DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

AD

Date July 24, 2007

From Mary E. Shackelford, PhD
Division of Food Contact Notifications, Toxicology Group 2

Subject Acceptance of Final TDERs for review of NTP's Carcinogenesis Bioassay of Bisphenol A in F344 rats and B6C3F1 mice (Feed Study) (NTP TR 215).

To Food Additive Master File 580

Through Michelle Twaroski, Ph.D.
Team Leader, Toxicology Group 1, DFCN

Following the Agency's instructions, ICF Consulting, the contractor, has reviewed the National Toxicology Program's (NTP) Carcinogenesis Bioassay of Bisphenol A (Cas No. 80-05-7) in F344 rats and B6C3F1 mice (Feed Study) (NTP TR 215). ICF has generated two comprehensive Toxicological Data Evaluation Reports (TDERs), one for the carcinogenesis bioassay in F344 Rats (Task Number 1) and one for the carcinogenesis bioassay in B6C3F1 mice (Task Number 2). Dr. Mary E Shackelford has reviewed and accepted the final TDERs for Tasks Number 1 and 2. The final TDERs (Contract No. 223-96-2302, Work Assignment Number 2000-17, dated May 17, 2001 for Tasks Number 1 and 2) can be incorporated into the file for Food Additive Master File 580

/s/

/s/

Mary E. Shackelford, Ph.D.

Attachments: Copy of final TDERs for Tasks Number 1 and 2, Work Assignments Number 2000-17 Under Contract Number 223-96-2302

MAY 17 2001

/s/

/s/

Contract Number

223-96-2302

Work Assignment Number

2000-17
(ICF 417)

Task Number

01

FDA Study Identification Number

NTP-80-35
NIH Publication No. 82-1771

Signature of Program Manager

/s/

/s/

Date

5-11-01

STUDY TITLE

Carcinogenesis Bioassay of Bisphenol A (CAS No. 80-05-7) in F344 Rats and B6C3F$_1$ Mice (Feed Study)

[Carcinogenesis Bioassay in Rats]

TESTING LABORATORY

Litton Bionetics, Inc.
Kensington, Maryland

COMPLETION DATE OF STUDY [1]

March 1982

SPONSOR OF STUDY [2]

National Cancer Institute's Carcinogenesis Testing Program

STUDY SUMMARY

Compliance and Quality Assurance Statements

No compliance or quality assurance statement was provided with the study report. Our reviewers note that this study began in February 1977 before Good Laboratory Practice regulations were codified.

Protocol

No protocol was included with the study report. The study authors did not discuss any protocol amendments or deviations that occurred during the conduct of the study.

Study Objective

The objective of the study was to evaluate the carcinogenic potential of bisphenol A when administered in the diet to Fischer 344 rats for approximately two years

---

[1] March 1982 is the date listed on the cover page of the study report. Our reviewers note that the study authors stated in the text (page ix) that the study was completed in February 1979.

[2] This organization has since become part of the National Institute of Environmental Health Sciences, National Toxicology Program.

Test Article

A single batch (Lot No DC6-24-75) of bisphenol A (4,4'-isopropylidenediphenol, CAS No. 80-05-7) was obtained from Dow Chemical Company. Midwest Research Institute conducted purity and identity analyses of the test article Based on information in Appendix E of the study report, these analyses consisted of an elemental analysis, a melting point determination, thin-layer chromatography, infrared spectroscopy, ultraviolet/visible spectroscopy, gas chromatography, and nuclear magnetic resonance spectroscopy The study authors concluded that the results of these analyses were consistent with the structure of the test article, but did note that four minor impurities were observed with thin-layer chromatography, and five minor impurities were observed with gas chromatography. The impurities were not identified by the study authors The test article was stored at 4°C over the course of the study.

Test Diets

Test diets were prepared by adding a small amount of basal diet (i.e., Purina® Lab Chow) to the appropriate amount of test article and then adding the concentrated pre-mix to the appropriate amount of basal diet Test diets were stored at 4°C in the dark for a maximum of two weeks. Our reviewers note that no homogeneity analyses were conducted to ensure that the mixing procedure produced homogeneous test diets.

The study authors noted that stability tests were conducted on a test diet with a concentration of 100,000 ppm of bisphenol A Based on information in Appendix F of the study report, samples were taken, stored at -20°C, 5°C, 25°C, and 45°C, and then analyzed after two weeks for the test article.[3] No statistically significant differences were found in test article concentration between samples. Therefore, the study authors concluded that the test diets were stable for two weeks at a maximum temperature of 45°C Our reviewers agree with the study authors' conclusion, but note that the basal diet used in the stability analysis (i e , Wayne Lab-Blox® Rodent Feed) differed from that used in the carcinogenicity study (i e., Purina® Lab Chow)

The study authors also noted that concentrations of the test article in "selected" samples of the test diets were analyzed during the course of the study. The results of these analyses indicated that concentrations of the test article in the test diets were "usually" within ±10% of the target concentrations. Based on data in Appendix G of the study report (see Table 1 below), our reviewers note that the mean concentrations were within 2% to 3% of the target concentrations. However, the upper end of the range of the high-concentration test diet deviated 29% from the target concentration, while the lower ends of the ranges of the low- and high-concentration test diets deviated 11% to 13%. Our reviewers note that no information was provided on when these samples were taken and believe that more samples should have been taken over the course of the study and analyzed for test article.

[3] Analyses were conducted by Midwest Research Institute.

Table 1. Concentrations of Bisphenol A in the Test Diets

Target Concentration (ppm)	Number of Samples	Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
1000	4	978	11.7	872 - 1057
2000	13	2061	9 4	1780 - 2575

Test Animals

F344 (Fischer) rats were received from NCI Frederick Cancer Research Center (Frederick, Maryland) when rats were three weeks old. The animals were observed for two weeks and then randomly assigned to test groups.[4] Graphical presentation of the group mean body weights of the males and females indicated that group mean body weights across groups of the same sex were approximately equal (see Attachment 1).

Animals were group caged (i.e., 5/cage) in polycarbonate cages. Absorb-dri® hardwood chips were used as bedding, and non-woven polyester filter sheets were used as cage covers. Cages, bedding, and water bottles were replaced twice each week. Fresh diets were provided once each week. Racks and filters were replaced every two weeks Temperature was maintained at 22 to 26°C, and relative humidity was maintained at 30 to 70%. Room air was filtered and exchanged 10 times per hour, and a 12 hour light/dark cycle was employed The study authors noted that the animals were housed with animals on a toxicology study for caprolactam

Food and water were available *ad libitum*. The study authors noted that water was acidified to a pH of 2 5. Our reviewers believe that this statement must have been a mistake as the animals would not have been to be maintained on water with a pH of 2.5

Experimental Design

Groups of rats (50/sex/group) were administered bisphenol A in the diet at target concentrations of 0, 1000, or 2000 ppm for at least 103 weeks The study authors noted that the test concentrations were selected based on decreases in weight gain observed in a subchronic toxicity study at concentrations of 1000 ppm or more.

Clinical Observations

The study authors noted that clinical observations were conducted twice each day, but that clinical signs were documented monthly. No other information was provided. Our reviewers note that no indication

[4] Table 7 of the study report indicates that the time observed was five weeks, which conflicts with the observation time noted in the study text Although the study report is unclear on this topic, our reviewers are assuming that the animals were observed two weeks prior to dosing, dosed for 103 weeks, and then observed for five weeks after dosing as study termination was listed as week 108.

was provided that the animals were examined or palpated weekly to monitor tumor development (e.g , location, dimensions, appearance, progression), which is critical for a carcinogenicity study. Evidence that these examinations were not conducted is that time to tumor observations were based entirely on necropsy observations (i.e., the time point of tumor observations was recorded as the week during which the animal died or was killed).

Body Weight and Food Consumption

Body weights were measured every two weeks for the first 13 weeks and every month thereafter. The study authors noted that body weights were measured by cage, which implies that rats were not weighed individually. The study authors did not indicate that food consumption was recorded; however, data in Appendix H indicate that food consumption measurements were determined at least once each month.

Clinical Laboratory Tests

No clinical laboratory tests (i e., clinical chemistry, hematology, or urinalyses) were conducted. Although standard carcinogenicity guidelines do not typically recommend evaluation of clinical chemistry parameters or urinalysis parameters, evaluation of several hematology parameters (e.g , erythrocyte and leukocyte counts) is typically recommended at interim and terminal evaluation points (Semler 1992).

Ophthalmology

Consistent with standard carcinogenicity guidelines, no ophthalmic examinations were conducted.

Gross Pathology

Moribund animals or those surviving to terminal sacrifice were killed by CO_2 inhalation. All animals were necropsied unless precluded by excessive autolysis or cannibalization (i.e., those found dead). No details were provided regarding the gross examination

Organ Weights

Consistent with standard carcinogenicity guidelines, organ weights were not recorded.

Histopathology

Tissues for histologic examination were first preserved in 10% neutral buffered formalin, then embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The study authors did note that special stains were used when necessary, but no other details were provided

A standard list of tissues recommended for histopathology is provided below. A check in the list indicates that the tissue was noted by the study authors in the text as one that was examined microscopically In addition to the tissues indicated below, the study authors also stated that the larynx was examined microscopically. Although the study authors did not specify the animals in which the tissues were examined, data in Appendix C indicate that animals in all groups were examined similarly.

Our reviewers note that several discrepancies exist between the study text and the data in Appendix C of the study report. Specifically, the study text indicates that the mammary glands, eyes, and nasal cavity in males and females and the seminal vesicles in males were examined microscopically. However, data in Appendix C indicate that these tissues were only grossly examined. Furthermore, data in Appendix C indicate that the large intestine was examined microscopically, while the list in the study text does not include this tissue. Also, our reviewers are assuming that no non-neoplastic lesions were observed in those tissues that were not listed in Appendix C, but were noted by the study authors in the text.

Cardiovascular/Hematopoietic

aorta
✓ bone marrow
✓ heart
✓ lymph nodes [5]
✓ spleen
✓ thymus

Glandular

✓ adrenals
✓ mammary glands
✓ thyroid / parathyroid

Urogenital

epididymides
fallopian tubes
✓ kidneys
✓ ovaries
✓ prostate
✓ seminal vesicle(s)
✓ testes
✓ urinary bladder
✓ corpus and cervix uterus [6]
vagina

Musculoskeletal

✓ bone (rib)
✓ skeletal muscle (thigh)

Digestive System

cecum
✓ colon
✓ duodenum
✓ esophagus
✓ ileum
✓ jejunum
✓ liver
✓ pancreas
rectum
✓ salivary glands
✓ stomach

Neurologic/Special Senses

✓ brain (no levels specified by study authors)
✓ eyes
extraorbital lacrimal glands
✓ peripheral nerve (sciatic)
✓ pituitary
✓ spinal cord (no levels specified by study authors)

Respiratory

✓ lungs with bronchi
✓ nasal cavity
✓ trachea

Other

✓ all gross lesions and masses
✓ skin

---

[5] The study authors indicated that the mandibular, mesenteric, and abnormal lymph nodes were examined.

[6] The study authors simply specified "uterus."

The study authors noted that lesion incidence was expressed as the ratio of the number of animals exhibiting the lesion to the number of animals examined histologically. The only exceptions were for those sites in which the gross examination led to the microscopic examination. In these cases, the incidence was the ratio of the number of animals exhibiting the lesion to the number of animals examined grossly.

Statistical Evaluation

Probabilities of survival were calculated using the Kaplan and Meier (1958) product-limit procedure. Dose-related effects and trends on survival were statistically analyzed using methods of Cox (1972) and Tarone (1975) No statistical analysis of body weight or food consumption data appears to have been conducted

Several statistical methods were used to evaluate the tumor incidence data. Dose-related trends in incidence data were evaluated using the Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage 1971). Pairwise comparisons between the control and dose groups were made using the Fisher exact test (Cox 1970). Bonferroni inequality criterion was used to adjust the criteria for significance given multiple comparisons. Life table analyses were conducted to account for intercurrent mortality, while time-adjusted analyses were used to make crude adjustments for competing risk. Lastly, the 95% confidence interval for relative risk was also calculated and presented with the upper and lower limits of the confidence interval.

Appraisal of the Study Design

Several significant deficiencies in the study design were noted by our reviewers. First, only two treatment groups were evaluated in this study. Standard test guidelines recommend at least three treatment groups be evaluated Second, some uncertainty exists regarding the age of the animals at initiation of treatment The study authors stated that the animals were three weeks old on receipt and were then observed for two weeks, which indicates that the animals were five weeks old at initiation of treatment. However, Table 7 of the study report notes that the animals were observed for five weeks, which indicates that either the animals were eight weeks old at initiation of treatment, or the animals were five weeks old at initiation of treatment with a five week observation period following termination of treatment as study termination was listed as week 108. If the animals were actually eight weeks old at initiation of treatment, then the bioassay may not have been sufficient to evaluate fully the carcinogenic potential of the test article as administration of the test article should begin when the animals are no older than six weeks old. Third, limited analyses were conducted on the test diets to ensure that the actual concentrations were within acceptable limits of the target concentrations Fourth, limited observations were made throughout the study Weekly physical examinations do not appear to have been conducted, body weights were measured only once every two weeks for the first 13 weeks instead of once each week for the first 13 weeks, and food consumption appears to have been measured only once each month instead of once each week for the first 13 weeks and then monthly thereafter. Also, no hematology parameters were evaluated at any time point during the study Total and differential leukocyte counts and erythrocyte counts should have been measured at interim and final evaluation points. Lastly, our reviewers were concerned that the animals in this study were housed with animals on another toxicity study, which is generally not recommended

STUDY RESULTS

Clinical Observations

The only treatment-related "clinical sign" that the study authors noted was a decrease in body weight of treated animals compared to control animals (see below) No data on clinical observations were included with the study report. Therefore, our reviewers could not verify the study authors' conclusion.

Survival

The Kaplan and Meier survival curves are provided in Attachment 2, and the survival at study termination for males and females is summarized below in Table 2 The study authors noted that no differences in survival between control and treated groups were observed. Based on the data provided in the study report, our reviewers agree with the study authors and note that no excessive early mortality was observed in the study (i e., survival was nearly 100% at one year) and that survival over the entire study was high (i e., survival was greater than 50% in all groups except the control males).

Table 2. Survival at Study Termination (Week 108)

Sex	Dose Group		
	0	1000	2000
Male	23/50 (46%)	30/50 (60%)	27/50 (54%)
Female	35/50 (70%)	35/50 (70%)	37/50 (74%)

Body Weight

The study authors plotted mean body weights of males and females over the course of the study. These graphs are provided in Attachment 1. Body weight data provided in Appendix H of the study report are summarized below in Table 3. The study authors simply stated that body weights of treated males and females were decreased compared to those of the control animals over the course of the study.

Our reviewers generally agree with the study authors and note that dose-related decreases were observed over the *majority* of the study for both males and females (i.e , until week 91 for the males and until week 83 for the females) The average decreases from control values over the study for low and high-dose males were 5% and 9%, respectively, and the average decreases from controls over the study for low- and high-dose females were 10% and 13% respectively These results are somewhat consistent with the subchronic toxicity study discussed by the study authors in which body weight gain was decreased at least 18% in males and at least 10% in females administered test diets with concentrations of 1000 to 4000 ppm of bisphenol A.

Table 3. Body Weights (g) of Males and Females over Study [a]

Week	Male Dose Groups			Female Dose Groups		
	0	1000	2000	0	1000	2000
4	207	195 (6%)	194 (6%)	148	139 (6%)	134 (9%)
8	268	255 (5%)	250 (7%)	175	161 (8%)	160 (9%)
12	304	289 (5%)	276 (9%)	187	173 (7%)	169 (10%)
16	324	304 (6%)	290 (10%)	196	182 (7%)	177 (10%)
20	334	318 (5%)	301 (10%)	209	190 (9%)	183 (12%)
24	360	340 (6%)	323 (10%)	216	202 (6%)	196 (9%)
28	367	347 (5%)	329 (10%)	220	206 (6%)	200 (9%)
32	385	360 (6%)	342 (11%)	226	210 (7%)	205 (9%)
36	390	362 (7%)	351 (10%)	231	212 (8%)	204 (12%)
40	397	373 (6%)	360 (9%)	238	215 (10%)	209 (12%)
44	406	382 (6%)	367 (10%)	243	225 (7%)	218 (10%)
48	408	388 (5%)	371 (9%)	249	230 (8%)	217 (13%)
52	413	391 (5%)	373 (10%)	253	229 (9%)	219 (13%)
56	414	398 (4%)	377 (9%)	255	227 (11%)	219 (14%)
59	420	396 (6%)	385 (8%)	264	233 (12%)	224 (15%)
63	423	394 (7%)	384 (9%)	273	237 (13%)	225 (18%)
67	413	395 (4%)	378 (8%)	278	241 (13%)	228 (18%)
71	429	401 (7%)	383 (11%)	286	247 (14%)	233 (19%)
75	423	402 (5%)	381 (10%)	289	251 (13%)	237 (18%)
79	418	395 (6%)	368 (12%)	297	243 (18%)	232 (22%)
83	416	399 (4%)	379 (9%)	300	258 (14%)	240 (20%)
87	415	401 (3%)	383 (8%)	229	264	239
91	406	403 (0 7%)	379 (7%)	242	270	248
95	381	399	382	244	270	249
99	384	400	359 (7%)	236	278	248

a Decreases from the control are shown in parentheses No statistics appear to have been conducted on data

Our reviewers note, however, that an error was made in either the female body weight graph or the female body weight data in Appendix H The graph shows the control group with higher body weights than the treated groups for the last four measurements of the study, while the data in Appendix H show the control group with lower body weights than the treated groups for this time period. Our reviewers could not determine which values were correct as no individual animal data were provided in the study report.

Food Consumption

Although the study authors did not state that food consumption was measured over the course of the study, food consumption data at monthly intervals were provided in Appendix H of the study report. Also, the study authors noted that food consumption of treated females was decreased 20% to 30% from control values and that of treated males was decreased 10% from control values The decreases for the treated females were observed as soon as week 12 and were dose-related. The study authors noted that the decreases in food consumption for the treated females were consistent with the decreases in body weight observed

Based on a review of the data in Appendix H, our reviewers partially agree with the study authors' evaluation of the data. The decreases in food consumption probably contributed to the decreases in body weights observed. However, in the early weeks of the study, the body weight decreases preceded the decreases in food consumption For example, in week 4 of the study, the body weight of high-dose males was 94% of the control value, while food consumption of the high-dose males was 100% of the control value. Also, the 20% to 30% decreases in food consumption observed for the females were not observed until week 12, while body weights of these females were decreased 6% to 9% by this time. Therefore, our reviewers believe that some other factor (e.g , a treatment-related effect on fat metabolism or efficiency of food utilization) was also contributory to the decreases in body weight observed. This view is supported by the subchronic toxicity study discussed by the study authors where body weights were decreased, but no effect on food consumption was observed. As a final note, our reviewers do not believe that any absolute conclusions can be made due to the limited number of measurements taken for both body weight and food consumption, especially during the first 13 weeks of the study.

Test Article Consumption

The study authors did not discuss test article consumption, but did provide data in Appendix H. The range of doses and the average dose over the course of the study for males and females are provided below in Table 4.

Table 4. Test Article Consumption (mg/kg/day)

Parameter	Dose group (ppm)	
	1000	2000
Males		
Average (±SD)	74±14	148±28
Range	55-111	110-246
Females		
Average (±SD)	74±12	135±29
Range	57-109	97-232

Clinical Laboratory Tests

No clinical pathology tests were conducted.

Ophthalmology

No ophthalmic examinations were conducted.

Organ Weights

No organ weights were measured

Gross Pathology

No data on gross lesions were provided in the study report. Our reviewers note that no observations of cecal enlargement in treated animals were recorded This result was different from previous observations in the subchronic toxicity study discussed by the study authors. The study authors stated that cecal enlargement was observed in 60 to 100% of males exposed to doses of 250 to 4000 ppm of bisphenol A and females exposed to doses of 500 to 4000 ppm of bisphenol A in the subchronic study. No histologic correlate was observed in the subchronic study.

Histopathology

Non-neoplastic Lesions

The study authors stated that no treatment-related non-neoplastic lesions were observed. Based on a review of Appendix C of the study report, our reviewers agree with the study authors, but provide the following observations (see Table 5 for a summary of selected non-neoplastic lesions). The incidences of testicular atrophy and mineralization and the incidence of prostatic inflammation (acute and chronic) were decreased in treated males compared to values of control males Our reviewers note that these lesions are generally more prevalent in obese animals, and that the decreased incidences in the treated

males are consistent with the reductions in body weight observed in these animals. The incidences of other common lesions (e.g , nephropathy in males) were unaffected by bisphenol A administration. We could not account for the apparent decreases in incidence of nephropathy in treated females. Similarly, we had no explanation for the apparent decreases in pituitary cysts and spleen hemosiderosis or the slight increases in bile duct inflammation and hyperplasia in treated females Also, we had no explanation for the apparent dose-related decreases in basophilic cytoplasmic change and chronic inflammation in the liver of treated males and females. Lastly, our reviewers note that no observations of hyaline masses in the urinary bladder of males were noted. This result is different from previous observations in the subchronic toxicity study discussed by the study authors in which hyaline masses were observed in the urinary bladder of males administered test diets with concentrations of 250 to 4000 ppm of bisphenol A. However, no clear dose-related increase in incidence was observed in the subchronic study.

Table 5. Incidences of Selected Non-neoplastic Lesions in Males and Females [a]

Finding	Dose Group (ppm)		
	0	1000	2000
Males			
Liver			
Basophilic cytoplasmic change	10/50	6/49	3/50
Inflammation, chronic focal	6/50	2/49	1/50
Prostate			
Inflammation, acute & chronic	6/50	0/50	1/50
Testis			
Mineralization	13/49	9/50	6/49
Atrophy, diffuse	25/49	5/50	11/49
Females			
Bile duct			
Inflammation, chronic	1/50	5/49	6/50
Hyperplasia, NOS	2/50	5/49	9/50
Kidney			
Nephropathy	28/50	11/50	5/50

Table 5. Incidences of Selected Non-neoplastic Lesions in Males and Females [a] (cont.)

Finding	Dose Group (ppm)		
	0	1000	2000
Liver			
Basophilic cytoplasmic change	16/50	12/49	9/50
Inflammation, chronic focal	28/50	19/49	17/50
Pituitary			
Cysts	9/48	3/49	3/50
Spleen			
Hemosiderosis	14/50	5/49	2/50

[a] Statistical analyses were not provided or conducted to allow statistical comparison of the incidences of non-neoplastic findings in treated versus control animals.

Neoplastic Lesions

The study authors noted that generally the incidences and types of tumors were similar between control and treated rats, but did note several tumors with incidences that were elevated in treated males and/or females compared to the controls (see Table 6 below) [7] Specifically, leukemia in males exhibited a statistically significant positive trend with the incidence elevated in the high-dose group. Pairwise comparison of the control and high-dose group values using the Fisher exact test yielded a p-value of 0.030, which was not considered statistically significant with application of the Bonferroni inequality criterion Life table analyses did not yield statistically significant results (i e., p=0.074 for trend analysis and p=0.141 for pairwise comparison of high-dose group and control). Although the incidences of leukemia were slightly elevated in the treated females, the increases were not statistically significant. The study authors concluded that these results suggested an association between exposure to the test article and cancers of the hematopoietic system, but did not provide convincing evidence.

The study authors also noted that interstitial cell tumors in males exhibited a statistically significant positive trend with the incidences in the treated males statistically significantly increased compared to that of the control. Although the study authors considered these results suggestive of a carcinogenic potential, they did not consider the evidence convincing because this tumor type has a high background rate, and the statistical significance may have resulted from an abnormally low incidence in the concurrent control group. Therefore, the study authors concluded that these findings were not "clearly" related to administration of test article.

---

[7] The study authors stated on page 17 of the study report that leukemia and testicular tumors were more common in high-dose males and females and in high-dose males, respectively Our reviewers note that leukemia in females and interstitial cell tumors in males were elevated at both the low dose and the high dose.

Finally, the study authors noted a statistically significant positive trend in mammary gland fibroadenomas in males with the incidence elevated in high-dose males However, the increase in high-dose males was not statistically significant based on the Fisher exact test. Our reviewers note that the incidence was within background range (i e , 0 to 12%) (Haseman et al 1990).

The study authors did note several tumors with incidences that were decreased in treated males and/or females. Specifically, adrenal pheochromocytomas in males and adrenal cortical adenomas in females exhibited statistically significant negative trends (p=0.031 and p=0.016 in males and females, respectively). The decreased incidences in the treated males and females were not statistically significant using the Fisher exact test with application of the Bonferroni inequality criterion. Also, endometrial stromal polyps in females exhibited a statistically significant (p=0 015) negative trend with the decreased incidence at the high dose statistically significant (p=0.018).

Table 6. Incidences of Selected Neoplastic Lesions in Males and Females [a]

Finding	Dose Group (ppm)		
	0	**1000**	**2000**
Males			
Multiple organs [b]			
Leukemia, NOS [c]	13/50	12/50 (NS)	23/50 (p=0.030)
Testis			
Interstitial cell tumor [d]	35/49	48/50 (p=0 001)	46/49 (p=0 003)
Mammary gland [b]			
Fibroadenoma [e]	0/50	0/50 (NS)	4/50 (NS)
Adrenal			
Cortical adenoma	1/48	3/50 (NS)	4/47 (NS)
Cortical adenoma or carcinoma	1/48	3/50 (NS)	5/47 (NS)
Females			
Multiple organs [b]			
Leukemia, NOS	7/50	13/50 (NS)	12/50 (NS)

[a] Only total incidence was provided in the study report (i e , no data were provided to determine incidences of unscheduled death animals and incidences of terminal sacrifice animals) Values provided in parentheses are the p-levels resulting from the Fisher exact test. NS indicates a p-level greater than 0 05
[b] The denominator represents the number of animals necropsied.
[c] Statistically significant (p=0 021) positive trend observed using Cochran-Armitage test
[d] Statistically significant (p=0.001) positive trend observed using Cochran-Armitage test with a departure from linear trend (p=0 021)
[e] Statistically significant (p=0 015) positive trend observed using Cochran-Armitage test

Based on a review of Appendix A in the study report, our reviewers generally agree with the evaluation of the data by the study authors, but add the following observations First, adrenal cortical adenomas were slightly increased in treated males (see Table 6 above) However, the increases were not statistically significant and were at the upper end of the background range for this type of tumor (i.e., 0 to 8%) (Haseman et al. 1990). Second, our reviewers note that the total numbers of animals with malignant tumors and the total number of malignant tumors in males exhibited dose-related trends and that these data provide further suggestive evidence of a carcinogenic potential of bisphenol A (see Table 7 below). Slight trends were also observed in these parameters for females, but the female data were less convincing than the male data

Table 7. Overall Tumor Incidence in Males and Females

Finding	Dose Group (ppm)		
	0	1000	2000
Males			
No. of animals with primary tumors [a]	48	50	50
No. of primary tumors [a]	104	116	120
No of animals with benign tumors	44	49	48
No of benign tumors	80	84	84
No of animals with malignant tumors	18	22	28
No of malignant tumors	19	25	33
Females			
No of animals with primary tumors [a]	47	45	38
No of primary tumors [a]	91	82	67
No. of animals with benign tumors	39	39	33
No. of benign tumors	72	59	49
No of animals with malignant tumors	13	15	17
No of malignant tumors	14	16	18

[a] The study authors defined primary tumors as all tumors except secondary tumors, which were defined as "*metastatic tumors or tumors invasive into an adjacent organ.*"

Overall, our reviewers note that the neoplasms of most concern as potentially treatment-related were all neoplasms that are common in Fischer 344 rats on carcinogenicity studies (i e., neoplasms that have relatively high background incidences). Since the study was conducted and reported, NTP has established the "gold standard" at a p-level of less than 0.01 for biologically important increased incidences of common neoplasms using the Fisher exact test or equivalent Our reviewers note that only

interstitial cell tumors met this criterion in the present study; however, this neoplasm did remain within the historical control incidence of 81% to 98% (Goodman et al. 1980, Maekawa et al 1983, Solleveld et al. 1984, Maekawa and Hayashi 1992). Furthermore, although the incidences of leukemia in treated animals were generally elevated compared to control values, the incidences remained essentially within the historical control reference ranges for Fischer 344 rats with the exception of the incidence of the high-dose males (see Table 8 below).

Table 8. Historical Control Values for Incidence of Leukemia in Male and Female Fischer Rats [a]

References	Historical Control Values for Males	Historical Control Values for Females
Moloney et al (1970)	Not Provided	21/86 (24 4%) 14-30 months of age
Cardy (1979), Cardy et al. (1979), Coate et al (1979), Coleman et al (1977), Dalbey et al. (1980), Goodman et al (1980), Jacobs and Huseby (1967), Sacksteder (1976), Sass et al. (1975) [b]	358/2360 (15.2%)	318/2372 (13 4%)
Haseman (1983) [c]	127/573 (22 2%)	117/572 (20 5%)
Stromberg and Vogtsberger (1983) [c]	322/1129 (28 5%)	230/1176 (20 0%)
Stromberg (1992)	311/1019 (30.5%)	223/1012 (22.0%)
Batelle Laboratories [d]	583/1977 (29 5%)	376/2021 (18 6%)

[a] Table was constructed from summary data provided by Stromberg (1992)
[b] Data for Fischer 344 rats on toxicology studies conducted from 1967 to 1980
[c] Data from NTP chronic studies.
[d] The only reference provided for these data in the primary source (Stromberg 1992) was "Batelle Laboratories " Our reviewers assume that these data were from studies conducted at Batelle, and note that Dr Stromberg was affiliated with Batelle Laboratories for many years.

As a final evaluation, our reviewers considered potential treatment-related effects on neoplasms with lower background incidence as the neoplasms of most concern were common neoplasms with relatively high background incidence. We selected mesothelioma of the tunica vaginalis as a common neoplasm with a lower incidence in Fischer 344 males (Maekawa et al. 1983). The incidence in all groups was 1/50 animals. Therefore, no treatment-related effect was observed on mesothelioma incidence in the present study Given all the data, our reviewers do not believe that this study provides definitive evidence of a carcinogenic potential of bisphenol A, but do believe that this study provides suggestive evidence.

ASSESSMENT

Our reviewers note that this study was conducted more than 20 years ago and is limited due to its age. As noted above in the *Appraisal of the Study Design*, the most significant limitations were the limited number of dose groups, the uncertainty regarding the age of the animals on initiation of treatment, and the limited observations made throughout the study (e.g., lack of weekly physical examinations). Also, the lack of an interim sacrifice in this study was a major limitation as one of the tumors that appeared to be treatment-related occurred with a high background incidence at study termination. Although the study did provide information on the carcinogenic potential of bisphenol A, our reviewers do not believe that this study can serve as the definitive carcinogenicity bioassay due to the limitations noted

CONCLUSIONS

No treatment-related effects on survival were noted. No data were included in the study report on clinical observations or gross pathology. Therefore, our reviewers cannot comment on any effects observed on these parameters

Treatment-related decreases were observed on body weight in both males and females. The average decreases from control values over the study for low and high-dose males were 5% and 9%, respectively, and the average decreases from controls over the study for low- and high-dose females were 10% and 13%, respectively. Decreases in food consumption provided some explanation for the decreases in body weight observed However, our reviewers note that in the early weeks of the study, the body weight decreases preceded the decreases in food consumption Therefore, we believe that some other factor (e.g , a treatment-related effect on fat metabolism or efficiency of food utilization) was also contributory to the decreases in body weight observed. The analysis of these results is hindered by the limited number of both body weight and food consumption measurements taken in the first 13 weeks of the study.

Although no treatment-related non-neoplastic lesions were observed, potentially treatment-related neoplastic lesions were observed. Specifically, the incidence of leukemia was elevated in high-dose males and slightly elevated in low- and high-dose females. The trend in males was statistically significant, but pairwise comparison of the control and high-dose group values did not yield a statistically significant result with application of Bonferroni inequality criterion. Analysis of the female data did not yield statistically significant results. Also, the incidence of interstitial cell tumors in males exhibited a statistically significant positive trend with the incidences in the treated males statistically significantly increased compared to that of the control. The study authors did not consider these results definitive evidence of a treatment-related effect as this tumor has a high background rate, and the statistical significance may have been due to an abnormally low incidence in the concurrent control group.

The study authors concluded that the study provided no "convincing" evidence regarding the carcinogenicity of bisphenol A, but noted that the results of the study suggested an association between exposure to bisphenol A and cancers of the hematopoietic system, as well as interstitial cell tumors in males Our reviewers agree with the study authors that the evidence for carcinogenicity of the test article is not definitive, but believe that the evidence is suggestive and cannot be discounted on the basis of this study alone.

EXECUTIVE SUMMARY

Groups of rats (50/sex/group) were administered bisphenol A in the diet at target concentrations of 0, 1000, or 2000 ppm for at least 103 weeks. These concentrations corresponded to average doses of 74 and 148 mg/kg/day for low- and high-dose males, respectively, and to average doses of 74 and 135 mg/kg/day for low- and high-dose females, respectively

No treatment-related effects were noted in survival. Body weight, food consumption, and histopathology appeared to be at least potentially affected by treatment. Specifically, treatment-related decreases were observed on body weight in both males and females The average decreases from control values over the study for low and high-dose males were 5% and 9%, respectively, and the average decreases from controls over the study for low- and high-dose females were 10% and 13%, respectively. Decreases in food consumption provided some explanation for the decreases in body weight observed. However, our reviewers note that in the early weeks of the study, the body weight decreases preceded the decreases in food consumption Therefore, we believe that some other factor (e.g., a treatment-related effect on fat metabolism or efficiency of food utilization) was also contributory to the decreases in body weight observed The analysis of these results is hindered by the limited number of both body weight and food consumption measurements taken in the first 13 weeks of the study. Although no treatment-related non-neoplastic lesions were observed, potentially treatment-related neoplastic lesions were observed. Specifically, the incidence of leukemia was elevated in high-dose males and slightly elevated in low- and high-dose females The trend in males was statistically significant, but pairwise comparison of the control and high-dose group values did not yield a statistically significant result with application of Bonferroni inequality criterion Analysis of the female data did not yield statistically significant results. Also, the incidence of interstitial cell tumors in males exhibited a statistically significant positive trend with the incidences in the treated males statistically significantly increased compared to that of the control The study authors did not consider these results definitive evidence of a treatment-related effect as this tumor has a high background rate, and the statistical significance may have been due to an abnormally low incidence in the concurrent control group

The study authors concluded that the study provided no "convincing" evidence regarding the carcinogenicity of bisphenol A, but noted that the results of the study suggested an association between exposure to bisphenol A and cancers of the hematopoietic system, as well as interstitial cell tumors in males Our reviewers agree with the study authors that the evidence for carcinogenicity of the test article is not definitive, but believe that the evidence is suggestive and cannot be discounted on the basis of this study alone, especially considering the limitations due to the age of the present study.

REFERENCES

Armitage P *Statistical Methods in Medical Research*, John Wiley & Sons, Inc., New York, 1971, pp 362-265. [As cited in NTP TR 215]

Cardy RH 1979. Carcinogenicity and chronic toxicity of 2,4-toluenediamine. *J Natl Cancer Inst* 62. 1107-1116 [As cited in Stromberg 1992]

Cardy RH, Renne RA, Warner JW, and Cypher RL 1979. Carcinogenesis bioassay of technical-grade piperonyl butoxide in F344 rats. J Natl Cancer Inst 62· 569-578. [As cited in Stromberg 1992]

Coate WB, Uland BM, Lewis TR 1979 Chronic exposure to low concentrations of halothane-nitrous oxide: Lack of carcinogenic effect in the rat. *Anesthesiology* 50· 306-390. [As cited in Stromberg 1992]

Coleman GL, Barthold SW, Osbaldiston GW, Foster SJ, Jonas AM. 1977 Pathological changes during aging in barrier-reared Fischer 344 male rats *J Gerontol* 32: 258-278 [As cited in Stromberg 1992]

Cox DR. *Analysis of Binary Data*, Methuen & Co., Ltd., London, 1970, pp. 48-52. [As cited in NTP TR 215]

Cox DR 1972 Regression models and life tables. *J R Stat Soc* B34: 187-220. [As cited in NTP TR 215]

Dalbey WE, Nettesheim P, Griesemer R, Caton JE, Guerin MR. 1980. Chronic inhalation of cigarette smoke by F344 rats. *J Natl Cancer Inst* 64 383-390. [As cited in Stromberg 1992]

Goodman DG, Ward JM, Squire RA, Paxton MB, Reichardt WD, Chu KC, Linhart MS. 1980. Neoplastic and nonneoplastic lesions in aging F344 rats *Toxicol Appl Pharmacol* 48: 237-248

Haseman JK, Arnold J, Eustis SL. Tumor Incidences in Fischer 344 Rats: NTP Historical Data. In: *Pathology of the Fischer Rat. Reference and Atlas*, Boorman GA, Eustis SL, Elwell MR, Montgomery CA, MacKenzie WF (eds), Academic Press, Inc., New York, 1990, pp 555-564.

Haseman JK. 1983. Patterns of tumor incidence in two-year cancer bioassay feeding studies in Fischer 344 rats *Fundam Appl Toxicol* 3: 1-9. [As cited in Stromberg 1992]

Jacobs BB, Huseby RA. 1967. Neoplasms occurring in aged Fischer rats, with special reference to testicular, uterine, and thyroid tumors. *J Natl Cancer Inst* 39· 303-309. [As cited in Stromberg 1992]

Kaplan EL and Meier P. 1958. Nonparametric estimation from incomplete observations. *J Amer Stat Assoc* 53: 457-481. [As cited in NTP TR 215]

Maekawa A, Hayashi Y Neoplastic lesions of the testis. In: *Pathobiology of the Aging Rat, Volume 1*, Mohr U, Dungworth DL, Capen CC (eds), ILSI Press, Washington, DC, 1992, pages 414-426.

Maekawa A, Kurokawa Y, Takahashi M, Kokubo T, Ogiu T, Onodera H, Tanigawa H, Ohno Y, Furukawa F, Hayashi Y. 1983. Spontaneous tumors in F344/DuCrj rats. *Gann* 74: 365-372

Moloney WC, Boschetti AE, King VP. 1970. Spontaneous leukemia in Fischer rats *Cancer Res* 30: 41-43 [As cited in Stromberg 1992]

Sacksteder MR 1976. Brief communication: Occurrence of spontaneous tumors in the germfree F344 rat. *J Natl Cancer Inst* 57· 1371-1373. [As cited in Stromberg 1992]

Sass B, Rabstein LS, Madison R, Nims RM, Peters RL, Kelloff GJ 1975 Incidence of spontaneous neoplasms in F344 rats throughout the natural life-span. *J Natl Cancer Inst* 54: 1449-1453. [As cited in Stromberg 1992]

Semler DE. The Rat: Toxicology. In *Animal Models of Toxicology*, Gad SC, Chengelis CP (eds), Marcel Dekker, Inc , New York, 1992, pp. 21-76.

Solleveld HA, Haseman JK, McConnell EE. 1984 Natural history of body weight gain, survival, and neoplasia in the F344 rat. *J Natl Cancer Inst* 72: 929-940.

Stromberg PC Changes in the hematologic system. In: *Pathobiology of the Aging Rat, Volume 1*, Mohr U, Dungworth DL, Capen CC (eds), ILSI Press, Washington, DC, 1992, pages 14-24.

Stromberg PC, Vogtsberger LM 1983 Pathology of the mononuclear cell leukemia of Fischer rats. I. Morphologic studies. *Vet Pathol* 20: 698-708. [As cited in Stromberg 1992]

Tarone RE. 1975. Tests for trend in life table analysis. *Biometrika* 62: 679-682. [As cited in NTP TR 215]

Carcinogenesis Bioassay of Bisphenol A (CAS No. 80-05-7) in F344 Rats and B6C3F$_1$ Mice (Feed Study) [Carcinogenesis Bioassay in Rats]

SIGNATURE AND DATE

Technical Reviewer

/s/ /s/ 4/29/01

Ellen Mantus, Ph.D. Date

QA/QC Reviewer

/s/ /s/ 5-10-01

Jennifer Rojko, D.V M, Ph.D , D.A.C.V.P. Date

ATTACHMENT 1

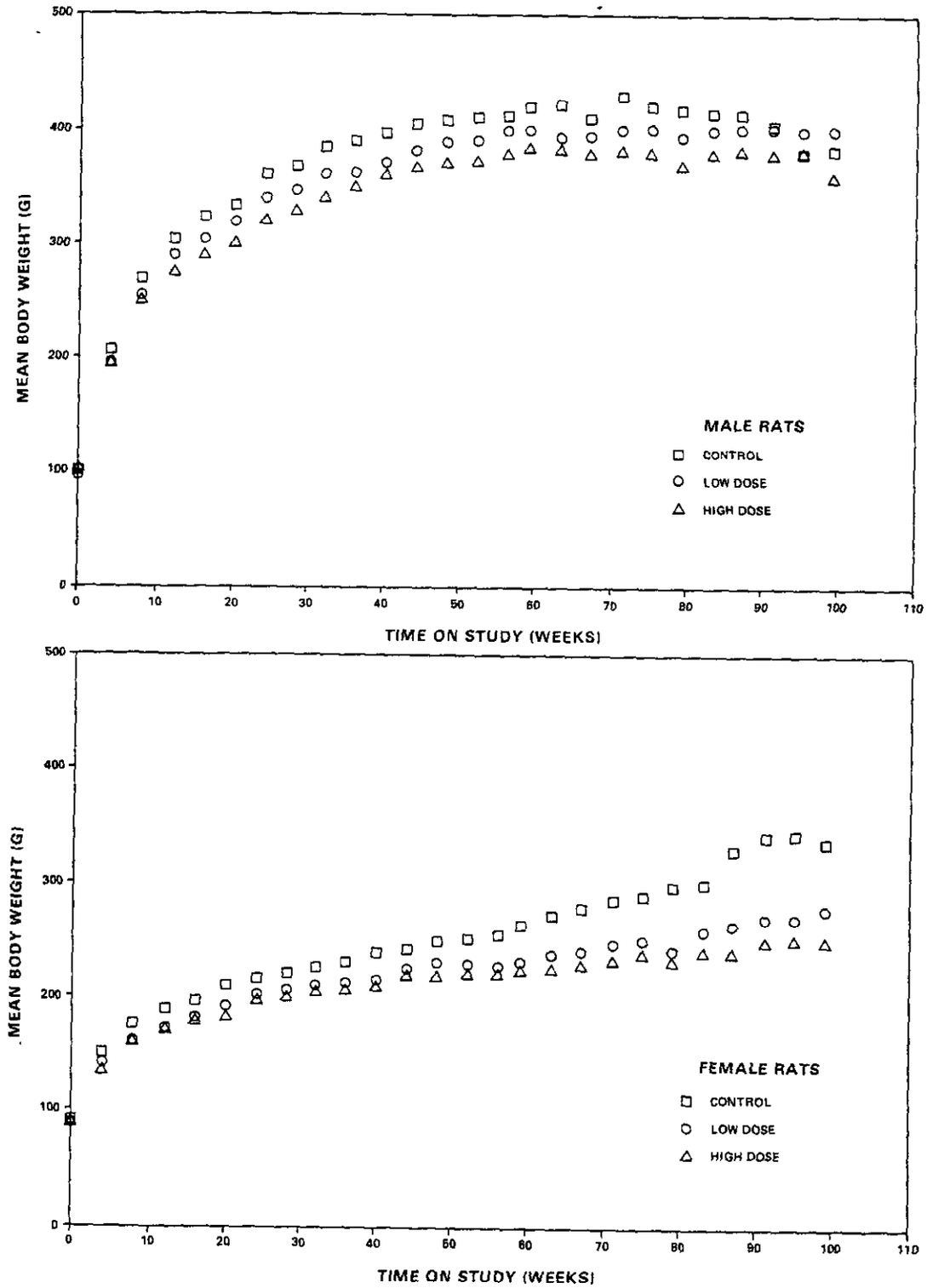

Figure 1. Growth Curves for Rats Fed Diets Containing Bisphenol A

ATTACHMENT 2

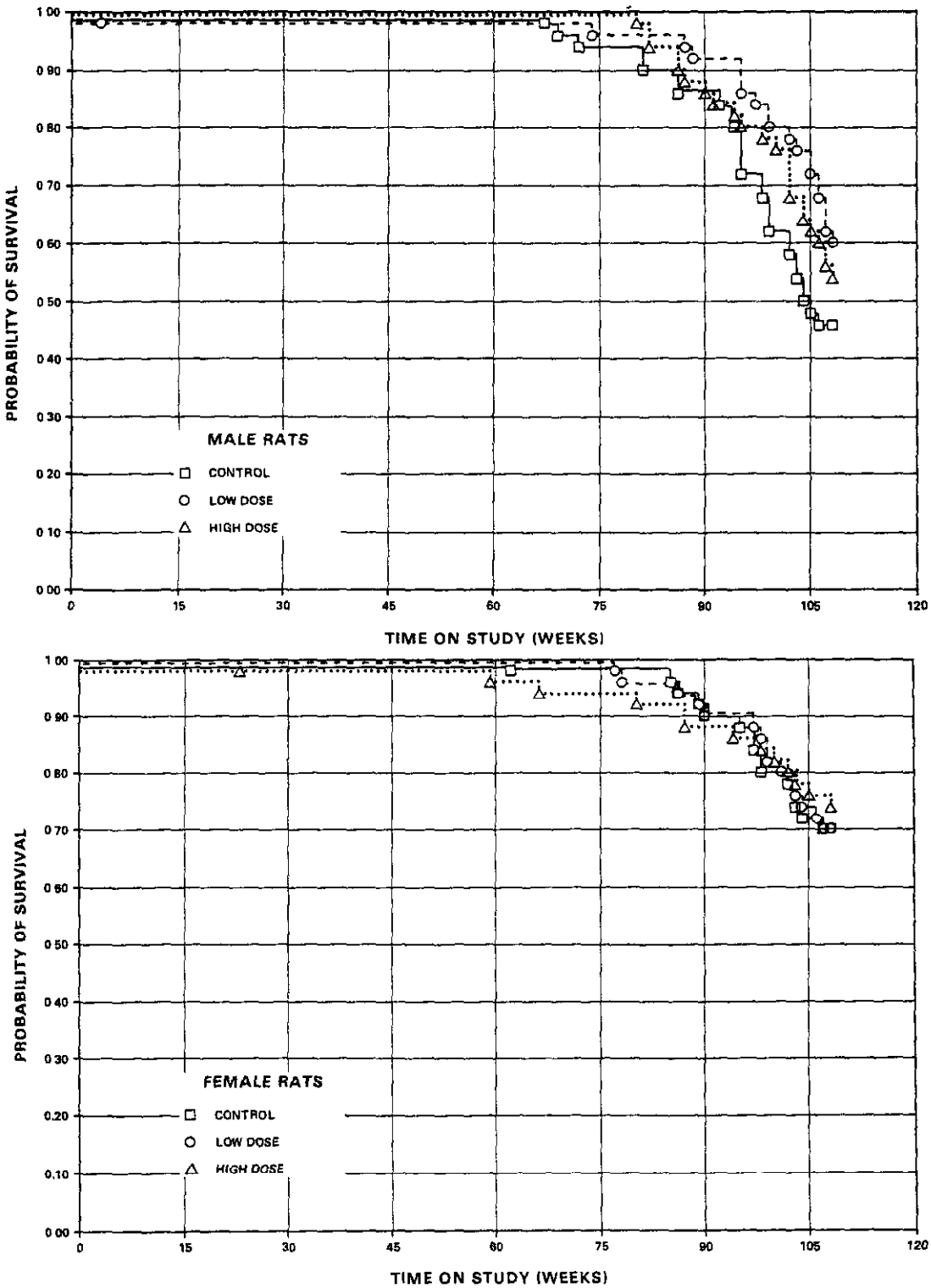

Figure 2. Survival Curves for Rats Fed Diets Containing Bisphenol A

MAY 17 2001

/s/

/s/

Contract Number

223-96-2302

Work Assignment Number

2000-17
(ICF 417)

Task Number

02

FDA Study Identification Number

NTP-80-35
NIH Publication No. 82-1771

Signature of Program Manager

/s/

/s/

Date

5-11-01

STUDY TITLE

Carcinogenesis Bioassay of Bisphenol A (CAS No. 80-05-7) in F344 Rats and B6C3F$_1$ Mice (Feed Study)

[Carcinogenesis Bioassay in Mice]

TESTING LABORATORY

Litton Bionetics, Inc.
Kensington, Maryland

COMPLETION DATE OF STUDY [1]

March 1982

SPONSOR OF STUDY [2]

National Cancer Institute's Carcinogenesis Testing Program

STUDY SUMMARY

Compliance and Quality Assurance Statements

No compliance or quality assurance statement was provided with the study report. Our reviewers note that this study began in February 1977 before Good Laboratory Practice regulations were codified.

Protocol

No protocol was included with the study report. The study authors did not discuss any protocol amendments or deviations that occurred during the conduct of the study.

Study Objective

The objective of the study was to evaluate the carcinogenic potential of bisphenol A when administered in the diet to B6C3F1 mice for approximately two years.

---

[1] March 1982 is the date listed on the cover page of the study report. Our reviewers note that the study authors stated in the text (page ix) that the study was completed in February 1979.

[2] This organization has since become part of the National Institute of Environmental Health Sciences, National Toxicology Program

Test Article

A single batch (Lot No DC6-24-75) of bisphenol A (4,4'-isopropylidenediphenol, CAS No. 80-05-7) was obtained from Dow Chemical Company. Midwest Research Institute conducted purity and identity analyses of the test article Based on information in Appendix E of the study report, these analyses consisted of an elemental analysis, a melting point determination, thin-layer chromatography, infrared spectroscopy, ultraviolet/visible spectroscopy, gas chromatography, and nuclear magnetic resonance spectroscopy. The study authors concluded that the results of these analyses were consistent with the structure of the test article, but did note that four minor impurities were observed with thin-layer chromatography, and five minor impurities were observed with gas chromatography. The impurities were not identified by the study authors. The test article was stored at 4°C over the course of the study.

Test Diets

Test diets were prepared by mixing a small amount of basal diet (i.e., Purina® Lab Chow) to the appropriate amount of test article and then adding the concentrated pre-mix to the appropriate amount of basal diet. Test diets were stored at 4°C in the dark for a maximum of two weeks. Our reviewers note that no homogeneity analyses were conducted to ensure that the mixing procedure produced homogeneous test diets

The study authors noted that stability tests were conducted on a test diet with a concentration of 100,000 ppm of bisphenol A. Based on information in Appendix F of the study report, samples were taken, stored at -20°C, 5°C, 25°C, and 45°C, and then analyzed after two weeks for the test article.[3] No statistically significant differences were found in test article concentration between samples. Therefore, the study authors concluded that the test diets were stable for two weeks at a maximum temperature of 45°C Our reviewers agree with the study authors' conclusion, but note that the basal diet used in the stability analysis (i e., Wayne Lab-Blox® Rodent Feed) differed from that used in the carcinogenicity study (i e., Purina® Lab Chow).

The study authors also noted that concentrations of the test article in "selected" samples of the test diets were analyzed during the course of the study The results of these analyses indicated that concentrations of the test article in the test diets were "usually" within ±10% of the target concentrations. Based on data in Appendix G of the study report (see Table 1 below), our reviewers note that the mean concentrations were within 0.5% to 2% of the target concentrations. Also, the upper and lower ends of the ranges were within 5% to 13% of the target concentrations. Our reviewers note that no information was provided on when these samples were taken and believe that more samples should have been taken over the course of the study and analyzed for test article.

[3] Analyses were conducted by Midwest Research Institute.

Table 1. Concentrations of Bisphenol A in the Test Diets

Target Concentration (ppm)	Number of Samples	Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
1000	4	978	11.7	872 - 1057
5000	10	4954	11 2	4436 - 5484
10000	3	9950	7 2	9466 - 10770

Test Animals

B6C3F1 mice were received from NCI Frederick Cancer Research Center (Frederick, Maryland) when mice were three weeks old. The animals were observed for two weeks and then randomly assigned to test groups.[4] Graphical presentation of the group mean body weights indicated that group mean body weight of the high-dose males was higher than that of the control and low-dose groups at initiation of treatment and that group mean body weight of low-dose females was slightly lower than that of control and high-dose females at initiation of treatment (see Attachment 1) [5]

Animals were group caged (i.e , 5/cage) in polycarbonate cages. Absorb-dri® hardwood chips were used as bedding, and non-woven polyester filter sheets were used as cage covers Cages, bedding, and water bottles were replaced twice each week. Fresh diets were provided once each week. Racks and filters were replaced every two weeks Temperature was maintained at 22 to 26°C, and relative humidity was maintained at 30 to 70%. Room air was filtered and exchanged 10 times per hour, and a 12 hour light/dark cycle was employed The study authors noted that the animals in this study were housed with animals on toxicology studies for caprolactam, 11-aminoundecanoic acid, and 2,6-dichloro-p-phenylenediamine.

Food and water were available *ad libitum*. The study authors noted that water was acidified to a pH of 2.5. Our reviewers believe that this statement must have been a mistake as the animals would not have been able to be maintained on water with a pH of 2.5.

---

[4] Table 7 of the study report indicates that the time observed was four weeks, which conflicts with the observation time noted in the study text. Although the study report is unclear on this topic, our reviewers are assuming that the animals were observed two weeks prior to dosing, dosed for 103 weeks, and then observed for four weeks after dosing as study termination was listed as week 107.

[5] Based on Figure 3 of the study report, group mean body weight of high-dose males appeared to be approximately 20 grams compared to a group mean body weight of approximately 17 grams for the control and low-dose males Group mean body weight of low-dose females appeared to be approximately 14 grams compared to a group mean body weight of approximately 15 grams for control and high-dose females. Tabulated summary and individual animal data were not available for review

Experimental Design

Groups of mice (50/sex/group) were administered bisphenol A in the diet at target concentrations of 0, 1000, or 5000 ppm for the males and at target concentrations of 0, 5000, or 10000 for the females for at least 103 weeks. The study authors noted that the test concentrations were selected based on results of a subchronic toxicity study Our reviewers assume that the dose selection was based on the decreased weight gain observed in females in the subchronic study, but was based on the histopathology observed in the male mice as no decrease in weight gain was observed at the dose selected as the high dose for the males in the carcinogenicity study.

Clinical Observations

The study authors noted that clinical observations were conducted twice each day, but that clinical signs were documented monthly. No other information was provided. Our reviewers note that no indication was provided that the animals were examined or palpated weekly to monitor tumor development (e g , location, dimensions, appearance, progression), which is critical for a carcinogenicity study. Evidence that these examinations were not conducted is that time to tumor observations were based entirely on necropsy observations (i.e., the time point of tumor observations was recorded as the week during which the animal died or was killed)

Body Weight and Food Consumption

Body weights were measured every two weeks for the first 13 weeks and every month thereafter. The study authors noted that body weights were measured by cage, which implies that mice were not weighed individually. The study authors did not indicate that food consumption was recorded; however, they did comment in the study report that food consumption was similar across groups indicating that some attempt was made to measure food consumption.

Clinical Laboratory Tests

No clinical laboratory tests (i.e., clinical chemistry, hematology, or urinalyses) were conducted. Although standard carcinogenicity guidelines do not typically recommend evaluation of clinical chemistry parameters or urinalysis parameters, evaluation of several hematology parameters (e.g , erythrocyte and leukocyte counts) is typically recommended at interim and terminal evaluation points (Andress 1992)

Ophthalmology

Consistent with standard carcinogenicity guidelines, no ophthalmic examinations were conducted.

Gross Pathology

Moribund animals or those surviving to terminal sacrifice were killed by CO_2 inhalation. All animals were necropsied unless precluded by excessive autolysis or cannibalization (i.e., those found dead). No details were provided regarding the gross examination

Organ Weights

Consistent with standard carcinogenicity guidelines, organ weights were not recorded.

Histopathology

Tissues for histologic examination were first preserved in 10% neutral buffered formalin, then embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The study authors did note that special stains were used when necessary, but no other details were provided.

A standard list of tissues recommended for histopathology is provided below. A check in the list indicates that the tissue was noted by the study authors in the text as one that was examined microscopically. In addition to the tissues indicated below, the study authors also stated that the gallbladder and larynx were examined microscopically Although the study authors did not specify the animals in which the tissues were examined, data in Appendix D indicate that animals in all groups were examined similarly.

Our reviewers note that a few discrepancies exist between the study text and the data in Appendix D of the study report. Specifically, the study text indicates that the skin in males and females and the mammary glands in females were examined microscopically. However, data in Appendix D indicate that these tissues were only grossly examined. Also, our reviewers are assuming that no non-neoplastic lesions were observed in those tissues that were not listed in Appendix D, but noted by the study authors in the text

The study authors noted that lesion incidence was expressed as the ratio of the number of animals exhibiting the lesion to the number of animals examined histologically. The only exceptions were for those sites in which the gross examination led to the microscopic examination In these cases, the incidence was the ratio of the number of animals exhibiting the lesion to the number of animals examined grossly.

Cardiovascular/Hematopoietic

aorta
✓ bone marrow
✓ heart
✓ lymph nodes [6]
✓ spleen
✓ thymus

Glandular

✓ adrenals
✓ mammary glands
✓ thyroid / parathyroid

Urogenital

epididymides
fallopian tubes
✓ kidneys
✓ ovaries
✓ prostate
✓ seminal vesicle(s)
✓ testes
✓ urinary bladder
✓ corpus and cervix uterus [7]
vagina

Musculoskeletal

✓ bone (rib)
✓ skeletal muscle (thigh)

Digestive System

cecum
✓ colon
✓ duodenum
✓ esophagus
✓ ileum
✓ jejunum
✓ liver
✓ pancreas
rectum
✓ salivary glands
✓ stomach

Neurologic/Special Senses

✓ brain (no levels specified by study authors)
✓ eyes
extraorbital lacrimal glands
✓ peripheral nerve (sciatic)
✓ pituitary
✓ spinal cord (no levels specified by study authors)

Respiratory

✓ lungs with bronchi
✓ nasal cavity
✓ trachea

Other

✓ all gross lesions and masses
✓ skin

Statistical Evaluation

Probabilities of survival were calculated using the Kaplan and Meier (1958) product-limit procedure. Dose-related effects and trends on survival were statistically analyzed using methods of Cox (1972) and Tarone (1975)

---

[6] The study authors indicated that the mandibular, mesenteric, and abnormal lymph nodes were examined.

[7] The study authors simply specified "uterus "

Several statistical methods were used to evaluate the tumor incidence data. Dose-related trends in incidence data were evaluated using the Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage 1971). Pairwise comparisons between the control and dose groups were made using the Fisher exact test (Cox 1970) Bonferroni inequality criterion was used to adjust the criteria for significance given multiple comparisons. Life table analyses were conducted to account for intercurrent mortality, while time-adjusted analyses were used to make crude adjustments for competing risk. Lastly, the 95% confidence interval for relative risk was also calculated and presented with the upper and lower limits of the confidence interval.

Appraisal of the Study Design

Several significant deficiencies in the study design were noted by our reviewers. First, only two treatment groups were evaluated in this study Standard test guidelines recommend at least three treatment groups be evaluated. Second, some uncertainty exists regarding the age of the animals at initiation of treatment. The study authors stated that the animals were three weeks old on receipt and were then observed for two weeks, which indicates that the animals were five weeks old at initiation of treatment However, Table 7 of the study report notes that the animals were observed for four weeks, which indicates that either the animals were seven weeks old at initiation of treatment, or the animals were five weeks old at initiation of treatment with a four week observation period following termination of treatment as study termination was listed as week 107. If the animals were actually seven weeks old at initiation of treatment, then the bioassay may not have been sufficient to evaluate fully the carcinogenic potential of the test article as administration of the test article should begin when the animals are no older than six weeks old. Third, limited analyses were conducted on the test diets to ensure that the actual concentrations were within acceptable limits of the target concentrations. Fourth, limited observations were made throughout the study Weekly physical examinations do not appear to have been conducted, body weights were measured only once every two weeks for the first 13 weeks instead of once each week for the first 13 weeks, and no information was provided regarding the frequency of food consumption measurements, if taken. Also, no hematology parameters were evaluated at any time point during the study Total and differential leukocyte counts and erythrocyte counts should have been measured at interim and final evaluation points. Lastly, our reviewers were concerned that the animals in this study were housed with animals on several other toxicity studies, which is generally not recommended.

STUDY RESULTS

Clinical Observations

The only treatment-related "clinical sign" that the study authors noted was a decrease in body weights of high-dose males and treated females compared to control animals (see below). No data on clinical observations were included with the study report. Therefore, our reviewers could not verify the study authors' conclusion.

Survival

The Kaplan and Meier survival curves are provided in Attachment 2, and the survival at study termination for males and females is summarized below in Table 2. The study authors stated that

survival was similar across groups for both males and females. Based on the data provided, our reviewers agree with the study authors and note that no excessive early mortality occurred, and that survival at study termination was high

Table 2. Survival at Study Termination (Week 107)

Sex	Dose Group		
	Control	Low	High
Male	42/49 (86%)[a]	37/50 (74%)	38/50 (76%)
Female	39/50 (78%)	37/48 (77%)[b]	41/48 (85%)[c]

[a] The study authors noted in the study text that two control male mice were accidentally killed and then provided the incidence noted in the table Our reviewers note that either the study authors incorrectly reported the number of males accidentally killed or the survival incidence provided
[b] The study authors gave no indication why the denominator for the low-dose females should be 48 instead of 50.
[c] The study authors noted that two female high-dose mice were accidentally killed

Body Weight

The study authors plotted mean body weights of males and females over the course of the study These graphs are included in Attachment 1. The study authors simply stated that body weights of high-dose males and treated females were decreased compared to those of the controls.

Our reviewers note that evaluation of the data was difficult as only the graphs were provided in the study report (i.e , no tabulated data were provided) However, our reviewers note that body weights of high-dose males began to diverge from control values by week 10 and appeared to be approximately 8% decreased from control values over the majority of the study Body weights of low- and high-dose females began to diverge from control values by week 12 and appeared to be approximately 22% decreased from control values over the majority of the study. Therefore, our reviewers agree that treatment-related decreases were observed in body weights of high-dose males and treated females.

Our reviewers note that these results are somewhat consistent with the subchronic toxicity study discussed by the study authors in which body weight gain was decreased at least 17% in female mice administered test diets with concentrations of 5000 to 25000 ppm of bisphenol A. The changes in the females were not dose-related, and no decreases in weight gain were observed in male mice administered test diets with concentrations as great as 10000 ppm of bisphenol A in the subchronic toxicity study.

Food Consumption

No data were provided on food consumption; however, the study authors did state that food consumption appeared to be similar across groups, although "data were incomplete due to excessive spillage of feed and could not be precisely evaluated."

Test Article Consumption

No information was provided in the study report regarding test article consumption nor was information provided that would have allowed calculation of test article consumption by our reviewers (i e., tabulated body weight and food consumption data).

Clinical Laboratory Tests

No clinical pathology tests were conducted.

Ophthalmology

No ophthalmic examinations were conducted

Organ Weights

No organ weights were measured.

Gross Pathology

No data were provided on gross lesions.

Histopathology

Non-neoplastic Lesions

The study authors noted that the incidence of multinucleated giant hepatocytes was increased in treated males (i.e., the incidences of this lesion were 1/49, 41/49, and 41/50 for the control, low-dose, and high-dose males). The hepatocytes in treated males contained six to 20 small nuclei and were considered by the study authors to be "abnormal forms." However, the study authors noted that no increase in liver tumors was observed in the male mice. Our reviewers note that the significance of multinucleated giant hepatocytes in the liver of mice on subchronic toxicity or chronic toxicity/carcinogenicity studies is not known The alteration has been observed in treated and control mice in many studies, and is generally observed in males (Maronpot et al. 1999, Hall 2001) The alteration does not have any particular significance as a preneoplastic lesion.

The study authors noted that no other treatment-related non-neoplastic lesions were observed. Based on a review of Appendix D of the study report, our reviewers agree with the study authors' evaluation of the data, but add the following observations. Overall, the incidences of various non-neoplastic lesions were low, corresponding to the general health and high survival rates of the mice. For example, the incidence of a relatively common lesion – mineralization in the kidneys of females – was lower than anticipated with the incidences equivalent for control and treated females. The incidence of brain mineralization, however, was slightly higher in high-dose males than control males (i.e., 28/50 in high-dose males versus 18/49 in control males), but the trend was not dose-related, and the incidence was not increased in high-dose females compared to control females. As expected in cycling female B6C3F1 mice (Maronpot et al. 1999), there was a high background incidence of estrogen-related lesions in the female reproductive tract (i.e , ovarian cysts and cystic endometrial hyperplasia in the uterus) and in the sternum (i.e., fibrous

dysplasia). Also as expected, fibrous dysplasia was an infrequent diagnosis in males. Lastly, the lack of treatment-related lesions in the female mice was similar to the observations in the previous subchronic toxicity study discussed by the study authors that was conducted with the same strain of mice at the same testing facility

Neoplastic Lesions

The study authors stated that the incidences of neoplastic lesions were similar across groups with one exception. The combined incidences of lymphomas or leukemia were slightly higher in treated males compared to control males (see Table 3 below). The trend was not statistically significant, and pairwise comparison of the control and high-dose group did not yield a statistically significant result. Pairwise comparison of the control and the low-dose group did yield a p-value of 0.028, but this result was not considered to be statistically significant with application of the Bonferroni inequality criterion. No similar trend was observed in female mice, and the study authors concluded that these results were not convincing evidence of carcinogenicity of bisphenol A.

The study authors also mentioned a statistically significant positive trend in the incidence of pituitary chromophobe carcinomas in male mice and a statistically significant positive trend in the incidence of hepatocellular adenomas or carcinomas in female mice after adjustment for female mice that died prior to week 52 (see Table 3 below). Our reviewers note that these tumors were observed in few animals, and the incidences were within the background range Therefore, our reviewers agree with the study authors that these lesions were probably not related to test article administration.

Table 3. Incidences of Selected Neoplastic Lesions in Males and Females [a]

Finding	Dose Groups		
	Control	Low	High
Males			
Multiple organs			
Lymphomas or leukemia	2/49	9/50 (p=0 028)	5/50 (NS)
Pituitary			
Chromophobe carcinoma [b]	0/37	0/36 (NS)	3/42 (NS)
Females			
Liver			
Hepatocellular adenoma or carcinoma [c]	0/50	1/48 (NS)	3/48 (NS)

[a] Only total incidence data were provided in the study report (i.e., no data were provided to determine incidences of unscheduled death animals and incidences of terminal sacrifice animals). Values provided in parentheses are the p-levels resulting from the Fisher exact test NS indicates a p-level greater than 0.05

[b] Statistically significant (p=0 016) positive trend observed using Cochran-Armitage test

[c] Time-adjusted analyses of tumor incidence eliminating animals that died prior to week 52 (i e , one control female, five low-dose females, and five high-dose females) resulted in a statistically significant positive trend (p=0 05)

Based on a review of Appendix B of the study report, our reviewers agree with the study authors' evaluation of the data Our reviewers note that the total numbers of animals with primary, benign, and malignant tumors were similar across groups, and that the total numbers of primary, benign, and malignant tumors were also similar across groups. Furthermore, the incidences and types of neoplasms observed were typical of chronic/carcinogenicity studies in B6C3F1 mice (Maronpot et al. 1999) Although a few neoplastic lesions were slightly higher in treated males (i e , chromophobe carcinomas of the pituitary and lymphoma or leukemia of multiple organs), the incidences of these lesions were not higher in treated females, and the incidences in both cases were within accepted historical control ranges for B6C3F1 mice on NTP carcinogenicity studies (Maronpot et al. 1999).

ASSESSMENT

Our reviewers note that this study was conducted more than 20 years ago and is limited due to its age. As noted above in the *Appraisal of the Study Design*, the most significant limitations were the limited number of dose groups, the uncertainty regarding the age of the animals on initiation of treatment, and the limited observations made throughout the study (e.g., lack of weekly physical examinations) Although the study did provide information on the carcinogenic potential of bisphenol A, our reviewers do not believe that this study can serve as the definitive carcinogenicity bioassay due to the limitations noted.

As an aside, one peer-reviewer of the study report noted that the dose levels administered to the male mice might have been too low based on the small decrease in body weight observed in this study. However, our reviewers note that body weight decreases of approximately 8% were observed and histologic changes were noted at both dose levels. Therefore, the high-dose was most likely an MTD.

CONCLUSIONS

No treatment-related effects were observed on survival. No data were included in the study report on clinical observations, food consumption, or gross pathology. Therefore, our reviewers cannot comment on any effects observed on these parameters

Body weights of high-dose males and treated females were decreased compared to those of the controls. Body weights of high-dose males appeared to be approximately 8% decreased from control values over the majority of the study, and body weights of low- and high-dose females appeared to be approximately 22% decreased from control values over the majority of the study. The body weight decreases did not appear to be the result of decreased food consumption as the study authors stated that food consumption was similar across groups. However, our reviewers are concerned about this conclusion as the study authors stated that food consumption data were "incomplete due to excessive spillage of feed and could not be precisely evaluated."

One treatment-related non-neoplastic lesion was observed The incidences of multinucleated giant hepatocytes were increased in treated males (i.e., the incidences of this lesion were 1/49, 41/49, and 41/50 for the control, low-dose, and high-dose males). No increase in liver tumors, however, was observed.

One potentially treatment-related neoplastic lesion was noted. The combined incidences of lymphomas or leukemia were slightly higher in treated males than in controls (i.e., 2/49, 9/50, and 5/50 in control, low-dose, and high-dose males, respectively) However, the trend was not statistically significant, and pairwise comparisons of treated groups to controls did not yield statistically significant results with application of the Bonferroni inequality criterion. Therefore, the study authors concluded that these results were not convincing evidence of carcinogenicity of bisphenol A.

Our reviewers agree with the study authors that the study results do not provide conclusive evidence of the carcinogenicity of bisphenol A. However, our reviewers do not believe that the carcinogenic potential of bisphenol A can be completely discounted on the basis of this study alone because this study does provide suggestive evidence (i.e., the increased incidences of lymphomas and leukemia in the treated males) and is limited due to its age

EXECUTIVE SUMMARY

Groups of mice (50/sex/group) were administered bisphenol A in the diet at target concentrations of 0, 1000, or 5000 ppm for the males and at target concentrations of 0, 5000, or 10000 for the females for at least 103 weeks. No information was provided in the study report to allow calculation of the corresponding dose levels for the target concentrations

No treatment-related effects were noted on survival No data were included in the study report on clinical observations, food consumption, or gross pathology. Therefore, no comments can be made regarding these parameters. Body weights and histopathology appeared to be affected by administration of the test article. Specifically, body weights of high-dose males and treated females were decreased compared to those of the controls Body weights of high-dose males appeared to be approximately 8% decreased from control values over the majority of the study, and body weights of low- and high-dose females appeared to be approximately 22% decreased from control values over the majority of the study. One treatment-related non-neoplastic lesion was observed. The incidences of multinucleated giant hepatocytes were increased in treated males (i.e., the incidences of this lesion were 1/49, 41/49, and 41/50 for the control, low-dose, and high-dose males). No increase in liver tumors, however, was observed. Also, one potentially treatment-related neoplastic lesion was noted. The combined incidences of lymphomas or leukemia were slightly higher in treated males than in controls (i.e., 2/49, 9/50, and 5/50 in control, low-dose, and high-dose males, respectively). However, the trend was not statistically significant, and pairwise comparisons of treated groups to controls did not yield statistically significant results with application of the Bonferroni inequality criterion Therefore, the study authors concluded that these results were not convincing evidence of carcinogenicity of bisphenol A.

Our reviewers agree with the study authors that the study results do not provide conclusive evidence of the carcinogenicity of bisphenol A. However, our reviewers do not believe that the carcinogenic potential of bisphenol A can be completely discounted on the basis of this study alone because this study does provide suggestive evidence (i.e., the increased incidences of lymphomas and leukemia in the treated males) and is limited due to its age.

REFERENCES

Andress JM. The Mouse: Toxicology In: *Animal Models of Toxicology*, Gad SC, Chengelis CP (eds), Marcel Dekker, Inc., New York, 1992, pp. 165-232

Armitage P *Statistical Methods in Medical Research*, John Wiley & Sons, Inc., New York, 1971, pp 362-265. [As cited in NTP TR 215]

Cox DR. *Analysis of Binary Data*, Methuen & Co., Ltd., London, 1970, pp. 48-52. [As cited in NTP TR 215]

Cox DR. 1972 Regression models and life tables. *J R Stat Soc* B34: 187-220 [As cited in NTP TR 215]

Hall WC, PAI, personal communication, 2001

Kaplan EL and Meier P. 1958. Nonparameteric estimation from incomplete observations. *J Amer Stat Assoc* 53. 457-481. [As cited in NTP TR 215]

Maronpot RR, Boorman GA, Gawl BW (eds). *Pathology of the Mouse*. Cache River Press, Vienna (IL), 1999

Tarone RE. 1975. Tests for trend in life table analysis. *Biometrika* 62: 679-682. [As cited in NTP TR 215]

Carcinogenesis Bioassay of Bisphenol A (CAS No. 80-05-7) in F344 Rats and B6C3F$_1$ Mice (Feed Study) [Carcinogenesis Bioassay in Mice]

SIGNATURE AND DATE

Technical Reviewer

/s/ /s/ 4/29/01

Ellen Mantus, Ph.D. Date

QA/QC Reviewer

/s/ /s/ 5-10-01

Jennifer Rojko, D V M , Ph.D., D.A.C.V.P. Date

ATTACHMENT 1

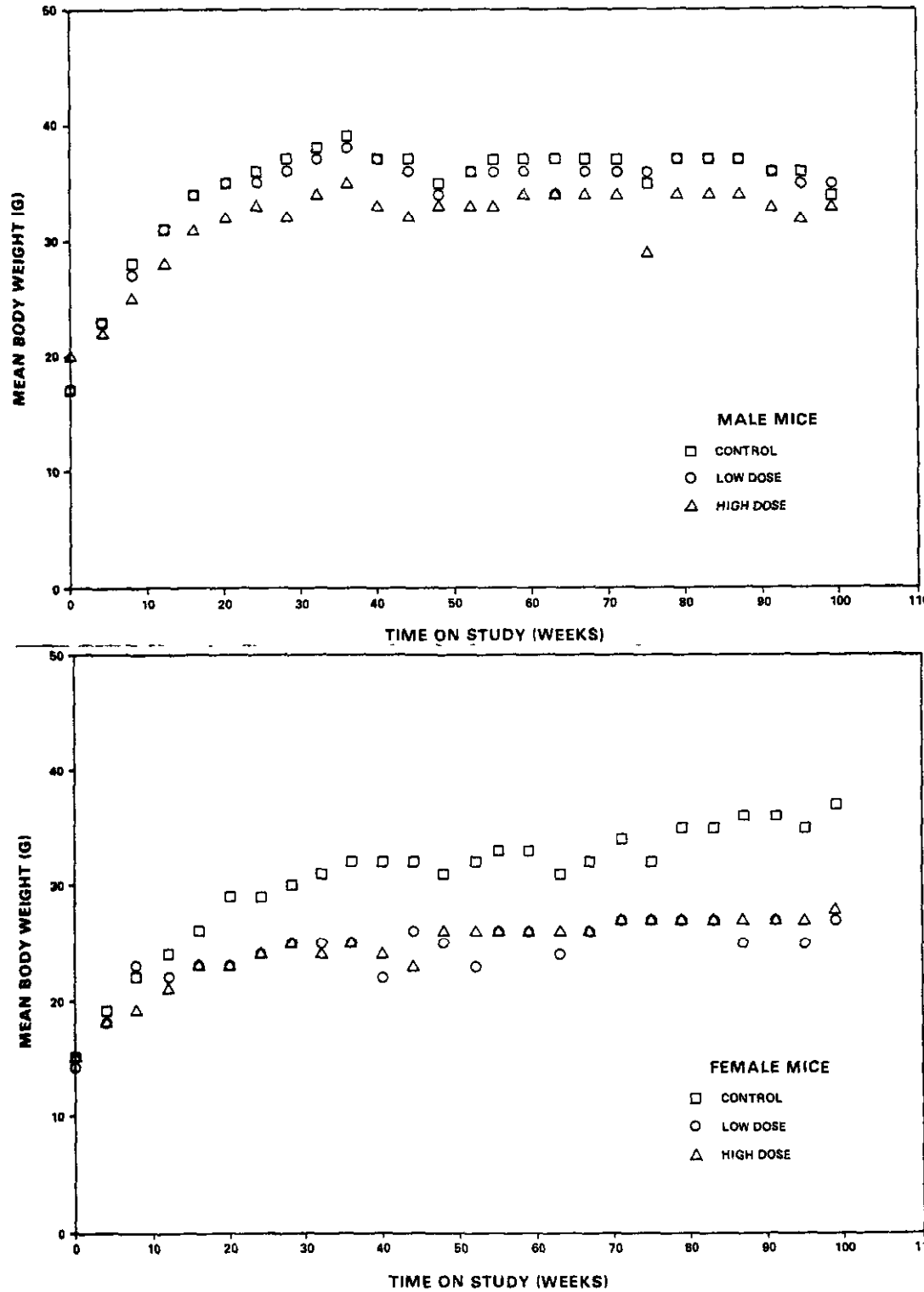

Figure 3. Growth Curves for Mice Fed Diets Containing Bisphenol A

ATTACHMENT 2

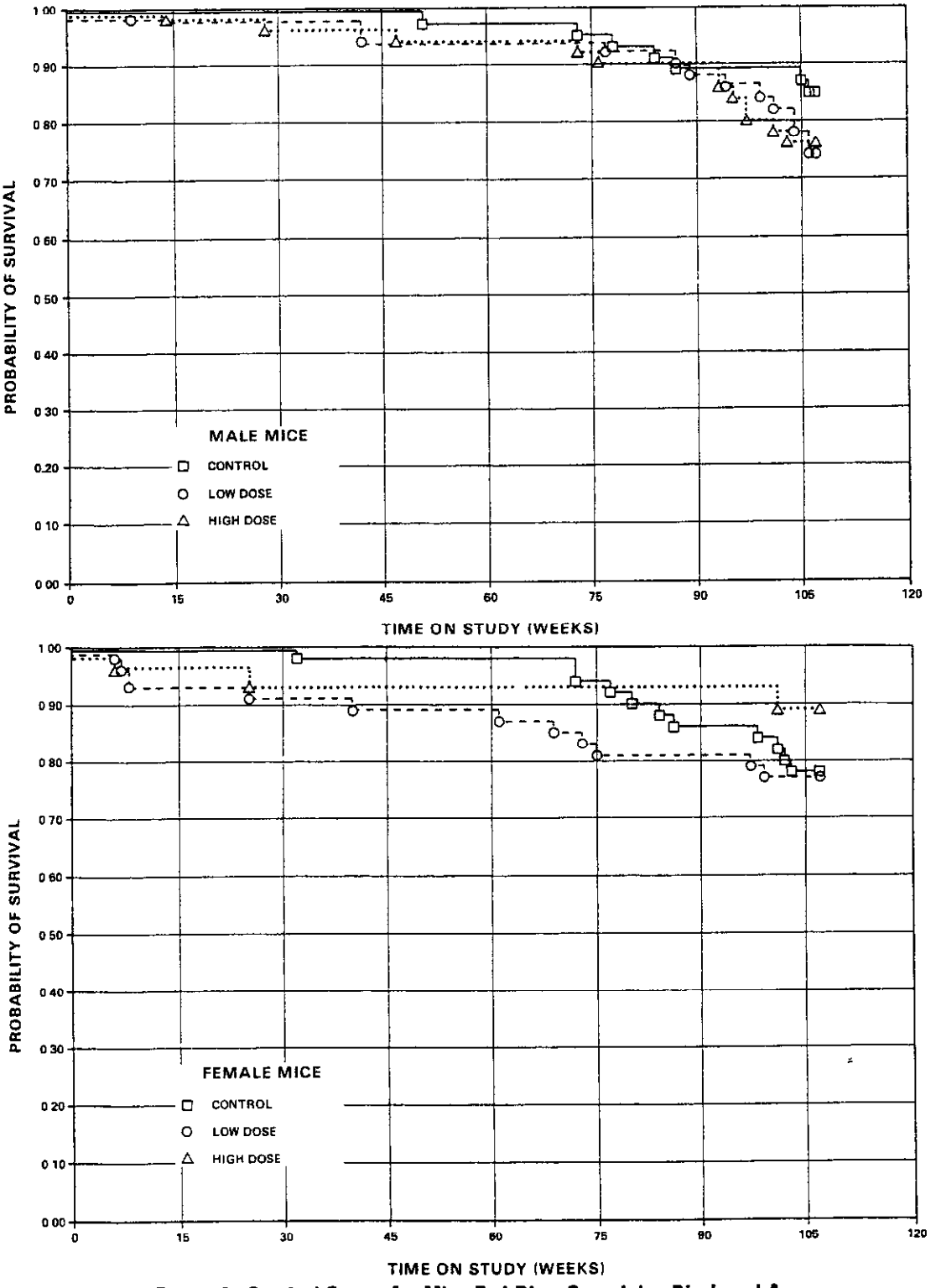

Figure 4. Survival Curves for Mice Fed Diets Containing Bisphenol A