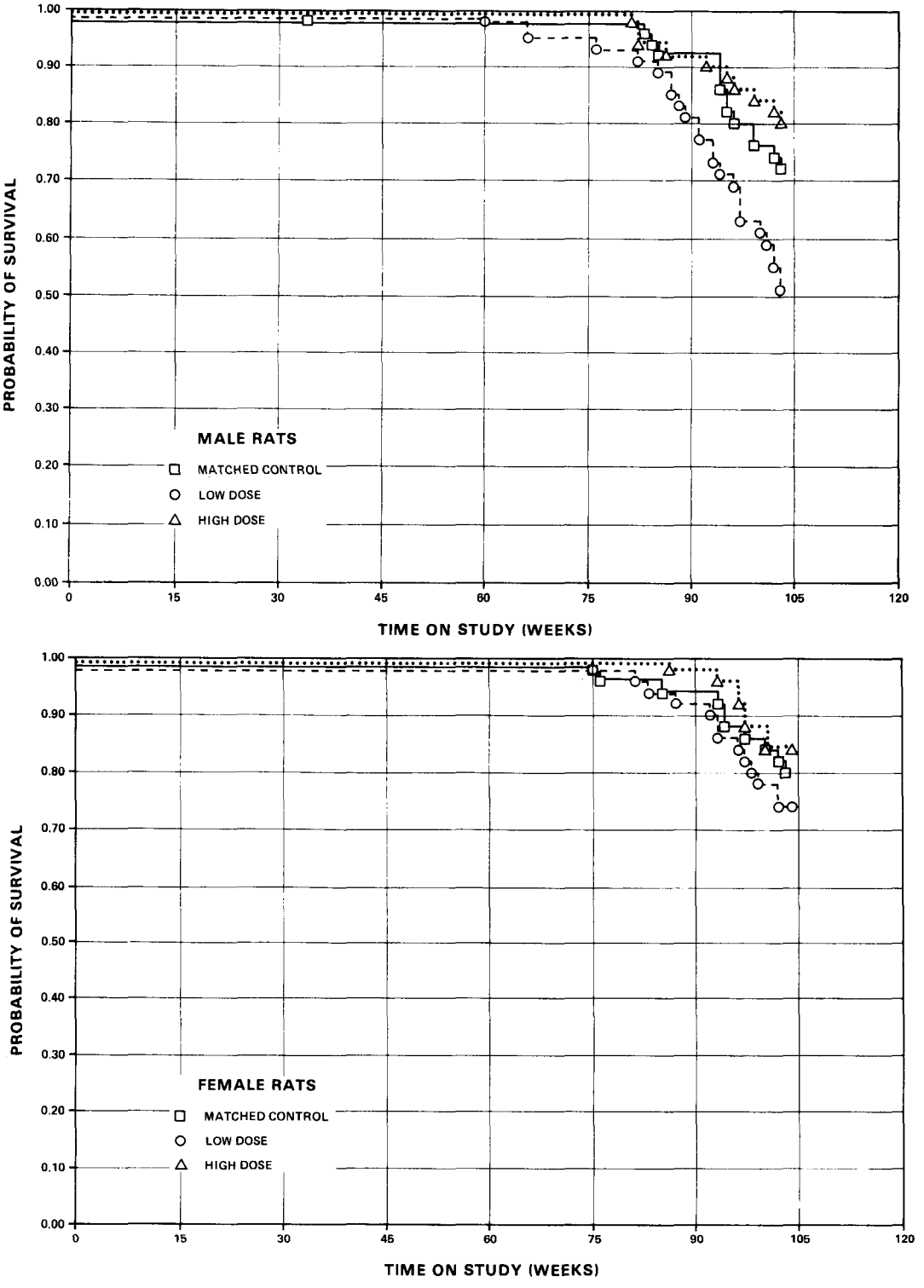


Figure 2. Survival Curves for Rats Administered Benzoin in the Diet

C. Pathology (Rats)

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

A variety of neoplasms were seen in both control and dosed rats. None was thought to be related to chemical administration. However, a dose-related increase in the incidence of adrenal medullary hyperplasia was observed in male rats:4/49 (8%) in matched-controls; 8/49 (16%) in low-dose males; and 19/50 (38%) in high-dose males. These foci were very small collections of medullary cells with basophilic cytoplasm and nuclei smaller than those of normal pheochromocytes. In contrast, pheochromocytomas (controls, 9/49; low-dose, 8/49; and high-dose, 6/50) were composed of large collections, nodules, or masses of cells with vesicular nuclei larger than normal pheochromocytes. The total number of rats with medullary hyperplasias or pheochomocytomas was 13/49 in controls, 16/49 in low-dose males, and 25/50 in high-dose males.

A dose-related increased incidence of chronic nephritis was noted in treated rats of each sex. The chronic inflammation observed in the kidneys was qualitatively similar to that usually observed in aging rats, but the incidence was increased. Chronic inflammation of the kidney was observed in 33/49 male controls, 41/49 low-dose males, and 45/50 high-dose males and in 7/50 control females, 19/49 low-dose females, and 29/50 high-dose females.

Other degenerative, proliferative, and inflammatory lesions observed were of the usual number and kind seen in aging F344 rats, and they occurred with essentially comparable incidences in control and treated rats.

Under the conditions of this bioassay, benzoin was not carcinogenic to F344 rats, but it was associated with an increased incidence of chronic inflammation in the kidneys in male and female rats and hyperplasias of the adrenal medulla in male rats.

D. Statistical Analyses of Tumor Incidences (Rats)

Tables 7 and 8 contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and at an incidence of at least 5% in one or more groups.

The Cochran-Armitage test indicates a significant (P=0.016) dose-related trend in the incidence of neoplastic nodules or hepatocellular carcinomas in the liver, but the results of the Fisher exact test comparing the tumor incidences in the control group with those in each dose group are not significant. A significant trend in the negative direction was observed in the incidence of lung tumors in female rats, but Fisher exact tests between the dosed groups and control groups were not significant. The incidence of male rats with lymphomas or leukemias increases with dose level, but the Cochran-Armitage test of trend does not provide a statistically significant result (P=0.063) nor are the Fisher exact tests significant. There were no significant differences in the times of observation of the lymphomas or leukemias in the male rat groups.

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

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Administered Benzoin in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System:			
Fibroma (b)	8/50 (16)	4/49 (8)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.510	0.500
Lower Limit		0.120	0.117
Upper Limit		1.771	1.737
Weeks to First Observed Tumor	84	87	99
Hematopoietic System:			
Lymphoma or Leukemia (b)	8/50 (16)	12/49 (24)	15/50 (30)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.531	1.875
Lower Limit		0.633	0.825
Upper Limit		3.935	4.631
Weeks to First Observed Tumor	83	82	81
Liver: Neoplastic Nodule or			
Hepatocellular Carcinoma (b)	0/50 (0)	0/48 (0)	4/50 (8)
P Values (c,d)	P=0.016	N.S.	N.S.
Relative Risk (e)		--	Infinite
Lower Limit		--	0.927
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	103
Pituitary: Adenoma, NOS			
or Carcinoma, NOS (b)	2/42 (5)	5/37 (14)	2/43 (5)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		2.838	0.977
Lower Limit		0.497	0.074
Upper Limit		28.308	12.937
Weeks to First Observed Tumor	95	88	103

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Administered Benzoin in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Pheochromocytoma (b)	9/49 (18)	8/49 (16)	6/50 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.889	0.653
Lower Limit		0.325	0.207
Upper Limit		2.378	1.895
Weeks to First Observed Tumor	94	103	103
Thyroid: C-cell Adenoma or Carcinoma (b)	5/47 (11)	2/48 (4)	5/50 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.392	0.940
Lower Limit		0.039	0.231
Upper Limit		2.259	3.832
Weeks to First Observed Tumor	84	102	103
Thyroid: C-cell Carcinoma (b)	2/47 (4)	2/48 (4)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.979	1.410
Lower Limit		0.074	0.169
Upper Limit		13.027	16.282
Weeks to First Observed Tumor	84	102	103
Pancreatic Islets: Islet-cell Carcinoma or Adenoma (b)	4/50 (8)	4/47 (9)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.064	0.250
Lower Limit		0.209	0.005
Upper Limit		5.393	2.411
Weeks to First Observed Tumor	84	101	103

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Administered Benzoin in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Preputial Gland: Carcinoma, NOS (b)	5/50 (10)	5/49 (10)	8/50 (16)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.020	1.600
Lower Limit		0.250	0.497
Upper Limit		4.161	5.808
Weeks to First Observed Tumor	102	87	95
Preputial Gland: Carcinoma, NOS or Adenoma, NOS (b)	7/50 (14)	5/49 (10)	8/50 (16)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.729	1.143
Lower Limit		0.195	0.392
Upper Limit		2.481	3.423
Weeks to First Observed Tumor	102	87	95
All Sites: Mesothelioma (b)	1/50 (2)	4/49 (8)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		4.082	4.000
Lower Limit		0.423	0.415
Upper Limit		196.665	196.805
Weeks to First Observed Tumor	103	102	82

(a) Dosed groups received doses of 125 or 250 ppm.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95% confidence interval of the relative risk between each dosed group and the control group.

Table 8. Analyses of the Incidence of Primary Tumors in Female Rats Administered Benzoin in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma (b)	3/49 (6)	0/49 (0)	0/49 (0)
P Values (c,d)	P = 0.037 (N)	N.S.	N.S.
Relative Risk (e)		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.662	1.662
Weeks to First Observed Tumor	94	--	--
Hematopoietic System: Leukemia (b)	9/50 (18)	9/49 (18)	7/50 (14)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.020	0.778
Lower Limit		0.392	0.267
Upper Limit		2.653	2.159
Weeks to First Observed Tumor	76	93	86
Pituitary: Adenoma, NOS or Carcinoma, NOS (b)	23/45 (51)	16/43 (37)	22/48 (46)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.728	0.897
Lower Limit		0.426	0.568
Upper Limit		1.225	1.424
Weeks to First Observed Tumor	76	93	96
Thyroid: C-cell Carcinoma (b)	1/48 (2)	4/48 (8)	2/48 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		4.000	2.000
Lower Limit		0.416	0.108
Upper Limit		192.630	115.535
Weeks to First Observed Tumor	103	103	104

Table 8. Analyses of the Incidence of Primary Tumors in Female Rats Administered Benzoin in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma or Adenoma (b)	4/48 (8)	6/48 (13)	6/48 (13)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.500	1.500
Lower Limit		0.381	0.381
Upper Limit		6.802	6.802
Weeks to First Observed Tumor	103	103	104
Mammary Gland: Fibroadenoma (b)	9/50 (18)	13/49 (27)	12/50 (24)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.474	1.333
Lower Limit		0.644	0.568
Upper Limit		3.539	3.258
Weeks to First Observed Tumor	94	102	104
Uterus: Endometrial Stromal Polyp (b)	10/48 (21)	10/47 (21)	5/47 (11)
P Values (c,e)	N.S.	N.S.	N.S.
Relative Risk (e)		1.021	0.511
Lower Limit		0.421	0.148
Upper Limit		2.475	1.506
Weeks to First Observed Tumor	103	75	104

(a) Dosed groups received doses of 250 or 500 ppm.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95% confidence interval of the relative risk between each dosed group and the control group.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of dosed males were similar to those of the corresponding control groups (Figure 3). No benzoin-related clinical signs were observed. After week 44, mean body weights of dosed females were lower (10% or less) than the corresponding controls.

B. Survival (Mice)

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

In male mice, 33/50 (66%) of the high-dose group, 34/50 (68%) of the low-dose group, and 38/50 (76%) of the matched-control group lived to the end of the bioassay. In females, 37/50 (74%) of the high-dose group, 42/50 (84%) of the low-dose group, and 39/50 (78%) of the control group lived to the end of the study.

A sufficient number of mice of each sex was at risk for the development of late-appearing tumors.

C. Pathology (Mice)

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

A moderate number of hematopoietic neoplasms and a low incidence of other neoplasms were observed in both control and treated mice. An increased incidence of a variety of neoplasms commonly seen in this strain was observed in some experimental groups.

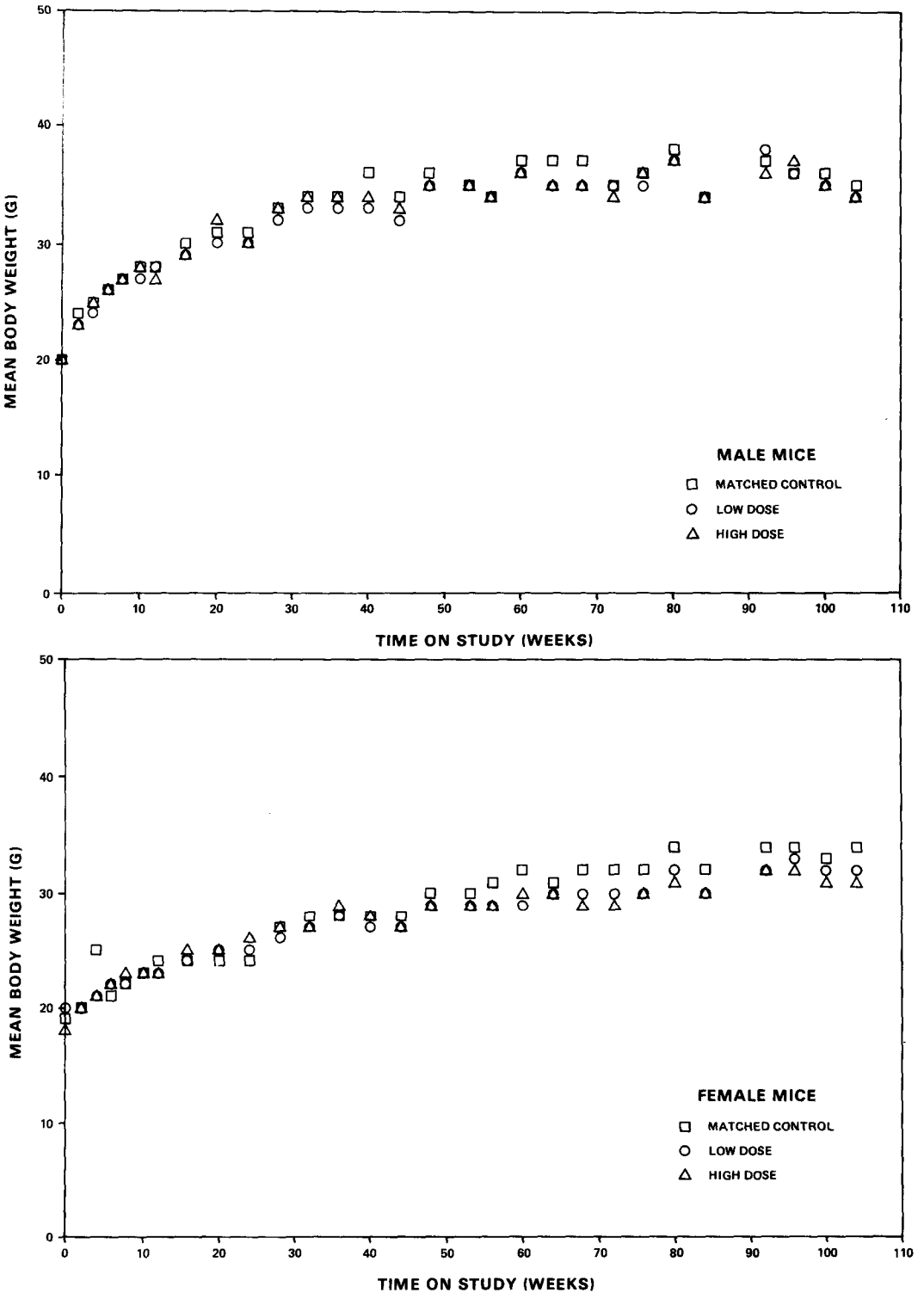


Figure 3. Growth Curves for Mice Administered Benzoin in the Diet

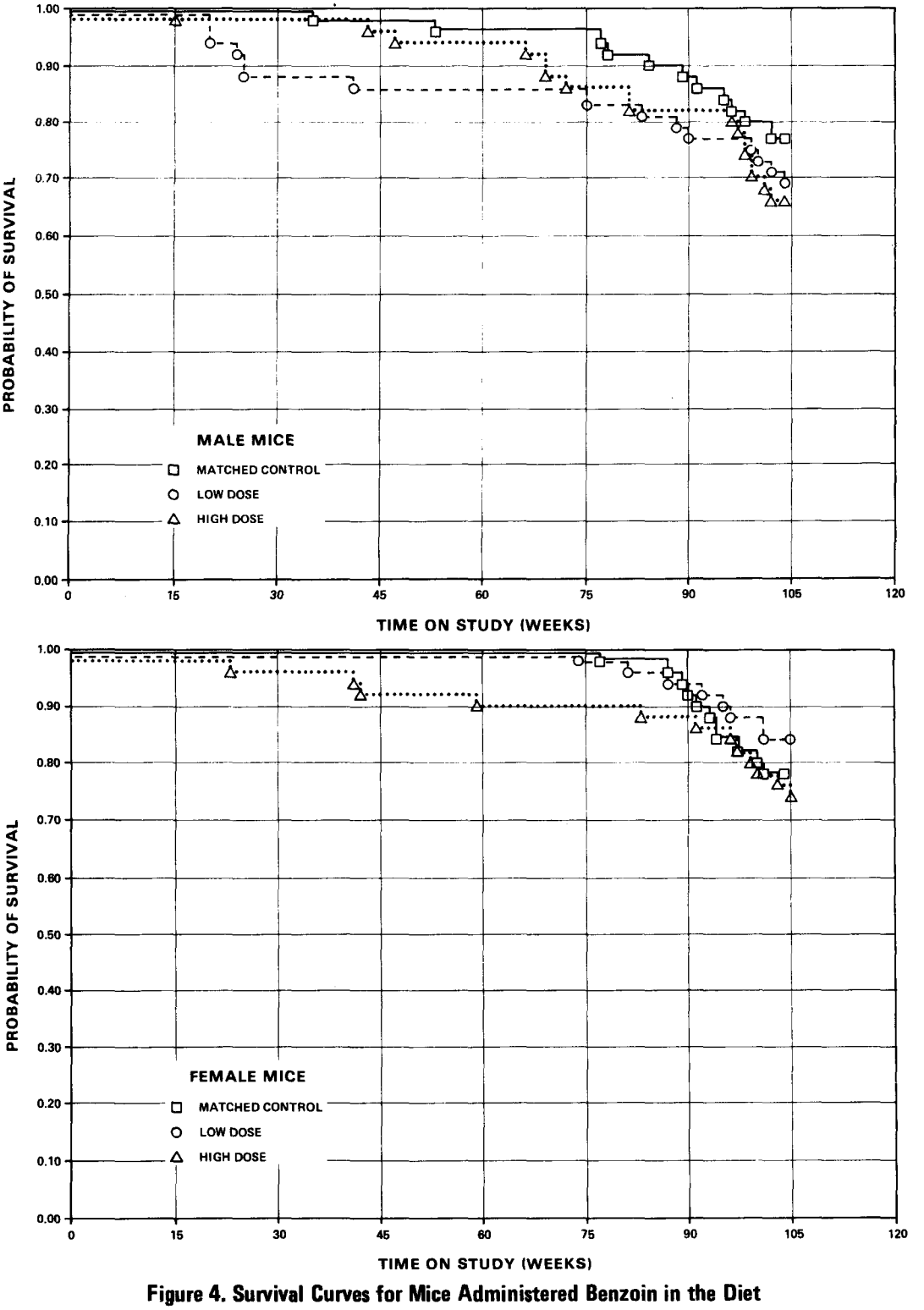


Figure 4. Survival Curves for Mice Administered Benzoin in the Diet

Other degenerative, proliferative, and inflammatory lesions were of the usual number and kind observed in aged B6C3F1 mice and occurred with essentially comparable incidences in control and treated mice.

In conclusion, there was no evidence of carcinogenicity of benzoin in B6C3F1 mice under the conditions of this bioassay.

D. Statistical Analyses of Tumor Incidences (Mice)

Tables 9 and 10 contain the results of statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and at an incidence of at least 5% in one or more groups.

In male mice, the result of the Cochran-Armitage test for positive dose-related trend in the incidence of lymphomas of the hematopoietic system is significant (P=0.041), but the results of the Fisher exact test are not significant. The result of the Cochran-Armitage test on the incidence of female mice with lymphomas or leukemias is not significant. The result of the Fisher exact test shows that the incidence in the low-dose mice is significantly higher (P=0.009) than that in the control group, but the incidence in the high-dose mice is not significant. The incidence to date of control B6C3F1 mice with lymphomas or leukemias across all NCI bioassay laboratories is 10% for the males (368/3,543) and 21% for females (764/3,617). At the laboratory where this study was done, the historical incidences of lesions in control groups were as high as 14% (7/50) in male mice and 31% (15/48) in females, as compared with the 8% in males and 22% in females in the matched control groups in this study.

The association of the administration of this compound with hematopoietic tumors is not clearly established because of the lack of significant results from the Fisher exact test in the high-dose group when compared with controls.

In each of the 95% confidence intervals for relative risk shown in the tables, except for the incidence of hematopoietic tumors in low-dose female mice, one is included: this indicates the absence of significant positive results. It should also be noted that each of the intervals has an upper limit greater than one indicating the theoretical possibility of tumor induction by benzoin, which could not be detected under the conditions of this test.

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System:			
Fibrosarcoma (b)	5/49 (10)	6/50 (12)	2/49 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.176	0.400
Lower Limit		0.320	0.040
Upper Limit		4.565	2.310
Weeks to First Observed Tumor	89	83	99
Integumentary System:			
Fibroma (b)	2/49 (4)	4/50 (8)	2/49 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.960	1.000
Lower Limit		0.296	0.075
Upper Limit		20.886	13.317
Weeks to First Observed Tumor	104	102	104
Lung: Alveolar/Bronchiolar			
Carcinoma, NOS (b)	1/49 (2)	4/50 (8)	3/48 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		3.920	3.063
Lower Limit		0.407	0.257
Upper Limit		188.989	157.336
Weeks to First Observed Tumor	104	104	101
Lung: Alveolar/Bronchiolar			
Carcinoma or Adenoma (b)	5/49 (10)	10/50 (20)	8/48 (17)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.960	1.633
Lower Limit		0.662	0.509
Upper Limit		6.803	5.913
Weeks to First Observed Tumor	95	104	69

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma (b)	4/49 (8)	3/50 (6)	10/49 (20)
P Values (c,d)	P = 0.041	N.S.	N.S.
Relative Risk (e)		0.735	2.500
Lower Limit		0.113	0.780
Upper Limit		4.120	10.230
Weeks to First Observed Tumor	104	24	66
Circulatory System: Hemangiosarcoma (b)	4/49 (8)	0/50 (0)	2/49 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.000	0.500
Lower Limit		0.000	0.047
Upper Limit		1.057	3.315
Weeks to First Observed Tumor	98	--	72
Liver: Hepatocellular Carcinoma (b)	14/49 (29)	10/50 (20)	18/48 (38)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.700	1.313
Lower Limit		0.309	0.700
Upper Limit		1.525	2.502
Weeks to First Observed Tumor	77	99	69
Liver: Hepatocellular Carcinoma or Adenoma (b)	16/49 (33)	12/50 (24)	18/48 (38)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.735	1.148
Lower Limit		0.357	0.631
Upper Limit		1.476	2.102
Weeks to First Observed Tumor	77	99	69

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

(continued)

(a) Dosed groups received doses of 2,500 or 5,000 ppm.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95% confidence interval of the relative risk between each dosed group and the control group.

Table 10. Analyses of the Incidence of Primary Tumors in Female Mice Administered Benzoin in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	6/49 (12)	5/49 (10)	3/49 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.833	0.500
Lower Limit		0.147	0.085
Upper Limit		3.059	2.198
Weeks to First Observed Tumor	90	95	105
Hematopoietic System: Lymphoma or Leukemia (b)	11/49 (22)	23/49 (47)	17/50 (34)
P Values (c,d)	N.S.	P = 0.009	N.S.
Departure from Linear Trend (f)	P = 0.024		
Relative Risk (e)		2.091	1.515
Lower Limit		1.113	0.751
Upper Limit		4.148	3.189
Weeks to First Observed Tumor	94	81	91
Liver: Hepatocellular Carcinoma (b)	2/49 (4)	3/49 (6)	4/49 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		1.500	2.000
Lower Limit		0.180	0.302
Upper Limit		17.316	21.298
Weeks to First Observed Tumor	104	104	97
Pituitary: Adenoma, NOS (b)	2/38 (5)	7/46 (15)	2/34 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		2.891	1.118
Lower Limit		0.594	0.085
Upper Limit		27.277	14.652
Weeks to First Observed Tumor	104	104	105

Table 10. Analyses of the Incidence of Primary Tumors in Female Mice Administered Benzoin in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Adenocarcinoma, NOS or Adenosquamous Carcinoma (b)	1/49 (2)	4/49 (8)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		4.000	3.920
Lower Limit		0.415	0.407
Upper Limit		192.766	188.989
Weeks to First Observed Tumor	91	104	100
Uterus: Endometrial Stromal Polyp (b)	3/49 (6)	2/49 (4)	4/48 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (e)		0.667	1.361
Lower Limit		0.058	0.243
Upper Limit		5.565	8.848
Weeks to First Observed Tumor	93	104	105

(a) Dosed groups received doses of 2,500 or 5,000 ppm.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P less than 0.05, otherwise; not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.
(f) The 95% confidence interval of the relative risk between each dosed group and the control group.

V. DISCUSSION

Dose-related increased incidences of chronic nephritis in male and female rats and adrenal medullary hyperplasia in male rats were the only effects of benzoin detected in the rats. Male rats usually have a higher incidence of chronic nephritis than female rats, and the numerical differences between the low- and high-dose males are not as striking as those in the low- and high-dose females. The incidence of male rats with lymphomas or leukemias increased with increasing dose, but the result of the Cochran-Armitage test was not significant (P=0.063).

Similarity of body weight gains and survival of rats and mice of either sex in the chronic study suggest that they probably could have tolerated higher doses.

In male mice, lymphomas occurred at incidences that may have been compound related (P=0.041), but in a direct comparison with the matched-control group the incidences were not significant. In female mice administered 2,500 ppm benzoin in the feed, lymphomas or leukemias occurred at an incidence that was significantly higher (P=0.009) when compared with the controls; however, in female mice receiving the high dose (5,000 ppm) the incidence of lymphomas or leukemias was not significantly different from that in control mice. Therefore, increased incidences of lymphomas in male B6C3F1 mice and lymphomas and leukemias in female B6C3F1 mice were not clearly related to administration of the test compound.

VI. CONCLUSIONS

Under the conditions of this bioassay, benzoin was not carcinogenic for F344 rats or B6C3F1 mice.

VII. BIBLIOGRAPHY

Armitage, P., Statistical Methods in Medical Research, John Wiley & Sons, Inc., New York, 1971, pp. 362-365.

Berenblum, I., ed., Carcinogenicity Testing: A Report of the Panel on Carcinogenicity of the Cancer Research Commission of UICC, Vol. 2, International Union Against Cancer, Geneva, 1969.

CFR, Synthetic flavoring substances and adjuvants. Code of Federal Regulations 121:1164, 1976.

Cox, D. R., Analysis of Binary Data, Methuen & Co., Ltd., London, 1970, pp. 48-52.

Cox, D. R., Regression models and life tables. J. R. Statist. Soc. B34:187-220, 1972.

Drinet, A. J. and DiBella, E. P., Benzoin. In: Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 17, Interscience Publishers, New York, 1969, pp 388-389.

Furia, E., Fenaroli's Handbook of Flavor Ingredients, 1975, p. 290.

Gart, J. J., The comparison of proportions: a review of significance tests, confidence limits and adjustments for stratification. Rev. Int. Stat. Inst. 39:148-169, 1971.

Kaji, K., and H. Nagashima, J. Pharm. Soc. Japan 76:1247-1250, 1956.

Kaplan E. L. and Meier, P., Nonparametric estimation from incomplete observations. J. Amer. Statist. Assoc. 53:457-481, 1958.

Linhart, M. S., Cooper, J. A., Martin, R. L., Page, N. P., and Peters, J. A., Carcinogenesis bioassay data system. Comp. and Biomed. Res. 7:230-248, 1974.

Miller, R. G., Jr., Simultaneous Statistical Inference, McGraw-Hill Book Co., New York, 1966, pp., 6-10.

Okuzumi, T., Nippon Kagaku Zasshi 82:1235, 1961.

Sadtler Standard Spectra, Sadtler Research Laboratories, Philadelphia, Pa., IR No. 2722, NMR No. 9476 M.

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F., and Kaufman, D. G., Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo(a)pyrene and ferric oxide. Cancer Res. 32:1073-1081, 1972.

Sharfstein, F., Anales fac. farm. y bioquim., Univ. nacl. mayor San Marcos, 5:577-82, 1954.

Sugihara, J. M. and Newman, S. R., J. Org. Chem. 21:1445, 1956.

Tarone, R. E., Tests for trends in life table analysis. Biometrika 62:679-682, 1975.

United States Food and Drug Administration, Bureau of Foods, Personal communication, 1979.

United States International Trade Commission, Synthetic Organic Chemicals - United States Production and Sales, 1978, USITC Publication 1001, U.S. Government Printing Office, Washington, D.C., 1979.

Ward, J. M., Goodman, D. G., Griesemer, R. A., Hardisty, J. F., Schueler, R. L., Squire, R. A., and Strandberg, J. D., Quality assurance for pathology in rodent carcinogenesis tests. J. Environ. Path. Toxicol. 2:371-378, 1978.

Appendix A

Summary of the Incidence of Neoplasms in Rats Administered Benzoin in the Diet

TABLE A1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	50	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(50)
SEBACEOUS ADENOCARCINOMA			1 (2%)
KERATOACANTHOMA	1 (2%)	1 (2%)	1 (2%)
FIBROMA		1 (2%)	
*SUBCUT TISSUE	(50)	(49)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
BASAL-CELL TUMOR		1 (2%)	
SEBACEOUS ADENOMA	1 (2%)		
KERATOACANTHOMA	1 (2%)		1 (2%)
SARCOMA, NOS		1 (2%)	
FIBROMA	8 (16%)	3 (6%)	4 (8%)
FIBROSARCOMA		1 (2%)	
OSTEOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(50)
NEOPLASM, NOS, MALIGNANT	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
SARCOMA, NOS, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
MYELOMONOCYTIC LEUKEMIA	1 (2%)		
MONOCYTIC LEUKEMIA	7 (14%)	11 (22%)	15 (30%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(50)	(48)	(50)
NEOPLASTIC NODULE			3 (6%)
HEPATOCELLULAR CARCINOMA			1 (2%)
#DUODENUM	(50)	(48)	(50)
LEIOMYOSARCOMA			1 (2%)
URINARY SYSTEM			
#KIDNEY	(49)	(49)	(50)
TUBULAR-CELL ADENOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(42)	(37)	(43)
CARCINOMA,NOS	1 (2%)		
ADENOMA, NOS	1 (2%)	5 (14%)	2 (5%)
#ADRENAL	(49)	(49)	(50)
CORTICAL CARCINOMA		1 (2%)	
PHEOCHROMOCYTOMA	9 (18%)	8 (16%)	6 (12%)
#THYROID	(47)	(48)	(50)
FOLLICULAR-CELL ADENOMA	2 (4%)	1 (2%)	
FOLLICULAR-CELL CARCINOMA		1 (2%)	2 (4%)
C-CELL ADENOMA	3 (6%)		2 (4%)
C-CELL CARCINOMA	2 (4%)	2 (4%)	3 (6%)
#PARATHYROID	(35)	(43)	(48)
C-CELL CARCINOMA, INVASIVE	1 (3%)		
#PANCREATIC ISLETS	(50)	(47)	(50)
ISLET-CELL ADENOMA	4 (8%)	3 (6%)	1 (2%)
ISLET-CELL CARCINOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(49)	(50)
FIBROADENOMA		1 (2%)	1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*PREPUTIAL GLAND	(50)	(49)	(50)
CARCINOMA,NOS	5 (10%)	5 (10%)	8 (16%)
ADENOMA, NOS	2 (4%)		
#TESTIS	(49)	(47)	(48)
INTERSTITIAL-CELL TUMOR	46 (94%)	42 (89%)	48 (100%)
NERVOUS SYSTEM			
#BRAIN	(49)	(49)	(50)
CARCINOMA, NOS, INVASIVE	1 (2%)		
EPENDYMOMA	1 (2%)		
ASTROCYTOMA	1 (2%)		1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*TUNICA VAGINALIS	(50)	(49)	(50)
MESOTHELIOMA, NOS	1 (2%)	1 (2%)	2 (4%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(49)	(50)
MESOTHELIOMA, NOS		1 (2%)	
MESOTHELIOMA, MALIGNANT		2 (4%)	2 (4%)
BACK			
FIBROMA	1		
THORACIC CAVITY			
NEOPLASM, NOS, MALIGNANT	1		

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	13	23	10
MORIBUND SACRIFICE	1	1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	25	40
ANIMAL MISSING		1	
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	50	47	49
TOTAL PRIMARY TUMORS	100	96	107
TOTAL ANIMALS WITH BENIGN TUMORS	47	45	48
TOTAL BENIGN TUMORS	79	67	67
TOTAL ANIMALS WITH MALIGNANT TUMORS	18	23	29
TOTAL MALIGNANT TUMORS	20	27	35
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	1	
TOTAL SECONDARY TUMORS	2	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	2	5
TOTAL UNCERTAIN TUMORS	1	2	5
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED BENZOIN IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
KERATOACANTHOMA		1 (2%)	
*SUBCUT TISSUE	(50)	(49)	(50)
CARCINOMA,NOS			1 (2%)
SQUAMOUS CELL CARCINOMA		1 (2%)	
SARCOMA, NOS	2 (4%)		
FIBROMA	2 (4%)		2 (4%)
FIBROSARCOMA	1 (2%)		1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(49)	(49)	(49)
CARCINOMA, NOS, METASTATIC			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (6%)		
SARCOMA, NOS, METASTATIC	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(50)
MYELOMONOCYTIC LEUKEMIA	1 (2%)		
MONOCYTIC LEUKEMIA	8 (16%)	9 (18%)	7 (14%)
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(50)	(49)	(50)
HEMANGIOPERICYTOMA, MALIGNANT		1 (2%)	
DIGESTIVE SYSTEM			
#JEJUNUM	(50)	(49)	(49)
MUCINOUS ADENOCARCINOMA		1 (2%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(50)
MIXED TUMOR, BENIGN		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(43)	(48)
CARCINOMA,NOS	1 (2%)	1 (2%)	1 (2%)
ADENOMA, NOS	22 (49%)	15 (35%)	21 (44%)
#ADRENAL	(48)	(49)	(50)
CORTICAL ADENOMA		2 (4%)	1 (2%)
PHEOCHROMOCYTOMA	2 (4%)		
GANGLIONEUROMA		1 (2%)	
#RIGHT ADRENAL GLAND	(48)	(49)	(50)
PHEOCHROMOCYTOMA		1 (2%)	
#LEFT ADRENAL GLAND	(48)	(49)	(50)
PHEOCHROMOCYTOMA, MALIGNANT		1 (2%)	
#THYROID	(48)	(48)	(48)
C-CELL ADENOMA	3 (6%)	2 (4%)	4 (8%)
C-CELL CARCINOMA	1 (2%)	4 (8%)	2 (4%)
#PANCREATIC ISLETS	(49)	(49)	(49)
ISLET-CELL ADENOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(49)	(50)
ADENOMA, NOS	1 (2%)		
ADENOCARCINOMA, NOS	1 (2%)	1 (2%)	
FIBROADENOMA	9 (18%)	13 (27%)	12 (24%)
*PREPUTIAL GLAND	(50)	(49)	(50)
CARCINOMA,NOS	1 (2%)		
*CLITORAL GLAND	(50)	(49)	(50)
CARCINOMA,NOS			1 (2%)
ADENOMA, NOS		2 (4%)	1 (2%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#UTERUS	(48)	(47)	(47)
ADENOCARCINOMA, NOS	1 (2%)		
ENDOMETRIAL STROMAL POLYP	10 (21%)	10 (21%)	5 (11%)
#OVARY	(46)	(47)	(47)
GRANULOSA-CELL TUMOR		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(50)	(47)	(50)
CARCINOMA, NOS, INVASIVE		1 (2%)	
ASTROCYTOMA			1 (2%)
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(49)	(50)
ADENOMA, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	8	13	8
MORIBUND SACRIFICE	2		
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	40	37	42
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	42	41	39
TOTAL PRIMARY TUMORS	71	69	60
TOTAL ANIMALS WITH BENIGN TUMORS	37	35	30
TOTAL BENIGN TUMORS	54	48	46
TOTAL ANIMALS WITH MALIGNANT TUMORS	17	18	12
TOTAL MALIGNANT TUMORS	17	20	14
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	1
TOTAL SECONDARY TUMORS	1	1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	
TOTAL UNCERTAIN TUMORS		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 B

Summary of the Incidence of Neoplasms in
Mice Administered Benzoin in the Diet

TABLE B1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	48
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(49)
SQUAMOUS CELL PAPILLOMA		1 (2%)	
SQUAMOUS CELL CARCINOMA	1 (2%)		
FIBROMA	1 (2%)	2 (4%)	2 (4%)
FIBROSARCOMA		1 (2%)	
NEUROFIBROSARCOMA		1 (2%)	
*SUBCUT TISSUE	(49)	(50)	(49)
FIBROMA	1 (2%)	2 (4%)	
FIBROSARCOMA	5 (10%)	5 (10%)	2 (4%)
RESPIRATORY SYSTEM			
#LUNG	(49)	(50)	(48)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	4 (8%)	7 (14%)	5 (10%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)	4 (8%)	3 (6%)
FIBROSARCOMA, METASTATIC		2 (4%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(49)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)	2 (4%)	2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	3 (6%)		5 (10%)
#BONE MARROW	(49)	(50)	(47)
FIBROSARCOMA, INVASIVE		1 (2%)	
#SPLEEN	(49)	(50)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#MESENTERIC L. NODE	(48)	(49)	(47)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)
#TESTIS	(49)	(45)	(46)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
#THYMUS	(17)	(18)	(18)
SARCOMA, NOS			1 (6%)
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(49)	(50)	(49)
HEMANGIOSARCOMA	1 (2%)		
#SPLEEN	(49)	(50)	(48)
HEMANGIOSARCOMA	2 (4%)		
#LIVER	(49)	(50)	(48)
HEMANGIOSARCOMA	1 (2%)		2 (4%)
*PREPUTIAL GLAND	(49)	(50)	(49)
HEMANGIOSARCOMA	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(48)
HEPATOCELLULAR ADENOMA	2 (4%)	2 (4%)	1 (2%)
HEPATOCELLULAR CARCINOMA	14 (29%)	10 (20%)	18 (38%)
*INTRAMUSCULAR ANAL G	(49)	(50)	(49)
ADENOMA, NOS		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(47)	(50)	(47)
CORTICAL ADENOMA	1 (2%)		

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
PHEOCHROMOCYTOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
#TESTIS	(49)	(45)	(46)
INTERSTITIAL-CELL TUMOR		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(49)	(50)	(49)
ADENOMA, NOS		1 (2%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
*STERNUM	(49)	(50)	(49)
FIBROSARCOMA, INVASIVE		1 (2%)	
*MUSCLE OF BACK	(49)	(50)	(49)
RHABDOMYOSARCOMA		1 (2%)	
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATHS	11	15	17
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	38	34	33
ANIMAL MISSING	1		
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	31	27	32
TOTAL PRIMARY TUMORS	40	42	45
TOTAL ANIMALS WITH BENIGN TUMORS	10	13	9
TOTAL BENIGN TUMORS	10	17	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	24	20	29
TOTAL MALIGNANT TUMORS	30	25	36
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	2	1
TOTAL SECONDARY TUMORS	1	4	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	49	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(49)	(49)	(50)
FIBROSARCOMA	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(49)	(49)	(49)
ADENOCARCINOMA, NOS, METASTATIC	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	5 (10%)	4 (8%)	3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)	1 (2%)	
ADENOSQUAMOUS CARCINOMA, METASTA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(49)	(50)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (2%)		1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	6 (12%)	16 (33%)	8 (16%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	3 (6%)	4 (8%)	7 (14%)
GRANULOCYTIC LEUKEMIA	1 (2%)	2 (4%)	1 (2%)
#MESENTERIC L. NODE	(49)	(48)	(49)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(49)	(49)	(50)
HEMANGIOSARCOMA		1 (2%)	
#SPLEEN	(48)	(48)	(49)
HEMANGIOSARCOMA			1 (2%)
HEMANGIOSARCOMA, METASTATIC		1 (2%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#OVARY/OVIDUCT	(49)	(49)	(48)
HEMANGIOSARCOMA	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(49)	(49)	(49)
HEPATOCELLULAR CARCINOMA	2 (4%)	3 (6%)	4 (8%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(38)	(46)	(34)
ADENOMA, NOS	2 (5%)	7 (15%)	2 (6%)
#ADRENAL	(49)	(49)	(48)
PHEOCHROMOCYTOMA	1 (2%)		
#THYROID	(48)	(47)	(42)
FOLLICULAR-CELL ADENOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(49)	(50)
ADENOCARCINOMA, NOS	1 (2%)	4 (8%)	2 (4%)
ADENOSQUAMOUS CARCINOMA			2 (4%)
#UTERUS	(49)	(49)	(48)
ENDOMETRIAL STROMAL POLYP	3 (6%)	2 (4%)	4 (8%)
ENDOMETRIAL STROMAL SARCOMA			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(49)	(49)	(50)
PAPILLARY ADENOMA		1 (2%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH∂	11	7	13
MORIBUND SACRIFICE		1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	39	42	37
ANIMAL MISSING			

∂ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	27	35	28
TOTAL PRIMARY TUMORS	29	46	36
TOTAL ANIMALS WITH BENIGN TUMORS	12	12	9
TOTAL BENIGN TUMORS	12	14	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	17	29	26
TOTAL MALIGNANT TUMORS	17	32	27
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	1
TOTAL SECONDARY TUMORS	1	1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

Appendix C

Summary of the Incidence of Nonneoplastic Lesions in Rats Administered Benzoin in the Diet

TABLE C1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	50	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(50)
ULCER, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE		2 (4%)	
FIBROSIS	4 (8%)	1 (2%)	
*SUBCUT TISSUE	(50)	(49)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
ABSCESS, NOS		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC	1 (2%)		
RESPIRATORY SYSTEM			
#TRACHEA	(50)	(49)	(50)
RUPTURE		1 (2%)	
INFLAMMATION, SUPPURATIVE		2 (4%)	
#TRACHEAL SUBMUCOSA	(50)	(49)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
#TRACHEAL GLAND	(50)	(49)	(50)
DISTENTION	1 (2%)		
CYST NOS		1 (2%)	
#LUNG	(50)	(49)	(50)
CONGESTION, NOS	3 (6%)	5 (10%)	
HEMORRHAGE	4 (8%)	1 (2%)	1 (2%)
INFLAMMATION, SUPPURATIVE	1 (2%)		
PNEUMONIA, CHRONIC MURINE	3 (6%)	8 (16%)	5 (10%)
HYPERPLASIA, ADENOMATOUS		1 (2%)	2 (4%)
METAPLASIA, OSSEOUS		1 (2%)	
#ALVEOLAR WALL	(50)	(49)	(50)
EPITHELIALIZATION	2 (4%)	1 (2%)	3 (6%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(50)	(49)	(50)
NECROSIS, FOCAL		1 (2%)	
HYPOPLASIA, NOS	1 (2%)	3 (6%)	1 (2%)
#SPLEEN	(50)	(49)	(50)
CONGESTION, NOS	1 (2%)		
FIBROSIS	1 (2%)	3 (6%)	2 (4%)
FIBROSIS, FOCAL		1 (2%)	
FIBROSIS, MULTIFOCAL	1 (2%)		
INFARCT, NOS		1 (2%)	
PIGMENTATION, NOS		3 (6%)	3 (6%)
ATROPHY, NOS	4 (8%)	4 (8%)	1 (2%)
HEMATOPOIESIS	2 (4%)	2 (4%)	3 (6%)
#CERVICAL LYMPH NODE	(49)	(48)	(50)
HYPERPLASIA, LYMPHOID	1 (2%)	3 (6%)	1 (2%)
#MESENTERIC L. NODE	(49)	(48)	(50)
HISTIOCYTOSIS			1 (2%)
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	
#LUNG	(50)	(49)	(50)
LEUKOCYTOSIS, NOS	1 (2%)	1 (2%)	1 (2%)
#LIVER	(50)	(48)	(50)
HEMATOPOIESIS		1 (2%)	
#ADRENAL	(49)	(49)	(50)
LEUKOCYTOSIS, NOS			1 (2%)
#THYMUS	(27)	(25)	(25)
CYST, NOS	1 (4%)		
CIRCULATORY SYSTEM			
#BRAIN	(49)	(49)	(50)
EMBOLUS, SEPTIC	1 (2%)		
*MEDIASTINUM	(50)	(49)	(50)
PERIARTERITIS			2 (4%)
#LYMPH NODE	(49)	(48)	(50)
LYMPHANGIECTASIS			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#CERVICAL LYMPH NODE	(49)	(48)	(50)
LYMPHANGIECTASIS			4 (8%)
#MESENTERIC L. NODE	(49)	(48)	(50)
LYMPHANGIECTASIS	1 (2%)		1 (2%)
#LUNG	(50)	(49)	(50)
THROMBOSIS, NOS	1 (2%)		1 (2%)
#HEART	(50)	(49)	(50)
MINERALIZATION	1 (2%)		
INFLAMMATION, CHRONIC		2 (4%)	
FIBROSIS	23 (46%)	21 (43%)	34 (68%)
ARTERIOSCLEROSIS, NOS			1 (2%)
FIBROELASTOSIS ENDOCARDIAL	1 (2%)		
NECROSIS, FOCAL		1 (2%)	
CALCIFICATION, NOS			1 (2%)
#HEART/ATRIUM	(50)	(49)	(50)
THROMBOSIS, NOS	1 (2%)	1 (2%)	
#AURICULAR APPENDAGE	(50)	(49)	(50)
THROMBOSIS, NOS	1 (2%)		1 (2%)
#MYOCARDIUM	(50)	(49)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
#CARDIAC VALVE	(50)	(49)	(50)
THROMBOSIS, NOS	1 (2%)		
*AORTA	(50)	(49)	(50)
INFLAMMATION, NOS	1 (2%)		
ARTERIOSCLEROSIS, NOS		2 (4%)	1 (2%)
*CORONARY ARTERY	(50)	(49)	(50)
PERIARTERITIS	2 (4%)	1 (2%)	
#PANCREAS	(50)	(47)	(50)
PERIARTERITIS			3 (6%)
#STOMACH	(50)	(49)	(50)
EMBOLUS, SEPTIC	1 (2%)		
*MESENTERY	(50)	(49)	(50)
PERIARTERITIS	2 (4%)	1 (2%)	3 (6%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ARTERIOSCLEROSIS, NOS			2 (4%)
#KIDNEY	(49)	(49)	(50)
EMBOLUS, SEPTIC	1 (2%)		
#URINARY BLADDER	(48)	(45)	(48)
PERIARTERITIS	1 (2%)		
#PROSTATE	(50)	(45)	(49)
PERIARTERITIS	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(48)	(50)
BILE STASIS	1 (2%)	1 (2%)	1 (2%)
CONGESTION, NOS		1 (2%)	
CHOLANGIOFIBROSIS		3 (6%)	1 (2%)
PELIOSIS HEPATIS		2 (4%)	
NECROSIS, NOS		1 (2%)	
NECROSIS, COAGULATIVE			1 (2%)
INFARCT, NOS	1 (2%)	2 (4%)	
INFARCT, FOCAL			1 (2%)
METAMORPHOSIS FATTY	3 (6%)	1 (2%)	1 (2%)
FOCAL CELLULAR CHANGE	9 (18%)	3 (6%)	4 (8%)
#LIVER/CENTRILOBULAR	(50)	(48)	(50)
NECROSIS, NOS	1 (2%)	6 (13%)	3 (6%)
NECROSIS, COAGULATIVE		1 (2%)	
#LIVER/PERIPORTAL	(50)	(48)	(50)
NECROSIS, NOS	1 (2%)		
#BILE DUCT	(50)	(48)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
FIBROSIS			1 (2%)
HYPERPLASIA, NOS	17 (34%)	7 (15%)	6 (12%)
#PANCREAS	(50)	(47)	(50)
ECTOPIA	1 (2%)		
DILATATION/DUCTS			1 (2%)
EDEMA, INTERSTITIAL	2 (4%)	1 (2%)	1 (2%)
INFLAMMATION, SUPPURATIVE	1 (2%)		
NECROSIS, FOCAL		1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC DUCT	(50)	(47)	(50)
INFLAMMATION PROLIFERATIVE		1 (2%)	
#PANCREATIC ACINUS	(50)	(47)	(50)
DEGENERATION, NOS		1 (2%)	
NECROSIS, NOS		1 (2%)	
ATROPHY, NOS	2 (4%)	2 (4%)	2 (4%)
ATROPHY, FOCAL		7 (15%)	13 (26%)
#STOMACH	(50)	(49)	(50)
ULCER, FOCAL	1 (2%)	3 (6%)	
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
AMYLOIDOSIS			1 (2%)
#GASTRIC MUCOSA	(50)	(49)	(50)
MINERALIZATION	1 (2%)		1 (2%)
HEMORRHAGE		1 (2%)	
CALCIFICATION, NOS			3 (6%)
#GASTRIC SUBMUCOSA	(50)	(49)	(50)
EDEMA, NOS		1 (2%)	
#LARGE INTESTINE	(48)	(48)	(50)
NEMATODIASIS	6 (13%)	4 (8%)	4 (8%)
URINARY SYSTEM			
*GENITOURINARY TRACT	(50)	(49)	(50)
INFLAMMATION, ACUTE HEMORRHAGIC	1 (2%)		
#KIDNEY	(49)	(49)	(50)
MINERALIZATION	1 (2%)	1 (2%)	
CONGESTION, NOS		1 (2%)	
PYELONEPHRITIS SUPPURATIVE			1 (2%)
INFLAMMATION, CHRONIC	33 (67%)	41 (84%)	45 (90%)
DEGENERATION, NOS			1 (2%)
CALCIFICATION, NOS			1 (2%)
PIGMENTATION, NOS	1 (2%)		
#KIDNEY/CORTEX	(49)	(49)	(50)
CYST, NOS			3 (6%)
#RENAL PAPILLA	(49)	(49)	(50)
VESICLE			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#KIDNEY/PELVIS	(49)	(49)	(50)
INFLAMMATION, SUPPURATIVE		1 (2%)	
*URETER	(50)	(49)	(50)
HYPERPLASIA, EPITHELIAL	1 (2%)		
#URINARY BLADDER	(48)	(45)	(48)
INFLAMMATION, SUPPURATIVE		1 (2%)	
HYPERPLASIA, EPITHELIAL	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(42)	(37)	(43)
CYST, NOS	3 (7%)		3 (7%)
HEMORRHAGE		1 (3%)	
HYPERPLASIA, FOCAL	1 (2%)		
HYPERPLASIA, CHROMOPHOBE-CELL	3 (7%)	1 (3%)	
#ADRENAL	(49)	(49)	(50)
NECROSIS, FOCAL		1 (2%)	
ANGIECTASIS			1 (2%)
#ADRENAL CORTEX	(49)	(49)	(50)
DEGENERATION, NOS		3 (6%)	4 (8%)
#ADRENAL MEDULLA	(49)	(49)	(50)
HYPERPLASIA, NOS	3 (6%)	2 (4%)	14 (28%)
HYPERPLASIA, FOCAL	1 (2%)	6 (12%)	5 (10%)
#THYROID	(47)	(48)	(50)
HYPERPLASIA, C-CELL	2 (4%)	4 (8%)	3 (6%)
HYPERPLASIA, FOLLICULAR-CELL			1 (2%)
#PARATHYROID	(35)	(43)	(48)
HYPERPLASIA, NOS	2 (6%)	5 (12%)	6 (13%)
#PANCREATIC ISLETS	(50)	(47)	(50)
HYPERPLASIA, NOS		3 (6%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(49)	(50)
LACTATION	1 (2%)	2 (4%)	1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*PREPUTIAL GLAND	(50)	(49)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)	1 (2%)	2 (4%)
ABSCESS, NOS	1 (2%)		
INFLAMMATION ACTIVE CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
#PROSTATE	(50)	(45)	(49)
INFLAMMATION, SUPPURATIVE	3 (6%)	4 (9%)	1 (2%)
INFLAMMATION, ACUTE		1 (2%)	
ABSCESS, NOS	2 (4%)		1 (2%)
HYPERPLASIA, EPITHELIAL	1 (2%)		
*SEMINAL VESICLE	(50)	(49)	(50)
ABSCESS, NOS	1 (2%)		
ATROPHY, NOS	1 (2%)		
HYPERPLASIA, EPITHELIAL		1 (2%)	
#TESTIS	(49)	(47)	(48)
GRANULOMA, SPERMATIC		1 (2%)	
DEGENERATION, NOS	43 (88%)	39 (83%)	42 (88%)
ATROPHY, NOS		1 (2%)	
HYPERPLASIA, INTERSTITIAL CELL	7 (14%)	13 (28%)	5 (10%)
*EPIDIDYMIS	(50)	(49)	(50)
INFLAMMATION, CHRONIC		3 (6%)	2 (4%)
GRANULOMA, SPERMATIC		1 (2%)	2 (4%)
NERVOUS SYSTEM			
#BRAIN	(49)	(49)	(50)
COMPRESSION	1 (2%)		
HEMORRHAGE	1 (2%)		
MALACIA	1 (2%)		
SPECIAL SENSE ORGANS			
*EYE	(50)	(49)	(50)
PHTHISIS BULBI			1 (2%)
*EYE/CORNEA	(50)	(49)	(50)
INFLAMMATION, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(49)	(50)
NECROSIS, FAT	5 (10%)	3 (6%)	2 (4%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(49)	(50)
MINERALIZATION		1 (2%)	
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY		1	

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

TABLE C2.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(50)
ULCER, NOS	2 (4%)	1 (2%)	
INFLAMMATION, SUPPURATIVE	1 (2%)		
INFLAMMATION, NECROTIZING	1 (2%)		
INFLAMMATION, ACUTE		1 (2%)	
*SUBCUT TISSUE	(50)	(49)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
RESPIRATORY SYSTEM			
#TRACHEA	(50)	(49)	(49)
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, SUPPURATIVE			1 (2%)
#LUNG	(49)	(49)	(49)
CONGESTION, NOS	2 (4%)		1 (2%)
HEMORRHAGE	4 (8%)		2 (4%)
INFLAMMATION, ACUTE FIBRINOUS		1 (2%)	
PNEUMONIA, CHRONIC MURINE	9 (18%)	2 (4%)	2 (4%)
HYPERPLASIA, ADENOMATOUS			6 (12%)
#ALVEOLAR WALL	(49)	(49)	(49)
EPITHELIALIZATION		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(49)	(49)	(50)
HYPOPLASIA, NOS	1 (2%)	1 (2%)	3 (6%)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
#SPLEEN	(49)	(49)	(49)
PIGMENTATION, NOS	7 (14%)	7 (14%)	12 (24%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ATROPHY, NOS	2 (4%)		2 (4%)
HEMATOPOIESIS	1 (2%)	2 (4%)	
#SPLENIC CAPSULE	(49)	(49)	(49)
INFLAMMATION WITH FIBROSIS		1 (2%)	
#CERVICAL LYMPH NODE	(50)	(48)	(49)
PIGMENTATION, NOS	1 (2%)		
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	
#BRONCHIAL LYMPH NODE	(50)	(48)	(49)
HYPERPLASIA, LYMPHOID		1 (2%)	
#MESENTERIC L. NODE	(50)	(48)	(49)
HISTIOCYTOSIS	1 (2%)		
HYPERPLASIA, LYMPHOID	1 (2%)		
#LUNG	(49)	(49)	(49)
LEUKOCYTOSIS, NOS		1 (2%)	
#LIVER	(50)	(49)	(50)
HEMATOPOIESIS		1 (2%)	
#PEYER'S PATCH	(50)	(49)	(49)
HYPERPLASIA, LYMPHOID	1 (2%)		
CIRCULATORY SYSTEM			
*MEDIASTINUM	(50)	(49)	(50)
PERIARTERITIS	1 (2%)		
ARTERIOSCLEROSIS, NOS		1 (2%)	
#LUNG	(49)	(49)	(49)
THROMBOSIS, NOS		1 (2%)	
#HEART	(49)	(49)	(49)
THROMBOSIS, NOS	1 (2%)		
ENDOCARDITIS, BACTERIAL	1 (2%)		
INFLAMMATION, FOCAL		1 (2%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, CHRONIC		3 (6%)	2 (4%)
FIBROSIS	11 (22%)	17 (35%)	8 (16%)
CALCIFICATION, FOCAL		1 (2%)	
#AURICULAR APPENDAGE	(49)	(49)	(49)
THROMBOSIS, NOS		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#MYOCARDIUM	(49)	(49)	(49)
INFLAMMATION, FOCAL	1 (2%)		
*AORTA	(50)	(49)	(50)
INFLAMMATION, NOS	2 (4%)		
*CORONARY ARTERY	(50)	(49)	(50)
PERIARTERITIS		1 (2%)	
#KIDNEY	(50)	(49)	(50)
EMBOLUS, SEPTIC		1 (2%)	
ARTERIOSCLEROSIS, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(48)	(48)	(48)
DILATATION/DUCTS	1 (2%)		
INFLAMMATION, SUPPURATIVE			1 (2%)
#LIVER	(50)	(49)	(50)
BILE STASIS		1 (2%)	1 (2%)
CONGESTION, NOS	1 (2%)		
CONGESTION, PASSIVE		1 (2%)	
INFARCT, NOS	1 (2%)		
METAMORPHOSIS FATTY	2 (4%)	5 (10%)	2 (4%)
FOCAL CELLULAR CHANGE	15 (30%)	23 (47%)	17 (34%)
ANGIECTASIS	2 (4%)		
NODULAR REGENERATION	1 (2%)		
#LIVER/CENTRILOBULAR	(50)	(49)	(50)
DEGENERATION, NOS		1 (2%)	
NECROSIS, NOS	3 (6%)	1 (2%)	1 (2%)
#BILE DUCT	(50)	(49)	(50)
CYST, NOS			1 (2%)
HYPERPLASIA, NOS	5 (10%)	12 (24%)	2 (4%)
#PANCREAS	(49)	(49)	(49)
EDEMA, INTERSTITIAL		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
ATROPHY, FOCAL	1 (2%)		
#PANCREATIC DUCT	(49)	(49)	(49)
INFLAMMATION PROLIFERATIVE		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC ACINUS	(49)	(49)	(49)
NECROSIS, DIFFUSE			1 (2%)
ATROPHY, NOS	1 (2%)	3 (6%)	
ATROPHY, FOCAL	5 (10%)	1 (2%)	6 (12%)
ATROPHY, DIFFUSE		1 (2%)	
HYPERTROPHY, FOCAL	1 (2%)		
#ESOPHAGUS	(48)	(48)	(49)
INFLAMMATION, SUPPURATIVE			1 (2%)
#STOMACH	(50)	(49)	(50)
INFLAMMATION, NOS			1 (2%)
ULCER, NOS	1 (2%)	1 (2%)	
ULCER, FOCAL		1 (2%)	
INFLAMMATION, ACUTE	1 (2%)		
EROSION	1 (2%)	1 (2%)	
HYPERKERATOSIS	1 (2%)	1 (2%)	1 (2%)
ACANTHOSIS	1 (2%)	1 (2%)	1 (2%)
#GASTRIC MUCOSA	(50)	(49)	(50)
MINERALIZATION		1 (2%)	
CALCIFICATION, NOS		1 (2%)	
#GASTRIC SUBMUCOSA	(50)	(49)	(50)
EDEMA, NOS		2 (4%)	
#LARGE INTESTINE	(49)	(49)	(50)
NEMATODIASIS	6 (12%)	2 (4%)	
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(50)
MINERALIZATION	2 (4%)	4 (8%)	4 (8%)
CONGESTION, NOS	1 (2%)		
INFLAMMATION, CHRONIC	7 (14%)	19 (39%)	29 (58%)
NECROSIS, MEDULLARY		1 (2%)	
LIPOIDOSIS		1 (2%)	
CALCIFICATION, FOCAL		1 (2%)	
#KIDNEY/CORTEX	(50)	(49)	(50)
CYST, NOS	1 (2%)		
#KIDNEY/TUBULE	(50)	(49)	(50)
PIGMENTATION, NOS	1 (2%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#URINARY BLADDER	(42)	(43)	(42)
CRYSTALS, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(43)	(48)
CYST, NOS	5 (11%)	9 (21%)	7 (15%)
MULTIPLE CYSTS	1 (2%)		
HEMORRHAGE	1 (2%)		
HYPERPLASIA, CHROMOPHOBE-CELL	7 (16%)	7 (16%)	3 (6%)
ANGIECTASIS	1 (2%)		4 (8%)
#ADRENAL	(48)	(49)	(50)
NECROSIS, CORTICAL	1 (2%)		
ANGIECTASIS			2 (4%)
#ADRENAL CORTEX	(48)	(49)	(50)
DEGENERATION, NOS	13 (27%)	5 (10%)	8 (16%)
LIPOIDOSIS			1 (2%)
#ADRENAL MEDULLA	(48)	(49)	(50)
HYPERPLASIA, NOS		1 (2%)	
#THYROID	(48)	(48)	(48)
FIBROSIS	1 (2%)		
HYPERPLASIA, FOCAL			1 (2%)
HYPERPLASIA, C-CELL	8 (17%)	3 (6%)	3 (6%)
#PANCREATIC ISLETS	(49)	(49)	(49)
HYPERPLASIA, NOS	2 (4%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(49)	(50)
DILATATION/DUCTS	1 (2%)		
GALACTOCELE	14 (28%)	7 (14%)	7 (14%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
HYPERPLASIA, NOS	1 (2%)		
LACTATION	24 (48%)	28 (57%)	34 (68%)
*PREPUTIAL GLAND	(50)	(49)	(50)
CYST, NOS	1 (2%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, SUPPURATIVE	1 (2%)		
HYPERPLASIA, NOS	1 (2%)		
#UTERUS	(48)	(47)	(47)
HYDROMETRA	3 (6%)	6 (13%)	5 (11%)
HEMORRHAGIC CYST		2 (4%)	
HEMATOMETRA	1 (2%)		
#UTERUS/ENDOMETRIUM	(48)	(47)	(47)
INFLAMMATION, SUPPURATIVE	1 (2%)		
INFARCT, NOS	1 (2%)	1 (2%)	
HYPERPLASIA, CYSTIC	1 (2%)		3 (6%)
#ENDOMETRIAL GLAND	(48)	(47)	(47)
CYST, NOS	2 (4%)	2 (4%)	2 (4%)
#OVARY	(46)	(47)	(47)
PAROVARIAN CYST		3 (6%)	1 (2%)
HEMORRHAGIC CYST		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(50)	(47)	(50)
COMPRESSION	3 (6%)	1 (2%)	4 (8%)
HYDROCEPHALUS, NOS		1 (2%)	
HYDROCEPHALUS, INTERNAL		2 (4%)	6 (12%)
SPECIAL SENSE ORGANS			
*EYE	(50)	(49)	(50)
PUS	1 (2%)		
SYNECHIA, ANTERIOR	1 (2%)		1 (2%)
SYNECHIA, POSTERIOR			1 (2%)
CATARACT			2 (4%)
*EYE/CORNEA	(50)	(49)	(50)
ULCER, NOS	1 (2%)		
INFLAMMATION, SUPPURATIVE	1 (2%)		
*EYEBALL TUNICA VASCU	(50)	(49)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
*EYE/RETINA	(50)	(49)	(50)
ATROPHY, NOS			2 (4%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*TARSAL GLAND	(50)	(49)	(50)
CYST, NOS	1 (2%)		
*HARDERIAN GLAND	(50)	(49)	(50)
INFLAMMATION, CHRONIC	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*STERNUM	(50)	(49)	(50)
ECTOPIA			1 (2%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(49)	(50)
NECROSIS, FAT	4 (8%)	2 (4%)	2 (4%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY/HISTO PERF	1		1
AUTOLYSIS/NO NECROPSY		1	

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

Appendix D

Summary of the Incidence of Nonneoplastic Lesions
in Mice Administered Benzoin in the Diet

TABLE D1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	48
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(49)
EPIDERMAL INCLUSION CYST		3 (6%)	
EDEMA, NOS	1 (2%)		
INFLAMMATION, NOS	1 (2%)		
ULCER, NOS			1 (2%)
INFLAMMATION, FOCAL		1 (2%)	
ULCER, FOCAL	1 (2%)		
INFLAMMATION, CHRONIC		7 (14%)	1 (2%)
ABSCESS, CHRONIC	1 (2%)		
FIBROSIS	1 (2%)		1 (2%)
FIBROSIS, FOCAL		1 (2%)	
HYPERPLASIA, EPITHELIAL		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(49)	(50)	(48)
INFLAMMATION, CHRONIC		1 (2%)	
#LUNG	(49)	(50)	(48)
CONGESTION, NOS	1 (2%)		1 (2%)
HEMORRHAGE	1 (2%)		
INFLAMMATION, FOCAL		1 (2%)	
INFLAMMATION, DIFFUSE			1 (2%)
PNEUMONIA, CHRONIC MURINE	7 (14%)	10 (20%)	20 (42%)
HYPERPLASIA, ADENOMATOUS	2 (4%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(49)	(50)	(47)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
#SPLEEN	(49)	(50)	(48)
ATROPHY, NOS	1 (2%)	3 (6%)	2 (4%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, LYMPHOID	1 (2%)		
HEMATOPOIESIS	2 (4%)	6 (12%)	1 (2%)
#MESENTERIC L. NODE	(48)	(49)	(47)
CONGESTION, NOS	4 (8%)	8 (16%)	4 (9%)
INFLAMMATION, NOS		1 (2%)	
MEGAKARYOCYTOSIS	1 (2%)		
HYPERPLASIA, LYMPHOID	12 (25%)	5 (10%)	8 (17%)
#LUNG	(49)	(50)	(48)
LEUKOCYTOSIS, NOS	1 (2%)	1 (2%)	
#PEYER'S PATCH	(49)	(50)	(48)
HYPERPLASIA, LYMPHOID	1 (2%)		2 (4%)
CIRCULATORY SYSTEM			
#MESENTERIC L. NODE	(48)	(49)	(47)
LYMPHANGIECTASIS		1 (2%)	1 (2%)
THROMBOSIS, NOS			1 (2%)
#HEART	(49)	(50)	(48)
MINERALIZATION	1 (2%)		
#AURICULAR APPENDAGE	(49)	(50)	(48)
THROMBOSIS, NOS		1 (2%)	
#LIVER	(49)	(50)	(48)
THROMBOSIS, NOS	3 (6%)	1 (2%)	2 (4%)
#PANCREAS	(49)	(50)	(47)
PERIARTERITIS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(48)
CONGESTION, NOS			1 (2%)
HEMORRHAGE			1 (2%)
INFLAMMATION, CHRONIC	1 (2%)		
FIBROSIS	1 (2%)		
NECROSIS, NOS	3 (6%)	2 (4%)	5 (10%)
NECROSIS, FOCAL	1 (2%)	1 (2%)	1 (2%)
INFARCT, NOS	3 (6%)	1 (2%)	1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
METAMORPHOSIS FATTY	1 (2%)		
FOCAL CELLULAR CHANGE			1 (2%)
ANGIECTASIS	1 (2%)		
#HEPATIC LOBULE	(49)	(50)	(48)
NECROSIS, NOS	1 (2%)		
#LIVER/CENTRILOBULAR	(49)	(50)	(48)
NECROSIS, NOS		2 (4%)	
#BILE DUCT	(49)	(50)	(48)
CYST, NOS	2 (4%)		
#PANCREAS	(49)	(50)	(47)
DILATATION/DUCTS			1 (2%)
CYSTIC DUCTS		1 (2%)	1 (2%)
EDEMA, INTERSTITIAL		2 (4%)	1 (2%)
#PANCREATIC ACINUS	(49)	(50)	(47)
ATROPHY, NOS			1 (2%)
#SMALL INTESTINE	(49)	(50)	(48)
INFLAMMATION, SUPPURATIVE	1 (2%)		
DIVERTICULITIS PERFORATED	1 (2%)		
#LARGE INTESTINE	(49)	(49)	(48)
NEMATODIASIS			2 (4%)
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(48)
HYDRONEPHROSIS	1 (2%)		
PYELONEPHRITIS, NOS	1 (2%)		
INFLAMMATION, SUPPURATIVE		1 (2%)	
PYELONEPHRITIS SUPPURATIVE		2 (4%)	
INFLAMMATION, CHRONIC	5 (10%)	5 (10%)	2 (4%)
#KIDNEY/PELVIS	(49)	(50)	(48)
INFLAMMATION, NOS		1 (2%)	1 (2%)
#URINARY BLADDER	(49)	(48)	(48)
EDEMA, NOS		2 (4%)	
HEMORRHAGE		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)	3 (6%)	1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, EPITHELIAL	1 (2%)	1 (2%)	
#U.BLADDER/SUBMUCOSA	(49)	(48)	(48)
EDEMA, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(33)	(42)	(39)
CYST, NOS			1 (3%)
#ADRENAL MEDULLA	(47)	(50)	(47)
HYPERPLASIA, NOS	3 (6%)		
HYPERPLASIA, FOCAL	1 (2%)		
#PANCREATIC ISLETS	(49)	(50)	(47)
HYPERPLASIA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
*PENIS	(49)	(50)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
#PROSTATE	(49)	(49)	(47)
INFLAMMATION, SUPPURATIVE		1 (2%)	
*SEMINAL VESICLE	(49)	(50)	(49)
DISTENTION			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
FIBROSIS			1 (2%)
#TESTIS	(49)	(45)	(46)
DEGENERATION, NOS	1 (2%)		
ATROPHY, NOS		4 (9%)	2 (4%)
*EPIDIDYMIS	(49)	(50)	(49)
HEMORRHAGE	1 (2%)		
INFLAMMATION, NOS		1 (2%)	
NECROSIS, FAT		1 (2%)	
NERVOUS SYSTEM			
NONE			

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(49)	(50)	(49)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(49)	(50)	(49)
NECROSIS, FAT	2 (4%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	2	4
ANIMAL MISSING/NO NECROPSY	1		
AUTO/NECROPSY/NO HISTO			1
AUTOLYSIS/NO NECROPSY			1

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

TABLE D2.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	49	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(49)	(49)	(49)
CONGESTION, NOS	1 (2%)		
HEMORRHAGE		1 (2%)	1 (2%)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, SUPPURATIVE	1 (2%)		
INFLAMMATION, ACUTE SUPPURATIVE	1 (2%)		
PNEUMONIA, CHRONIC MURINE	1 (2%)	9 (18%)	3 (6%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(47)	(49)	(47)
FIBROUS OSTEODYSTROPHY	31 (66%)	32 (65%)	30 (64%)
HYPERPLASIA, HEMATOPOIETIC	3 (6%)	1 (2%)	3 (6%)
#SPLEEN	(48)	(48)	(49)
ATROPHY, NOS		1 (2%)	
MONOCYTOSIS		1 (2%)	
HYPERPLASIA, LYMPHOID		2 (4%)	1 (2%)
HEMATOPOIESIS	1 (2%)	4 (8%)	1 (2%)
#CERVICAL LYMPH NODE	(49)	(48)	(49)
HYPERPLASIA, LYMPHOID		2 (4%)	
#BRONCHIAL LYMPH NODE	(49)	(48)	(49)
HYPERPLASIA, LYMPHOID	1 (2%)		
#MESENTERIC L. NODE	(49)	(48)	(49)
CONGESTION, NOS	1 (2%)	1 (2%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
LEUKOCYTOSIS, NOS			1 (2%)
HYPERPLASIA, LYMPHOID	2 (4%)	3 (6%)	4 (8%)
#LUNG	(49)	(49)	(49)
LEUKOCYTOSIS, NOS	1 (2%)		
#LIVER	(49)	(49)	(49)
LEUKOCYTOSIS, NOS	1 (2%)	1 (2%)	
HEMATOPOIESIS		1 (2%)	
#PEYER'S PATCH	(49)	(49)	(48)
HYPERPLASIA, LYMPHOID	2 (4%)		
#KIDNEY	(49)	(49)	(49)
HYPERPLASIA, LYMPHOID	1 (2%)		
CIRCULATORY SYSTEM			
#HEART	(49)	(49)	(48)
THROMBOSIS, NOS	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
#MYOCARDIUM	(49)	(49)	(48)
INFLAMMATION, SUPPURATIVE	2 (4%)		
*CORONARY ARTERY	(49)	(49)	(50)
PERIARTERITIS			2 (4%)
#KIDNEY	(49)	(49)	(49)
EMBOLUS, SEPTIC	1 (2%)		
PERIARTERITIS	1 (2%)	1 (2%)	
#OVARY	(47)	(48)	(45)
THROMBOSIS, NOS			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(49)	(47)	(48)
ATROPHY, NOS	1 (2%)		
#LIVER	(49)	(49)	(49)
BILE STASIS		1 (2%)	
INFLAMMATION, MULTIFOCAL	1 (2%)	1 (2%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, NOS	1 (2%)		1 (2%)
NECROSIS, FOCAL		1 (2%)	
NECROSIS, COAGULATIVE	1 (2%)		
INFARCT, NOS			1 (2%)
#LIVER/CENTRILOBULAR	(49)	(49)	(49)
NECROSIS, NOS			1 (2%)
#BILE DUCT	(49)	(49)	(49)
CYST, NOS	1 (2%)	1 (2%)	1 (2%)
#PANCREAS	(48)	(49)	(49)
DILATATION/DUCTS	2 (4%)	2 (4%)	
#PANCREATIC DUCT	(48)	(49)	(49)
FIBROSIS	1 (2%)		
NECROSIS, NOS	1 (2%)		
#PANCREATIC ACINUS	(48)	(49)	(49)
ATROPHY, NOS	1 (2%)	3 (6%)	1 (2%)
#STOMACH	(49)	(49)	(49)
ULCER, FOCAL		1 (2%)	1 (2%)
HYPERKERATOSIS	1 (2%)		
ACANTHOSIS	1 (2%)		
#ILEUM	(49)	(49)	(48)
DIVERTICULITIS PERFORATED	2 (4%)		
URINARY SYSTEM			
#KIDNEY	(49)	(49)	(49)
INFLAMMATION, CHRONIC	3 (6%)		1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(38)	(46)	(34)
CYST, NOS			1 (3%)
ANGIECTASIS			1 (3%)
#ADRENAL CORTEX	(49)	(48)	(48)
DEGENERATION, NOS		1 (2%)	
#THYROID	(48)	(47)	(42)
FOLLICULAR CYST, NOS		1 (2%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, FOCAL		1 (2%)	
HYPERPLASIA, FOLLICULAR-CELL		4 (9%)	2 (5%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(49)	(50)
DILATATION/DUCTS			1 (2%)
METAPLASIA, SQUAMOUS		1 (2%)	
LACTATION		1 (2%)	1 (2%)
#UTERUS	(49)	(49)	(48)
HYDROMETRA	1 (2%)		1 (2%)
INFARCT, NOS			1 (2%)
#UTERUS/ENDOMETRIUM	(49)	(49)	(48)
INFLAMMATION, SUPPURATIVE	1 (2%)		1 (2%)
HYPERPLASIA, CYSTIC	42 (86%)	39 (80%)	34 (71%)
#OVARY	(47)	(48)	(45)
CYSTIC FOLLICLES	5 (11%)		1 (2%)
FOLLICULAR CYST, NOS	1 (2%)	3 (6%)	4 (9%)
PAROVARIAN CYST	7 (15%)	6 (13%)	3 (7%)
HEMORRHAGIC CYST	1 (2%)	1 (2%)	
NERVOUS SYSTEM			
#CEREBRUM	(49)	(49)	(49)
HEMORRHAGE			1 (2%)
#BRAIN	(49)	(49)	(49)
HYDROCEPHALUS, INTERNAL		2 (4%)	
ABSCESS, NOS	1 (2%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL CAVITY	(49)	(49)	(50)
STEATITIS		1 (2%)	
NECROSIS, FAT		1 (2%)	
*PERITONEUM	(49)	(49)	(50)
INFLAMMATION, NOS	1 (2%)		
*PERITONEAL CAVITY	(49)	(49)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
*EPICARDIUM	(49)	(49)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1		2
AUTO/NECROPSY/NO HISTO			1
AUTOLYSIS/NO NECROPSY	1	1	

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

Appendix E

Analysis of Benzoin (Lot No. 034) - Midwest Research Institute

APPENDIX E

Analyses of Benzoin (Lot No. 034)
Midwest Research Institute

A. ELEMENTAL ANALYSIS

Element	C	H
Theory	79.22	5.70
Determined	79.31	5.51
	79.28	5.52

B. MELTING POINT

Determined	Literature Values
m.p. 134.4°-135.2°C (Du Pont 900 DTA) 130°-134°C (visual capillary)	129°C (Okuzumi, 1961) 134°C (Sharfstein, 1954) 131°C (Kaji and Nagashima, 1956) 131.6°-132.2°C (Sugihara and Newman, 1954.)

C. THIN-LAYER CHROMATOGRAPHY

Plates: Silica gel F-254
Amount Spotted: 100 and 300 μg
Ref. Standard: Phenol
Visualization: Ultraviolet and iodine vapor
System 1: Ethyl ether (100%)
R_f: 0.72, 0.78 (trace), origin (slight trace)
R_{st}: 0.92, 1.00, origin
System 2: Ethyl acetate: carbon tetrachloride (30:70)
R_f: 0.66, 0.85 (trace), 0.18 (slight trace), origin (slight trace)
R_{st}: 0.76, 0.98, 0.21, origin

D. HIGH-PRESSURE LIQUID CHROMATOGRAPHY
(Waters ALC 202)

Column: Porasil A-60, 2 ft x 1/8 in.
Solvent: 25% Chloroform:75% Hexane
Detection: Ultraviolet, 254 nm
Results: Main peak and one impurity
Retention times: 5.4 min (major), 1.7 min (impurity)

E. SPECTRAL DATA

1. Infrared: (Beckman IR-12)
   1.5% pellet in KBr
   vs: 3425, 3395, 1682, 757, 705 cm^{-1}
   s: 1595, 1451, 1265, 1209, 1070, 979, 695, 682, 675, 622, 609, 597, 511 cm^{-1}
   m: 3090, 3070, 3035, 1495, 1396, 1344, 1309, 1181, 1095, 1030, 1005, 930, 857, 836, 398, 245 (broad) cm^{-1}
   w: 1320 cm^{-1}

   Identical to literature spectrum (Sadtler Standard Spectra) and Lot No. 034

2. ULTRAVIOLET/VISIBLE: (Cary 118)

   $\epsilon max_{247.5} = 1.26 \pm 0.02$ (δ) x 10^4

   Solvent: 95% ethanol

   $\epsilon max_{248.0} = 1.1 \times 10^4$ (Rumpf and Gillois, 1955)

   Solvent: 95% ethanol
   $\log \epsilon = 4.1$ at 248 nm (Meisenheimer and Dorner, 1933) (1.2 - 1.4 x 10^4)

   Solvent: alcohol

3. NUCLEAR MAGNETIC RESONANCE:
   (Varian HA-100)
   Solvent: $CDCl_3$ with internal TMS
   Assignments:
   (a) 4.40δ, (b) 5.84δ,
   (c) 7.08-7.46δ,
   (d) 7.81δ, J = 8 cps.
   Integration Ratios: (a) 0.37, (b) 0.75, (c) 8.20, (d) 2.05

   Agrees with literature spectrum (Sadtler Standard Spectra)

Appendix F

Analysis of Benzoin (Lot No. 135) - Midwest Research Institute

APPENDIX F

Analyses of Benzoin (Stauffer Chemical; Lot No. 135)
Midwest Research Institute

A. ELEMENTAL ANALYSIS

Element	C	H
Theory	79.22	5.70
Determined	79.14	5.67
	79.19	5.70

B. MELTING POINT

Determined	Literature Values
m.p. 135°.5-136°C (Dupont 900 DTA)	129°C (Okuzumi, 1961)
m.p. 133°-135.5°C (visual capillary)	134°C (Sharfstein, 1954)
	131°C (Kaji and Nagashima, 1956)
	131.6°-132.2°C (Sugihara and Newman, 1956)

C. THIN-LAYER CHROMATOGRAPHY

Plates: Silica gel F-254
Amount Spotted: 100 and 300 μg
Ref. Standard: Phenol
Visualization: Ultraviolet (254 and 365 nm) and vanillin-sulfuric acid

System 1: Carbon tetrachloride: ethylacetate (70:30)	System 2: Diethyl ether: dibutyl ether (40:10)
R_f: 0.91 (minor), 0.77 (major), origin (slt. trace)	R_f: 0.73 (minor), 0.62 (major), origin (slt. trace)
R_{st}: 1.17, 0.99, origin	R_{st}: 0.99, 0.83

D. HIGH-PRESSURE LIQUID CHROMATOGRAPHY

Instrument: Waters ALC 202 with Model 660 Solvent Programmer
Column: Bondapak C_{18}, 30 cm x 4 mm
Solvent: Water to Acetonitrile, program 6, 15 min.
Flow rate: 1 ml/min

Detection: Ultraviolet, 254 nm
Results: Major peak and two impurities
Retention times and relative areas:
Impurity; 12.3 min; 1.1 ± 0.3%
Major peak; 13.4 min; 100%
Impurity; 15.4 min; 0.4 ± 0.2%

E. SPECTRAL DATA

1. Infrared
Instrument: Beckman IR-12
Cell: 1.03% KBr pellet
Results: See Figure 5

Identical to literature spectrum (Sadtler Standard Spectra) and spectrum of Lot # 034

2. ULTRAVIOLET/VISIBLE: (Cary 118)

$\epsilon max_{247.0} = (1.26 \pm 0.02\ (\delta) \times 10^4$

No absorbance from 800-350 nm at 4 mg/ml

Solvent: 95% Ethanol

$\epsilon max_{248.0} = 1.1 \times 10^4$ (Rumpf and Gillois, 1955)

Solvent: 95% ethanol
$\log\epsilon = 4.1$ at 248 nm (Meisenheimer and Dorner, 1933) (1.2-1.4 x 104)
Solvent: alcohol

3. NUCLEAR MAGNETIC RESONANCE:
Instrument: Varian HA-100

Solvent: DMSO-d_6 with internal TMS

Assignments: (See Figure 6)

(a) 5.98δ
(b) 6.00δ
(c)(d) 6.78-8.01δ

Integration Ratios:

(a) (b) 2.07
(c) (d) 8.40 + 1.53

Identical to literature spectrum (Sadtler Standard Spectra)

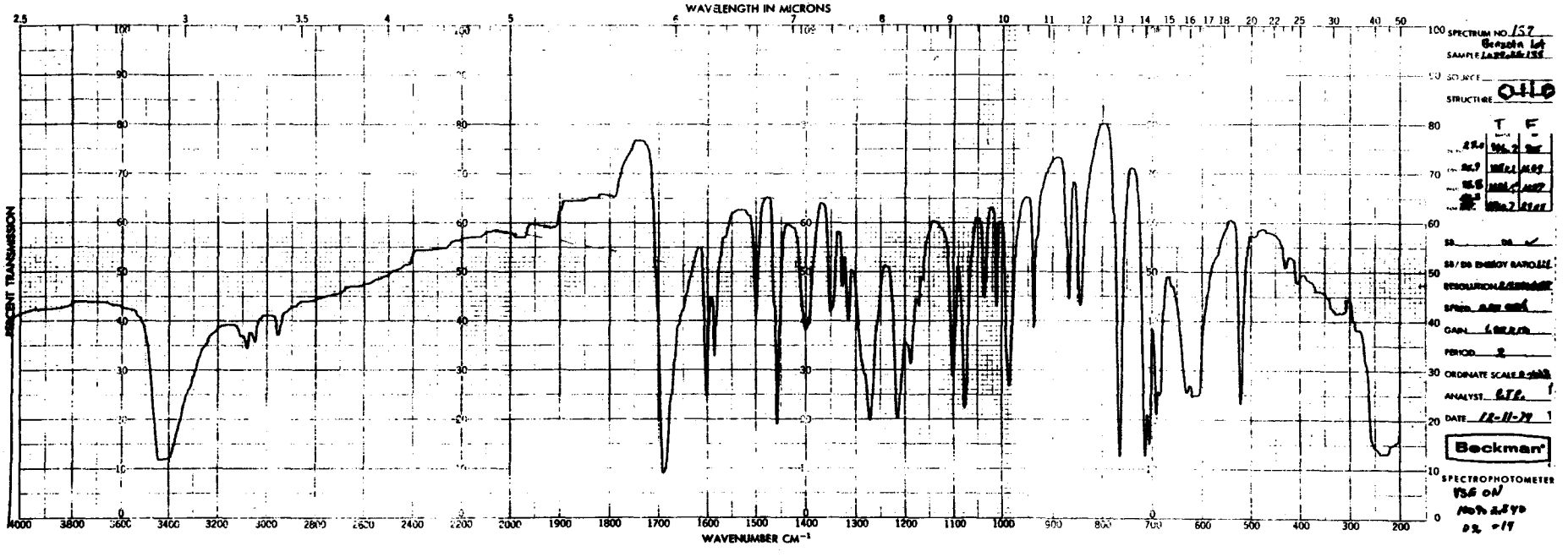


Figure 5. Infrared Absorption Spectrum of Benzoin

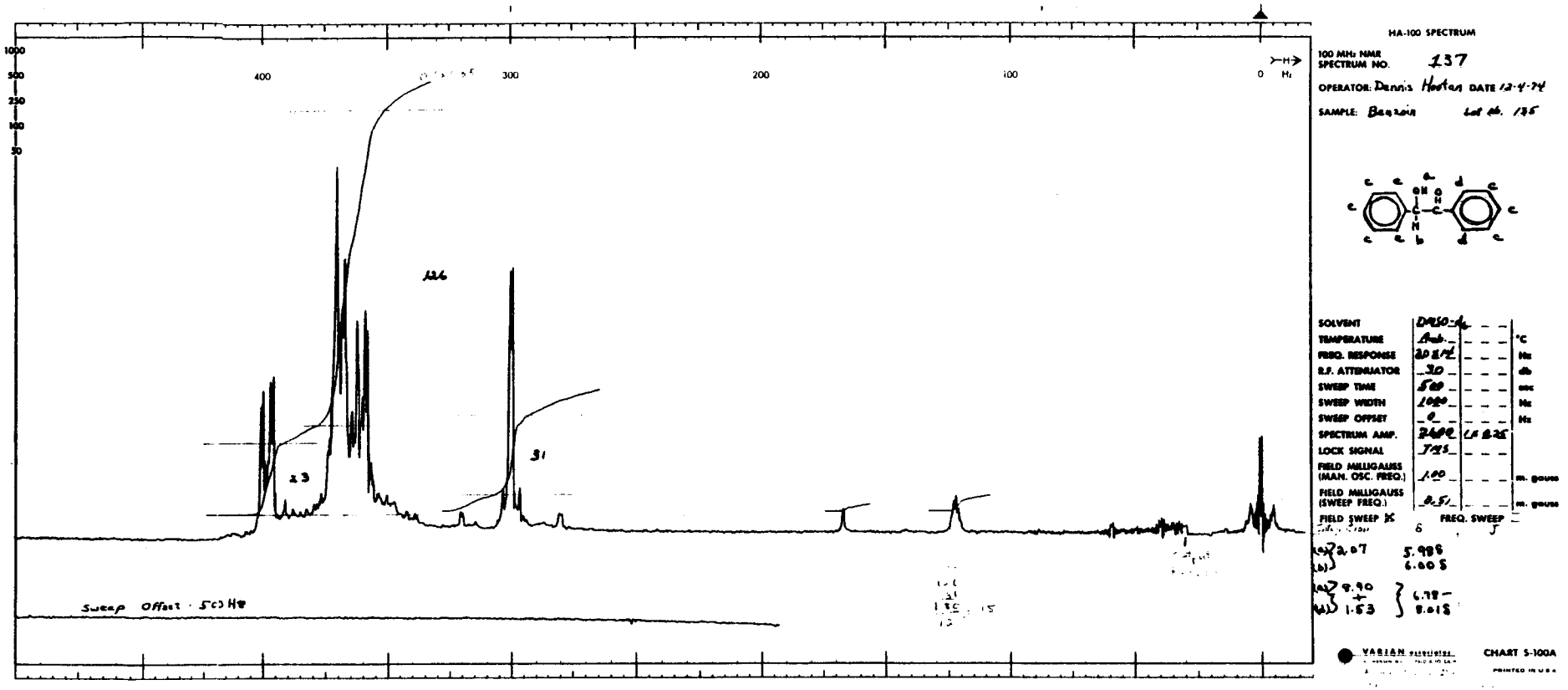


Figure 6. Nuclear Magnetic Resonance of Benzoin

Appendix G

Analysis of Formulated Diets for Stability of Benzoin

Analysis of Formulated Diets for Stability of Benzoin in the Diet

1. METHOD

Samples of diet mixtures containing 100,000 ppm benzoin were stored at -20, 5, 25, and 35°C for two weeks. Two-gram samples of the chemical-feed were mixed with chloroform (60 ml), blended for 1 minute on a Brinkman Polytron mixer, and then filtered through medium pore scintered glass filters. The residue was washed with 2-10 ml portions of chloroform. The undiluted filtrate was injected on the Waters ALC 202 liquid chromatograph and analyzed by the method described in Appendix E.

2. RESULTS

The area of the major peak was constant within the limit of error of the analysis. The impurity peak area approximately doubled in the 35°C sample. However, exact area measurements of the impurity could not be determined because of interference with a component in the feed.

Temperature (Degrees C)	Area of Major Peak
-20	14.0
5	nd
25	14.1
35	14.1
	Average 14.1±0.1

3. CONCLUSIONS

Feed/chemical mixture should be prepared at maximum intervals of 1 week and stored at refrigerator temperatures.

Appendix H

Analysis of Formulated Diets for Concentrations of Benzoin

APPENDIX H

Analysis of Formulated Diets for Concentrations of Benzoin

The feed samples were analyzed using 2 g samples for the 2,500 and 5,000 ppm levels, 10 g samples for the 125 and 250 ppm levels, and 5 g samples for the 500 ppm level. The feed samples were extracted with 50 ml of chloroform in a blender for 2 minutes. The extracts were filtered and analyzed for benzoin using high-pressure liquid chromatography (Spectra-Physics 3500B equipped with model 770 variable wavelength detector at 254 nm wavelength and Vydac adsorb SS2 500 column, with hexane chloroform (75%-25%) mobile phase).

Theoretical Concentration (ppm)	Number of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
125	6	119	17.9	105-162
250	8	231	11.6	182-260
500	8	498	11.5	422-592
2,500	6	2492	9.3	2,210-2800
5,000	6	4731	8.8	4,348-5387

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

February 15, 1980

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

The primary reviewer for the report on the bioassay of benzoin noted that the chemical is used as a polymerizing catalyst, chemical intermediate, and a flavoring agent. After a brief description of the experimental design, he commented on the increased incidence of lymphomas/leukemias in treated male rats and in both sexes of treated mice. In rats there was a dose-related trend, although the incidence was not statistically significant. In female mice, the incidence in the low-dose group was statistically significant. Given these results, the reviewer said he could not agree with the conclusion in the report that benzoin was not carcinogenic under the conditions of the bioassay. He said that, at the very least, the evidence was suggestive of carcinogenicity. The reviewer urged that a statement be added to the report indicating that the findings were equivocal and that further testing was warranted.

The NCI chemical manager for benzoin said that the evidence of a response at only one dose level in only one species was insufficient proof to classify the chemical as a carcinogen. A Program staff member noted that the significance of leukemias is often difficult to interpret because of the wide variation in the spontaneous incidence rate and time to onset. Because of this variation, to call the findings "suggestive" could be misleading. He added that the results were not equivocal when evaluated in the context of the range of variability of the background incidence of leukemia.

The secondary reviewer
stated in the report. The reviewer did not think that the term "suggestive" should be applied to the results, but did urge that data on the variability of leukemias be added to the report so that readers would be able to

judge the significance of the findings. If benzoin is to be retested, there is a need to develop a different kind of study that could give a more definitive conclusion.

A lengthy discussion ensued in which the primary reviewer argued that the results were sufficiently equivocal that some statement to that effect should be made in the report. A Program staff member responded, that in the staff's judgment, the results were not equivocal and that the report adequately spelled-out concerns regarding the study.

The primary reviewer moved that the report on the bioassay of benzoin be accepted as written. He further moved that the Subgroup regards the results as equivocal (i.e., under the condition of test, benzoin was neither shown to be carcinogenic or not carcinogenic) and recommends that the chemical be considered for retest. The motion was seconded and passed four votes to two.

Members present were:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
David B. Clayson, Eppley Institute for Research in Cancer
Joseph Highland, Environmental Defense Fund
William Lijinsky, Federick Cancer Research Center
Henry C. Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Louise Strong, University of Texas Health Sciences Center

---

* 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.

*U.S. GOVERNMENT PRINTING OFFICE : 1980 O-311-201/3148

NIH Publication No. 80-1760
August 1980