



**Memorandum**

Date July 24, 2007

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

Subject Acceptance of Final TDERs for studies reviewed under contract with ICF Consulting (Contract Number 223-96-2302) for Food Additive Master File No 580 under Work Assignment 2000-20 (ICF 020), Tasks Number 1, 2 and 3

To Food Additive Master File 580

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

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Following the Agency's instructions, ICF Consulting, the contractor, has generated comprehensive Toxicological Data Evaluation Reports (TDERs) for the studies which are listed below. Dr. Mary E Shackelford has reviewed and accepted the final TDERs for Work Assignment Number 2000-20, Task Number 1, 2, and 3. The final TDERs for Work Assignment Number 2000-20 for Task Number 1, 2, and 3 may be incorporated into the file for Food Additive Master File 580

- 1 Bisphenol A: 2-Week Aerosol Toxicity Study with Fischer 344 Rats (Work Assignment Number 2000-20, Task Number 1)
- 2 Ninety Day Oral Toxicity Study in Dogs (Work Assignment Number 2000-20, Task Number 2)
- 3 Bisphenol A: 13-Week Aerosol Toxicity Study with Fischer 344 Rats (Work Assignment Number 2000-20, Task Number 3)

/s/ 

Mary E. Shackelford, Ph.D.

Attachments: Copy of final TDERs Task Number 1, 2, and 3, Work Assignment Number 2000-20 under Contract Number 223-96-2302



AUG 16 2001

**Contract Number**

223-96-2302

**Work Assignment Number**

2000-20  
(ICF 020)

**Task Number**

01

**FDA Study Identification Numbers**

HSE-85-0047

**Signature of Program Manager**

/s/ [Redacted Signature]

**Date**

8-7-01

## **STUDY TITLE**

Bisphenol A 2-Week Aerosol Toxicity Study with Fischer 344 Rats

## **TESTING LABORATORY**

Health and Environmental Sciences Mammalian and Environmental Toxicology Research Laboratory,  
Dow Chemical U.S.A., Midland, MI

## **COMPLETION DATE OF STUDY**

June 06, 1985

## **SPONSORS OF STUDY**

Dow Chemical U S A., Midland, MI

## **STUDY SUMMARY**

### ***Compliance and Quality Assurance Statement***

A signed and dated quality assurance statement was included in the study report with a list of inspection dates. Based on the provided dates, the quality assurance findings were reported promptly to the study director and management. The quality assurance statement also indicated that the report was in compliance with FDA and EPA Good Laboratory Practice Regulations.

### ***Study Objective***

This study was conducted to determine the toxicity of polycarbonate grade bisphenol A when administered to Fischer 344 rats (6-hours per day, 5-days per week for a total of 9 exposures) at concentrations of 0, 10, 50, or 150 mg/m<sup>3</sup> for 2-weeks. Animals were observed over a 4-week reversibility period following treatment to determine reversibility of any test article related effects and to assess any residual effects resulting from bisphenol A treatment

### ***Test Article***

The test article, polycarbonate grade bisphenol A (lot TB 84071221), was supplied by the Dow Chemical Co, Freeport, Texas. Polycarbonate grade was chosen because it contains finer grade, more respirable particles than epoxy grade. Molar purity was 99.93% as determined by differential scanning calorimetry. Physical properties of the test article were not included in the study report, however, the study authors did indicate that polycarbonate grade bisphenol A is generally a white to light tan solid. Storage conditions and stability of the test article were not reported

### ***Test Atmosphere Generation and Exposure Chamber Conditions***

The exposure chambers were described as 1000 liter stainless steel and glass Rochester-type chambers. Air flow in the chambers was kept at a rate of approximately 200 liters/min. Bisphenol A particles were aerosolized in a modified Marple dust generator using approximately 90 liters/min of dry, compressed

air. Test article particle size was assessed weekly using aerodynamic measurement. Test article concentration was monitored one to three times during each exposure period by gravimetric capture measurement on Teflon filters. The remaining air supplied to the test chambers was controlled with temperature settings kept at 70° F and relative humidity settings at 50%. Actual maximum and minimum temperatures and actual relative humidity values were recorded for each exposure period.

### ***Test Animals***

Six- to eight-week old Fischer F-344 rats (20/sex/dose) were supplied by Charles River Breeding Laboratories, Kingston, NY. Rats were examined upon arrival to the performing laboratory by the lab veterinarian.<sup>1</sup> All animals were acclimated according to Standard Operating Procedures of the Sub-chronic and Chronic Toxicology Laboratory.<sup>2</sup> Duration of the acclimatization period was not provided in the study report. Our reviewers note that standard toxicity guidelines recommend a one-week acclimatization period.

All animals were stratified by body weight; animals with outlying body weights were removed and discarded until only the number of animals needed for the study remained. Animals selected for study were then assigned to treatment groups randomly based on body weight. All animals were individually housed in stainless steel wire cages and were identified using ear tags. Animals were housed in rooms at 72°F with relative humidity at 50%. A 12-hour light cycle was used. Male animals weighed between 171.3 and 196.7 grams and female animals weighed between 101.2 and 125.5 grams at study initiation.

### ***Diet***

Certified Rodent Chow<sup>3</sup> and municipal tap water was provided to the animals *ad libitum* except during exposure periods. The Ralston Purina Co. analyzed the food for nutritional value and for contaminants associated with the formulation process. The City of Midland, Michigan performed an analysis of the water supply. The results of these analyses were not included in the study report.

### ***Experimental Design***

Twenty rats/sex/group were exposed to aerosols of bisphenol A at target concentrations of 0, 10, 50, or 150 mg/m<sup>3</sup>. Rats were exposed for 6-hours/day, 5-days per week, for a total of nine exposures. The highest dose selected (150 mg/m<sup>3</sup>) approximated the maximal achievable concentration of polycarbonate grade bisphenol A in air. Half of the animals (10 rats/sex/group) were sacrificed one-day after the final bisphenol A exposure (terminal sacrifice group) while the remaining 10 rats/sex/group were allowed to recover for an additional 29-days without additional exposure to the test article, and then sacrificed (recovery sacrifice group).

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<sup>1</sup> The performing laboratory was fully accredited by the American Association for Accreditation of Laboratory Animal Care (A.A.A.L.A.C.)

<sup>2</sup> Standard operating procedure was not provided in the study text.

<sup>3</sup> Certified Rodent Chow was supplied by Ralston Purina Co., St. Louis, MO

***Clinical Observations***

After each exposure period, animals were observed and changes in appearance were noted. Also, observations were made daily to check for mortality and availability of food and water. On weekends, observations and monitoring consisted only of removing dead animals and ensuring the availability of food and water.

***Body Weights***

Body weight measurements were taken prior to exposure 1, 5, 6, and 9 during the treatment phase and weekly during the 4-week recovery phase.

***Food Consumption***

Food consumption was not measured.

***Clinical Laboratory Determinations***

Hematology

Prior to necropsy, blood samples were collected from the orbital sinus from methoxyflurane anesthetized animals. Standard hematology parameters are listed below. A check indicates that the corresponding parameter was evaluated in all non-recovery groups for this study. In addition to the parameters included below, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were determined. Blood smears were prepared for all animals, but were not evaluated. Differential leukocyte counts were quantified by machine (Honeywell ACS 1000) only for control and high dose animals. Hematology parameters were not measured in recovery animals.

- |   |   |   |                              |
|---|---|---|------------------------------|
| ✓ | hematocrit/packed cell volume           | ✓ | total leukocyte count        |
| ✓ | hemoglobin                              | ✓ | differential leukocyte count |
| ✓ | erythrocyte count                       | ✓ | --neutrophils                |
| ✓ | clotting potential                      | ✓ | --lymphocytes                |
|   | --clotting time                         | ✓ | --eosinophils                |
|   | --prothrombin time                      | ✓ | --basophils                  |
|   | --activated partial thromboplastin time | ✓ | --monocytes                  |
| ✓ | --platelet count                        |   |                              |

Clinical Chemistry

At necropsy, blood samples were collected from severed cervical blood vessels. All samples were refrigerated or kept on ice until clinical chemistry analyses were performed. Standard clinical chemistry parameters are listed below. A check indicates that the corresponding parameter was evaluated in all non-recovery groups for this study. In addition to the parameters listed below, the study authors determined globulin concentration. Clinical chemistry parameters were not measured in recovery animals.

*Electrolytes*

calcium  
chloride  
phosphorus  
potassium  
sodium

*Other*

✓ albumin  
bilirubin (total)  
creatinine  
✓ glucose  
✓ protein (total)  
✓ urea nitrogen

*Enzymes*

✓ alanine aminotransferase<sup>4</sup>  
✓ alkaline phosphatase  
✓ aspartate aminotransferase<sup>5</sup>  
γ-glutamyl transferase  
ornithine decarboxylase

Our reviewers note that there was no evaluation of serum or plasma electrolytes. Evaluation of electrolytes is a critical indicator of overall health and nutritional plane as well as the integrity of multiple organ systems (e.g., calcium/phosphorus levels are monitors of parathyroid, kidney, bone, and intestine, etc.) Furthermore, there was no evaluation of total bilirubin which is an important monitor of erythrocyte breakdown/clearance as well as hepatic function/obstruction. Additionally, there was no evaluation of creatinine which is an important adjunct to the BUN evaluation to assess renal versus pre-renal changes. For these reasons, we considered the overall clinical chemistry evaluation to be incomplete.

Urinalysis

Urine was collected before the final bisphenol A exposure. The method of urine collection was not discussed in the study text. The following urinalysis parameters were determined in non-recovery animals only.

bilirubin  
glucose  
ketones  
blood  
specific gravity  
pH

protein  
urobilinogen

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<sup>4</sup> The study authors used the former name of this enzyme (glutamic pyruvic transaminase)

<sup>5</sup> The study authors used the former name of this enzyme (glutamic oxaloacetic transaminase)

### ***Ophthalmology***

An ophthalmic examination was not performed. Our reviewers note that standard toxicity guidelines recommend that ophthalmic observations be performed for short-term toxicity tests.

### ***Gross Pathology***

Ten animals in each dose-group of each sex were sacrificed via decapitation under methoxyflurane anesthesia one-day after the last exposure (terminal sacrifice animals). Ten recovery animals were sacrificed in the same manner 29- days after the final exposure (recovery sacrifice animals). Prior to sacrifice, all animals were fasted overnight. An ACVP-board-certified veterinary pathologist examined the animals for gross alterations at necropsy. The eyes were examined *in situ*.

### ***Organ weights***

At necropsy, the following organs were weighed. Relative organ weights (per 100 g body weight) were also calculated.

brain	kidneys
heart	thymus
liver	testes

### ***Histopathology***

A complete set of tissues was collected from all terminal sacrifice and recovery sacrifice animals as indicated by a check on the standard toxicity guideline tissue list below. Additional tissues collected included oviduct, larynx, lacrimal/Hardarian gland, oral tissues, mesenteric tissues, cervix, tongue, auditory sebaceous gland, coagulating gland, vagina, and mediastinal tissues. Also, the stomach, small intestine, and large intestine were collected, but the site was not specified.

Tissues were preserved in 10% neutral buffered formalin. Immersion fixation was used with two exceptions. Lungs were filled with fixative by airway perfusion until the normal inspiratory volume was approximated. Nasal tissues were flushed with a fixative using the pharyngeal duct to facilitate rapid fixation of the mucosal surfaces. Histologic processing and histologic evaluation were performed on complete tissue sets<sup>6,7</sup> from control and high-dose terminal sacrifice animals and on a limited set of respiratory tract tissues (nasal tissues, larynx, trachea, and lungs) from all recovery sacrifice animals as well as intermediate-dose terminal sacrifice animals.

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<sup>6</sup> Auditory sebaceous gland was collected by not processed or evaluated

<sup>7</sup> The study authors indicated that histologic processing was performed using “conventional techniques”. Our reviewers interpreted this to mean that fixed tissues were trimmed, placed in tissue cassettes, dehydrated through alcohols, embedded in paraffin, sectioned at 4-6  $\mu\text{m}$ , rehydrated, and stained with hematoxylin-eosin.

*Cardiovascular/Hemopoietic*

- ✓ aorta
- ✓ bone marrow
- ✓ heart
- ✓ lymph node (mesenteric and mediastinal)
- ✓ spleen
- ✓ thymus

*Digestive system*

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

*Urogenital*

- ✓ epididymides
- ✓ kidneys
- ✓ ovaries (ovary)
- ✓ prostate
- ✓ seminal vesicle
- ✓ testes
- ✓ urinary bladder
- ✓ uterus

*Neurologic/Special Senses*

- ✓ brain
- ✓ eyes
- ✓ pituitary
- ✓ sciatic nerve (peripheral nerve)
- ✓ spinal cord

*Glandular*

- ✓ adrenals
- ✓ mammary gland
- ✓ thyroid/parathyroid

*Respiratory*

- ✓ lungs
- ✓ nasal turbinates (nasal tissue)
- ✓ trachea

*Musculoskeletal*

- ✓ bone
- ✓ skeletal muscle

*Other*

- all gross lesions
- ✓ skin

***Statistical Analysis***

Quantitative data (i.e., absolute and relative organ weights, clinical chemistry data, appropriate hematology data, and urine specific gravity) were evaluated by Bartlett's test for equal variance ( $p \leq 0.01$ ). A parametric or nonparametric analysis of variance (ANOVA) was then performed ( $p \leq 0.1$ ) based on the results of Bartlett's test. Dunnett's test ( $p \leq 0.05$ ) or the Wilcoxon Rank-Sum test ( $p \leq 0.05$ ) with a Bonferroni correction for multiple comparisons was then performed. Outliers were identified using a

sequential test ( $p \leq 0.02$ ) and excluded from analysis. Results of the statistical analyses were then interpreted for toxicological and biological significance. “Descriptive statistics” (i.e., means and standard deviations) were used to present white blood cell differential counts and red blood cell indices.

### *Appraisal of Experimental Design*

A study protocol was not included with the study report for review. Our reviewers feel that the use of polycarbonate test article and the concentrations employed to be well-considered and well-justified in the study report. However, we do note several important deficiencies. The clinical pathology assessment (hematology, clinical chemistry and urinalysis) was incomplete as several important components were omitted: (1) no evaluation of clotting factor function as an indicator of the ability to form a stable clot, (2) no evaluation of serum or plasma electrolytes (critical indicators of overall health and nutritional plane as well as the integrity of multiple organ systems), (3) no evaluation of total bilirubin (important monitor of erythrocyte turnover and hepatic function); and (4) no evaluation of creatinine (important adjunct to urea nitrogen evaluation). We also note that food consumption was not measured in the present study. Although the study was an inhalation study and not a feeding study, we consider measurement of food consumption a very useful component of most toxicity studies. Last, an ophthalmic examination was not performed as standard toxicity guidelines recommend. Because of these deficiencies, our reviewers consider that this study was only marginally sensitive to detect toxic effects of bisphenol A when administered to rats via whole body inhalation.

## RESULTS

### *Exposure Concentrations*

The study authors concluded that the mean analytical concentrations were similar to the desired exposure concentrations. Selected data concerning exposure concentrations and particle characteristics are reported in Table 1 below.

**Table 1. Exposure Concentrations and Mass Median Aerodynamic Diameter of the Test Article**

<i>Target Concentration (mg/m<sup>3</sup>)</i>	<i>Mean Measured Concentration (mg/m<sup>3</sup>)<sup>8</sup></i>	<i>Range of Daily Analytical Concentration (mg/m<sup>3</sup>)</i>	<i>Mass Median Aerodynamic Diameter</i>
0	0.2 ± 0.2	0.0 to 0.5	--
10	11.1 ± 1.6	8.8 to 14.6	6.2 ± 0.5
50	54.5 ± 21.1	19.6 to 93.7	2.6 ± 1.1
150	143.1 ± 33.6	73.8 to 183.2	3.4 ± 0.7

<sup>8</sup> Determined from the daily time weighted average concentration

Our reviewers note that while the mean measured concentration was similar to the target concentration in all dose groups, there is a wide range of daily analyzed concentrations as noted by the study authors. The study authors attributed this to mechanical problems. Our reviewers note that the wide range of daily exposure levels may have compromised the results of this study because, at times, animals in the high-dose group were being exposed to a lower concentration of test-article than animals in the mid-dose group. However, the study results were not likely drastically affected because the time-weighted mean measured concentration did approximate target concentrations.

Inside exposure chambers, the average temperature was between 21 and 26°C (69.8 - 78.8°F)<sup>9</sup> and average relative humidity was between 18 and 26%. Our reviewers note that the relative humidity measured in the exposure chambers was approximately half the target of 50%. Our reviewers note the chambered animals likely had increased insensible water losses compared to “caged” laboratory rodents, but that the losses should have been equivalent between test and control animals with minimal impact on the study.

### ***Clinical Observations***

A summary of clinical observations and individual animal data concerning clinical observations was not included in the study report, however, the study authors indicated that animals exposed to 10 mg/m<sup>3</sup> did not exhibit clinical effects significantly different from control animals. Animals exposed to 50 and 150 mg/m<sup>3</sup> of bisphenol A exhibited reddish material around the nose during the exposure period. The study authors noted that the reddish material may have been porphyrin. Our reviewers also note that hemorrhage or serosanguinous nasal discharge/exudate can give the appearance of “reddish material around the nose.”

Females in the 150 mg/m<sup>3</sup> exposure group also exhibited perineal soiling during the 2-week exposure period. The study authors attributed these effects to bisphenol A treatment. These effects were not seen several days after the recovery phase of the study began.

### ***Body Weights***

Group mean body weights of all treated males were slightly decreased at days 5, 8, and 11 (See Table 2 below). A dose dependent trend was evident at each time point and the difference was statistically significant at day 8 and at necropsy in the high-exposure group only. After treatment with bisphenol A was ceased, body weights were similar to control values in all exposure groups. A similar effect was not seen in female animals.

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<sup>9</sup> Conversion was made by our reviewers.

**Table 2. Mean Body Weights of Male Fischer Rats Exposed to Bisphenol A Aerosol for 2-Weeks**

Exposure Concentration	Experiment Day				
	1	5	8	11	At Necropsy <sup>a</sup>
0	186.1 ± 5.8	197.4 ± 7.2	209.3 ± 8.1	213.1 ± 8.7	191.5 ± 5.4
10	185.0 ± 5.7	196.4 ± 8.2	207.4 ± 9.5	211.6 ± 10.6	187.1 ± 9.8
50	183.9 ± 6.2	193.4 ± 6.9	203.6 ± 7.8	208.9 ± 8.7	187.3 ± 6.8
150	184.0 ± 5.5	192.6 ± 5.2	202.7 ± 5.3*	207.1 ± 5.8	182.8 ± 5.2*

\* Statistically significant at  $\alpha = 0.05$  using Dunnett's test

<sup>a</sup> Terminal sacrifice animals

### *Hematology*

Analysis of hematology parameters did not reveal treatment-related effects. No statistically significant values were seen and no dose-dependent relationships were evident in any hematology parameter.

### *Clinical Chemistry*

The study authors concluded that no treatment-related effects were detected with respect to clinical chemistry parameters. Our reviewers note that statistically significant ( $\alpha = 0.05$ ) changes were seen with respect to urea nitrogen, albumin, glutamic oxaloacetic transaminase, and glucose as indicated in Table 3 below. The changes were slight in all parameters and a dose-dependent relationship was not seen with the exception of albumin concentration in males. Our reviewers note that data from Charles River Laboratory<sup>10</sup> (6 - 8 weeks old male Fischer rats) indicate that the normal range of albumin is from 3.4 - 4.0 g/dL. The statistically significant value in the 150 mg/m<sup>3</sup> group is slightly outside of this range (Charles River Laboratories 1998). Therefore, our reviewers agree that changes in urea nitrogen, GOT, and glucose were not treatment-related. However, we do suggest that the decrease in albumin concentration seen in the high-dose group may be test-article related because the decrease was statistically significant, a dose-dependent trend was seen, and the value was outside of the normal range. A statistically significant value was also seen in female rats that was outside of the normal range for 6 - 8 week old female Fischer rats (3.3 - 4.3 g/dL); however, our reviewers do not consider this to be test-article-related because a dose-response relationship was not evident.

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<sup>10</sup> Charles River Laboratories, Technical Bulletin, Spring 1998

**Table 3. Statistically Significant Clinical Chemistry Parameters**

Exposure Concentration (mg/m <sup>3</sup> )	Clinical Chemistry Parameter							
	Blood Urea Nitrogen (mg/dL)		Glutamic Oxaloacetic Transaminase (mU/ml)		Glucose (mg/dL)		Albumin (g/dL)	
	<i>Males</i>	<i>Females</i>	<i>Males</i>	<i>Females</i>	<i>Males</i>	<i>Females</i>	<i>Males</i>	<i>Females</i>
0	16±1	16±1	107±12	101±15	133±12	126±13	3.5±0.1	3.1±0.1
10	17±2*	17±1	117±32	89±40*	134±22	119±7	3.4±0.1	3.1±0.1
50	16±1	26±26*	117±30	94±15	121±12	112±7*	3.4±0.1	3.2±0.1
150	17±1*	17±3	105±19	107±47	134±18	115±16	3.3±0.1*	3.0±0.2*

\* Statistically significant at p≤0.05

**Urinalysis**

The study authors indicated that no treatment-related effects were seen with respect to urinalysis parameters. Our reviewers note that specific gravity was statistically significantly elevated above control values in the 50 and 150 mg/m<sup>3</sup> exposure groups as indicated in Table 4 below. A clear dose-related trend was not evident, all values remained within normal reference ranges (1.001 - 1.075 [Hall 1992]), and the increase was slight; therefore, our reviewers agree that no treatment-related effects were evident with respect to urinalysis parameters. Our reviewers also note that a dose-related decrease in pH was seen in males and the mean urine pH in high-dose females was approximately half of a pH unit less than the mean control value. Our reviewers note that these decreases in urine pH could have been treatment related, however, we also note that bacterial growth and loss of CO<sub>2</sub> in standing urine samples may affect the urine pH (Hall 1992).

**Table 4. Urine Specific Gravity and pH Values from Male and Female Fischer Rats Exposed to Bisphenol A Aerosol for 2-Weeks**

Exposure Concentration (mg/m <sup>3</sup> )	Specific Gravity		pH	
	<i>Males</i>	<i>Females</i>	<i>Males</i>	<i>Females</i>
0	1.033±0.017	1.044±0.009	8.10±0.70	8.00±0.62
10	1.036±0.014	1.034±0.011	7.75±0.48	8.15±0.41
50	1.056±0.009*	1.034±0.011	7.72±0.62	7.95±0.55
150	1.055±0.012*	1.045±0.009	7.20±0.59	7.45±0.55

\* Statistically significant at p≤0.05

### ***Organ Weights***

The study authors indicated that exposure-related effects were not seen with respect to organ weights (absolute or relative). Our reviewers note that brain weights relative to body weights were statistically significantly increased in males exposed to 150 mg/m<sup>3</sup> bisphenol A. The study authors attributed the increase in relative brain weights to the statistically significant decrease in body weights of the same exposure group. Based on Table 7 in the study report, our reviewers agree. Our reviewers also note that a statistically significant increase in relative liver weights was seen in females in recovery animals of the high exposure group and a dose-dependent trend is evident. Because the increase was slight and only seen in females, our reviewers do not attribute this observation to treatment. Our reviewers also note that the body weights of this exposure group were the lowest of the four groups, which may have contributed to the relative increase in liver weights.

### ***Gross Pathology***

Facial soiling and decreased abdominal fat were observed in animals exposed to 150 mg/m<sup>3</sup> of bisphenol A upon gross examination. The study authors attributed these findings to test-article treatment. Incidence of decreased abdominal fat and facial soiling are presented in Table 5 below. Our reviewers agree that these observations were related to bisphenol A treatment because a dose-response relationship was clear. These changes were not seen in the 10 mg/m<sup>3</sup> group. We note that the observation of decreased abdominal fat was consistent with the statistically significant decreases in terminal body weights in terminal sacrifice, high-dose males and the trend to decreased terminal body weights in the terminal sacrifice, high-dose females.

**Table 5. Treatment-Related Gross Pathology Observations**

Exposure Concentration (mg/m <sup>3</sup> )	Gross Pathology Parameter			
	<i>Decreased fat in abdominal cavity</i>		<i>Facial soiling</i>	
	Males	Females	Males	Females
0	0/10 <sup>a</sup>	0/10	0/10	0/10
10	0/10	0/10	0/10	0/10
50	0/10	0/10	2/10	0/10
150	3/10	4/10	8/10	6/10

<sup>a</sup> Number affected/number examined

### ***Histopathology***

Treatment-related, microscopic pathology alterations were restricted to nasal tissues (Table 6 below). Male rats exposed to 150 mg/m<sup>3</sup> and female rats exposed to either 50 or 150 mg/m<sup>3</sup> bisphenol A exhibited very slight to slight inflammation in the vestibule region of the external nares and at the mucotaneous junction. Additionally, high-dose and intermediate-dose males and females developed low-grade hyperplasia of the squamous epithelium at the mucocutaneous junction in the anterior portion of the nasal cavity. The intensity of the nasal changes was generally very slight in intermediate-dose

animals and slight in high-dose animals, consistent with a dose-response. The study authors noted that hyperplastic change extended into the nasal cavity to involve the respiratory epithelium overlying the vomeronasal organ. The study authors also noted that the pathologic examination revealed no further tissue alterations that were attributed to treatment.

**Table 6. Treatment-Related Microscopic Pathology Observations**

Microscopic Finding	Sex	Exposure Concentration (mg/m <sup>3</sup> )			
		0	10	50	150
Nasal tissue hyperplasia, mucocutaneous junction, bilateral, slight or very slight	<i>Males</i>	0/10 <sup>a</sup>	0/10	7/10	10/10
	<i>Females</i>	0/10	0/10	9/10	10/10
Nasal tissue Inflammation, Subacute to chronic, external nares, bilateral, focal, slight or very slight	<i>Males</i>	2/10	0/10	1/10	10/10
	<i>Females</i>	0/10	0/10	3/10	8/10
Nasal tissue Inflammation, Subacute to chronic, mucocutaneous, bilateral, focal, slight or very slight	<i>Males</i>	0/10	0/10	1/10	10/10
	<i>Females</i>	2/10	0/10	6/10	10/10

<sup>a</sup> Number affected/number examined

No treatment-related effects were detected in the low-dose animals or in recovery animals.

### ASSESSMENT

Our reviewers believe this study's ability to evaluate short-term, multiple dose, inhalation toxicity associated with test-article exposure was limited by the following study deficiencies. First, food consumption was not measured in the present study. Although the study was an inhalation study and not a feeding study, food consumption data would have been useful information in light of the test article effects on decreased body weights and decreased abdominal fat. Second, the clinical pathology assessment (hematology, clinical chemistry and urinalysis) was incomplete as several important components were omitted: (1) no evaluation of clotting factor function as an indicator of the ability to form a stable clot, (2) no evaluation of serum or plasma electrolytes (critical indicators of overall health and nutritional plane as well as the integrity of multiple organ systems), (3) no evaluation of total bilirubin (important monitor of erythrocyte turnover and hepatic function), and (4) no evaluation of creatinine (important adjunct to urea nitrogen evaluation). Also, urine was collected prior to the final bisphenol A exposure, which may have compromised the sensitivity of the study results because the

urinalysis parameters were evaluated after only 8 bisphenol A exposures, while all other parameters were evaluated after 9 bisphenol A exposures. The shorter time duration may have compromised the urinalysis results. Additionally, technical problems prevented a consistent exposure concentration in the exposure chambers. This resulted in a wide variation of exposure concentrations within each exposure group. Although the mean daily exposures were similar to each group's respective target concentration, the wide variation of exposure concentrations may have compromised the study because the high dose group (150 mg/m<sup>3</sup>), at times, was receiving a lower concentration of test article than the mid-dose group (50 mg/m<sup>3</sup>). Similarly, the mid-dose group was, at times, being exposed to a similar concentration as the low-dose group. Last, clinical chemistry, hematology, and urinalysis parameters were not evaluated in recovery animals. Therefore, our reviewers feel that the study design was not sufficient to detect residual effects caused by bisphenol A treatment in recovery animals. Because of these limitations, our reviewers consider that this study was only marginally sensitive to detect toxic effects of bisphenol A aerosol when administered to Fisher rats for two-weeks via whole body inhalation exposure.

### CONCLUSIONS

Treatment-related effects are listed in Table 7 below and include a slight decrease in body weight gain (male rats exposed to 150 mg/m<sup>3</sup> [a non-statistically significant trend was also seen in females of this exposure group]), decreased abdominal fat, and histopathologic changes including anterior nasal inflammation and/or epithelial hyperplasia (male and female rats exposed to 50 and 150 mg/m<sup>3</sup>). No treatment-related effects were seen with respect to hematology, urinalysis, or organ weight data. No treatment-related effects were seen in recovery animals, no systemic effects were seen in any treated animals, and no treatment-related effects were seen in animals exposed to 10 mg/m<sup>3</sup>; thus the NOEL was 10 mg/m<sup>3</sup>. However, the sensitivity of the study to detect toxic effects was compromised by several factors including inadequate clinical chemistry evaluation and the wide range of daily exposure concentrations seen in each exposure group.

**Table 7. Selected Treatment Related Effects**

<b>Males</b>		
<i>Dose Group</i>	<i>Effect</i>	<i>Severity</i>
150 mg/m <sup>3</sup>	Decreased abdominal fat	Not reported
	Decrease in body weight gain	Slight
	Histopathologic changes in the anterior portion of the nasal cavity	Slight to very slight
50 mg/m <sup>3</sup>	Histopathologic changes in the anterior portion of the nasal cavity	Slight to very slight

<b>Females</b>		
150 mg/m <sup>3</sup>	Decreased abdominal fat	Not reported
	Histopathologic changes in the anterior portion of the nasal cavity	Slight to very slight
50 mg/m <sup>3</sup>	Histopathologic changes in the anterior portion of the nasal cavity	Slight to very slight to very slight

### **EXECUTIVE SUMMARY**

Fischer 344 rats (20/sex/dose) were administered bisphenol A via inhalation in whole body exposure chambers at target concentrations of 0, 10, 50, or 150 mg/m<sup>3</sup> for 14 days. Half the animals were sacrificed immediately after the 2-week exposure period and half the animals were sacrificed after a 4-week recovery period. Treatment-related effects included a slight decrease in body weight gain (male rats exposed to 150 mg/m<sup>3</sup> [a non-statistically significant trend was also seen in females of this exposure group]), decreased abdominal fat, and histopathologic changes including anterior nasal inflammation and/or epithelial hyperplasia (male and female rats exposed to 50 and 150 mg/m<sup>3</sup>). No treatment-related effects were observed in the following endpoints: mortality, hematology parameters, urinalysis, or organ weights. No systemic, treatment related effects were seen in any dose group. No treatment-related effects were seen after a 29 day recovery period. The NOEL was considered to be 10 mg/m<sup>3</sup>. The sensitivity of the study to detect toxic effect was compromised by inadequate clinical chemistry evaluation and the wide range of daily exposure concentrations seen in each exposure group.

### **REFERENCES**

Hall, RL. Clinical pathology of laboratory animals. In Gad SC, Chengelis CP (eds) Animal Models in Toxicology, Marcel-Dekker, Inc. 1992

**Bisphenol A: 2-Week Aerosol Toxicity Study with Fischer 344 Rats**

**Technical Reviewer**

/s/ [Redacted Signature]

Brian Pitner Anderson, M.E.M.

7-20-01

Date

**QA/QC Reviewer**

/s/ [Redacted Signature]

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

7-20-01

Date

## Appendix A: Data Validation

### Bisphenol A: 2-Week Aerosol Toxicity Study with Fischer 344 Rats

#### I. Animals Followed Throughout the Study

20% of all animals used in this study were selected for review using the random number generator in Microsoft EXCEL and are listed in the following table. Data for all animals selected for review were checked in all tables containing individual animal data. No abnormalities were noted in examining the individual animal data.

##### Males:

##### Females:

0 mg/m <sup>3</sup>	10 mg/m <sup>3</sup>	50 mg/m <sup>3</sup>	150 mg/m <sup>3</sup>	0 mg/m <sup>3</sup>	10 mg/m <sup>3</sup>	50 mg/m <sup>3</sup>	150 mg/m <sup>3</sup>
84A9443	84A9453	84A9488	84A9498	84A9515	84A9547	84A9570	84A9590
84A9431	84A9470	84A9484	84A9507	84A9518	84A9543	84A9561	84A9575
84A9433	84A9461	84A9490	84A9500	84A9519	84A9550	84A9567	84A9571
84A9448	84A9464	84A9479	84A9505	84A9528	84A9534	84A9562	84A9578

#### II. Critical Effects and Spot Checks

A study protocol was not included in the study report; therefore, a comparison of the study protocol to the materials and methods was not performed. Means and standard deviations were calculated and incident counts were determined where applicable. All findings are presented below.

- The symbol, #, in Table A-6 for female clinical chemistry data (pg. 41), was not defined. Because it was defined in later tables, our reviewers assume it is probably a statistical outlier which was not excluded from analysis.
- The footnotes in Table A-5 (male clinical chemistry data, pg. 40) and A-7 (urinalysis data, pg. 42) are illegible because the text was only partially xeroxed; therefore, this data could not be validated.
- In Table A-2 (female body weight data, pg. 34-35), the study authors noted that individual 84A9581 in the 150 mg/m<sup>3</sup> exposure group was excluded from analysis after day 1 because the rat was without water for an undetermined length of time. However, data for this animal were excluded only for body weight calculations and were included for all other calculations (e.g. organ weight).
- The study authors reported that only 19 animals were used to calculate the mean body weight for the 150 mg/m<sup>3</sup> exposure group females on day 1. However, all 20 animals were apparently used for this calculation.

Group mean values or incident counts were verified through calculation using individual animal data in the following tables:

- Table 3 (pg. 16): Body weight as indicated in the table below;

<b>Bisphenol A Concentration (mg/m<sup>3</sup>)</b>	<b>Day- Males</b>	<b>Day- Females</b>
0	11, 25	11, 18
10	5, 39	8, 39
50	1, 32	1, 18
150	11, 32	5, 18

- Table 4 (pg. 17): Hematological Values (all data for males, 50 and 150 mg/m<sup>3</sup> exposure group, and females, 0 and 50 mg/m<sup>3</sup> exposure group);
- Table 5 (pg. 18): Clinical Chemistry Data as indicated in the table below;

<b>Bisphenol A Concentration (mg/m<sup>3</sup>)</b>	<b>Parameter Validated*</b>	
	<b>Males:</b>	<b>Females:</b>
0	UN, GPT, AP, GOT	OPT, AP
10	Gluc, TP, Alb, Glob	GOT, Gluc
50	UN, GPT, AP, GOT	UN, GPT, AP
150	Gluc, TP, Alb, Glob	GOT, Gluc

\* Abbreviations found in Table 5 of the study text.

- Table 6 (pg. 19): Urinalysis Data (males, 0 and 150 mg/m<sup>3</sup> exposure group, and females, 10 and 50 mg/m<sup>3</sup> exposure group);
- Table 7 (pg. 20): Organ Weights for Animals Necropsied on Experiment Day 12 (males, 10 and 150 mg/m<sup>3</sup> exposure group, and females 0 and 50 mg/m<sup>3</sup> exposure group); and
- Table 8 (pg. 21): Organ Weights for Animals Necropsied on Experiment Day 40 (males, 0 and 150 mg/m<sup>3</sup> exposure group, and females 10 and 50 mg/m<sup>3</sup> exposure group).

The only finding noted was the mean thymus gland weights for females in the 50 mg/m<sup>3</sup> exposure group could not be calculated because of illegible individual data in table A-12 (pg. 51), female organ/body weight data. Otherwise, no inconsistencies were observed between the summary data tables and our calculations using individual animal data.

### III. Findings

As noted above, discrepancies or inconsistencies revealed in this data validation included unclear or illegible footnotes and data. These issues, however, do not affect the confidence in this data study report.

/s/ [Redacted Signature] /s/

Lila Chen, B.S.

6/28/06  
Date

AUG 16 2001

**Contract Number**

223-96-2302

**Work Assignment Number**

2000-20  
(ICF 020)

**Task Number**

02

**FDA Study Identification Numbers**

313-079

**Signature of Program Manager**

/s/ [Redacted Signature]

**Date**

8-7-01

## **STUDY TITLE**

Ninety Day Oral Toxicity Study in Dogs

## **TESTING LABORATORY**

International Research and Development Corporation

## **COMPLETION DATE OF STUDY**

August 3, 1976

## **SPONSORS OF STUDY**

International Research and Development Corporation

## **STUDY SUMMARY**

### ***Compliance and Quality Assurance Statement***

A compliance statement or a quality assurance statement was not included in the study report. A protocol was also not included in the study report. Our reviewers note that the study was conducted prior to the issuance of FDA Good Laboratory Practice regulations.

### ***Study Objective***

This study was conducted to assess the toxicity of bisphenol A when administered to beagle dogs in the diet for 90-days.

### ***Test Article***

Bisphenol A was described as a crystalline white powder. The test-article was received from the General Electric Company, Mt. Vernon, IN on January 19, 1976. There was no information regarding purity or stability of the test article.

### ***Test Animals***

The protocol required 32 beagle dogs (4/sex/dose-group). At the beginning of the study, male dogs weighed 6.6 to 13.4 kg and females weighed 6.5 to 10.7 kg. The ages and sources of the dogs were not provided. However, our reviewers compared the body weights to the growth curves provided by Haggerty (1992). This comparison suggested that the dogs ranged from 4 months to 8 months at the beginning of the study. Our reviewers considered that some of the dogs may have been older at study initiation than recommended by standard toxicity testing guidelines. However, we note that Haggerty (1992) indicates that dogs up to 12 months of age are acceptable.

All animals were individually housed in metal metabolism cages. Humidity and temperature were controlled, however, the target range of values for temperature and humidity were not reported. Our reviewers note that standard toxicity guidelines recommend that the temperature should be kept at a

range of 64.4 to 84.2° F (16 to 27 ° C) and relative humidity should be kept at a range of 30 to 70%. Temperature and relative humidity above this range may affect food consumption and susceptibility to toxic agents

Food (ground Purina Dog Chow) and water were available to the animals *ad libitum*. Medicine was administered to the animals to expel intestinal worms and vaccines against canine distemper, hepatitis, leptospirosis, and rabies were provided prior to study initiation. The study authors indicated that a conditioning period was present prior to study initiation, however, the study authors did not indicate the duration of the conditioning period. Our reviewers note that standard toxicity guidelines recommend a 7-day acclimatization period for sub-chronic toxicity tests in dogs. Lastly, there was no information to indicate whether an initial veterinary examination was conducted to ensure that only healthy dogs were placed on study.

### ***Diet***

Diet formulations were prepared by making a concentrated premix, then diluting and mixing the premix with basal diet in a blender. Homogeneity analysis was not performed on the diets. A concentration analysis was not performed on the test diets.

### ***Experimental Design***

Four dogs/sex/group were exposed to bisphenol A at target concentrations of 0, 1000, 3000, or 9000 ppm in the diet for 90 days. The data in Table 1 in the study report indicate the dogs were weight stratified prior to group assignment. There was no discussion of randomization.

### ***Clinical Observations***

Observations were made daily for changes in physical appearance and behavior. A general physical examination was conducted monthly during the study and once during the conditioning period.

### ***Body Weights and Food Consumption***

Body weights and food consumption were measured weekly. Spillage was not addressed; our reviewers note that it is particularly important to measure spillage in *ad libitum* feeding studies.

### ***Clinical Laboratory Determinations***

#### **Hematology**

Hematology parameters were determined one time each month during treatment. Standard hematology parameters are listed below. A check indicates that the corresponding parameter was evaluated in all groups for this study. In addition to the parameters included below erythrocyte sedimentation rate and reticulocyte count were also determined. The method of blood collection was not reported.

- |   |                                |
|---|--------------------------------|
| ✓ hematocrit/packed cell volume         | ✓ total leukocyte count        |
| ✓ hemoglobin                            | ✓ differential leukocyte count |
| ✓ erythrocyte count                     | ✓ --neutrophils                |
| clotting potential                      | ✓ --lymphocytes                |
| ✓ --clotting time (partial evaluation)  | ✓ --eosinophils                |
| ✓ --prothrombin time                    | ✓ --basophils                  |
| --activated partial thromboplastin time | ✓ --monocytes                  |
| --platelet count                        |                                |

Our reviewers note that the assessment of clotting potential was incomplete and that partial thromboplastin time and platelet counts were not measured

### Clinical Chemistry

Standard clinical chemistry parameters are listed below. A check indicates that the corresponding parameter was evaluated in all groups for this study. In addition to the parameters listed below, the study authors also determined globulin concentration, cholesterol, and albumin/globulin ratio. The method of blood collection was not reported. Our reviewers note that there was no evaluation of serum or plasma electrolytes. Evaluation of electrolytes is a critical indicator of overall health and nutritional plane as well as the integrity of multiple organ systems (e.g., calcium/phosphorus levels are monitors of parathyroid, kidney, bone, and intestine, etc.). There was also no evaluation of creatinine which is an important adjunct to the BUN evaluation to assess renal versus pre-renal changes. For these reasons, we considered the overall clinical chemistry evaluation to be incomplete.

#### *Electrolytes*

calcium  
chloride  
phosphorus  
potassium  
sodium

#### *Other*

✓ albumin  
✓ bilirubin  
creatinine  
✓ glucose (fasting)  
✓ protein (total)  
✓ urea nitrogen

#### *Enzymes*

✓ alanine aminotransferase<sup>1</sup>  
✓ alkaline phosphatase  
✓ aspartate aminotransferase<sup>2</sup>  
γ-glutamyl transferase  
ornithine decarboxylase

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<sup>1</sup> The study authors used the former name of this enzyme (glutamic pyruvic transaminase)

<sup>2</sup> The study authors used the former name of this enzyme (glutamic oxaloacetic transaminase).

## Urinalysis

The following urinalysis parameters were determined. Color and appearance of the urine were visually inspected and the sediment was examined microscopically.

volume	pH
bilirubin	specific gravity
glucose	occult blood
	albumin

## ***Ophthalmology***

An ophthalmic examination was conducted on all dogs prior to exposure and at 3 months using an indirect ophthalmoscope. Pupils were dilated with 1% Tropicamide solution. Initial evaluation established that clarity of the precorneal tear film, cornea, aqueous lens, and vitreous as well as fundic reflexes. Subsequently, the ocular adnexa and iris were viewed under magnification and the lens focal distance was altered to permit visualization of the fundus.

## ***Gross Pathology***

After the 90-day treatment period, all animals were exsanguinated under sodium pentobarbital anesthesia. At necropsy, selected organs were collected in buffered neutral 10% formalin from all animals. Further details regarding gross pathology procedure were not provided. The study authors did not indicate any procedure used to minimize gross pathology bias (e.g., randomization of animal order for necropsy). Our reviewers note that multiple personnel may be involved in recording gross lesions and may vary in their thoroughness, threshold for recording (e.g. what is red to one person may be pink to another), and terminology used. Therefore, procedures to minimize bias are necessary.

## ***Organ weights***

The following organs were weighed at necropsy as indicated in Table 18 of the study report.

spleen	testes
liver	ovaries
adrenals	heart
kidneys	thyroid/parathyroid
brain	pituitary

## ***Histopathology***

All tissues selected for histologic examination were fixed in 10% neutral buffered formalin. Tissues were then embedded in paraffin, sectioned and stained with hematoxylin and eosin. A standard list of tissues recommended for histologic examination is provided below. A check in the list indicates that the tissue was examined in all control and high-dose group animals. Details provided by the study authors are provided in parentheses. In addition to the tissues indicated below, the study authors reported that the gall bladder, rectum, and tongue were retained and examined. Based on findings in control and high-dose group animals, dogs exposed to 1000 or 3000 ppm were not examined histologically. Our reviewers

consider the histopathologic examination incomplete because of the failure to examine the heart, bone, and mammary gland

#### Standard List of Tissues for Histopathologic Examination

##### *Cardiovascular/Hemopoietic*

- ✓ aorta
- ✓ bone marrow
- heart
- ✓ lymph node (mesenteric)
- ✓ spleen
- ✓ thymus

##### *Digestive system*

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

##### *Urogenital*

- epididymides
- ✓ kidneys
- ✓ ovaries
- ✓ prostate
- seminal vesicle
- ✓ testes
- ✓ urinary bladder
- ✓ uterus

##### *Neurologic/Special Senses*

- ✓ brain
- ✓ eye (with optic nerve)
- ✓ pituitary
- ✓ nerve
- ✓ spinal cord

##### *Glandular*

- ✓ adrenals
- mammary gland
- ✓ thyroid/parathyroid

##### *Respiratory*

- ✓ lungs
- nasal turbinates
- trachea

##### *Musculoskeletal*

- bone
- ✓ skeletal muscle

##### *Other*

- ✓ all gross lesions
- ✓ skin

#### ***Statistical Analysis***

No methods for analyzing the data were reported. It appears that all data, if evaluated, were analyzed using Dunnett's test. It does not appear that any tests were performed that justified the use of Dunnett's test for comparison (e.g., homogeneous and normal data). Our reviewers note that if an appropriate

statistical analysis was not performed, statistically significant values are meaningless. Therefore, statistical methods used in this study should have been reported. Furthermore, an analysis for outliers was not performed. Our reviewers note that statistical outliers should be excluded from analysis as recommended by standard toxicity guidelines.

Our reviewers performed statistical analyses on absolute and relative organ weight data. The data were checked for normality and homogeneity using Shapiro-Wilk's test and Bartlett's test, respectively. The data for the treatment groups were then compared to the control group using Dunnett's test to compare the treatment group's mean values to the control group's mean value if the data were normal and homogeneous. If the assumption of normality and/or homogeneity were not met, then the non-parametric test of Kruskal-Wallis followed by Dunn's method for comparing the group means was used. Our reviewers note that the power of the statistical analyses was severely limited by the small number of animals in each exposure group (4 animals/sex/dose).

### ***Appraisal of Experimental Design***

Our reviewers note numerous deficiencies in the experimental design. First, male and female data were analyzed together for selected parameters (i.e., hematology and clinical chemistry parameters). Standard toxicity guidelines recommend that data for male and female animals be analyzed separately because male animals may be more or less sensitive than female animals to treatment. Grouping the data may mask test-article related effects if the effects are seen only in one sex. Second, the clinical pathology assessment (hematology, clinical chemistry and urinalysis) was incomplete as several important components were omitted: (1) no evaluation of serum or plasma electrolytes (critical indicators of overall health and nutritional plane as well as the integrity of multiple organ systems), (2) no evaluation of creatinine (important adjunct to urea nitrogen evaluation). Additionally, some dogs may have been older than standard toxicity guidelines recommend (up to approximately 8 months old), however, this was not likely to significantly affect the study results because animals up to 12-months of age may be used (Haggerty 1992). Also, means and standard deviations were not reported for organ weight data or urinalysis data. Furthermore, a compliance statement was not included in the study report and a quality assurance statement was also omitted. Regarding the test article, no concentration analyses were performed on the dietary mixtures. Without these analyses, it is not possible to assess the actual doses delivered to the animals. Homogeneity analyses were not performed. Therefore, whether the test article was adequately mixed and distributed throughout the diets administered to the animals is not known. Additionally, there was no justification for the selected doses and food was provided *ad libitum* rather than on a weight basis. Hence, there may have been differences in the actual quantity of test article consumed by each dog. Also, test article intake data were not provided. With regard to these deficiencies, our reviewers note the study was conducted prior to the issuance of FDA GLPs and many standard toxicity testing guidelines. Last, it was not clear as to what data were analyzed statistically. Therefore, our reviewers could not determine if appropriate data were correctly analyzed. Furthermore, the data tables did not indicate the presence of any statistically significant findings. Our reviewers note that it is unlikely that no statistically significant values were detected for any parameter in this sub-chronic study. Because of these deficiencies, our reviewers do not feel that this study was adequate to assess the sub-chronic toxicity of bisphenol A when orally administered to dogs in the diet.

## RESULTS

### *Mortality*

No animals died during the course of this study

### *Clinical Effects*

The study authors concluded that no behavioral changes or changes in appearance were related to test article administration. Our reviewers note that clinical data were not provided in the study report and could not, therefore, verify this claim

### *Ophthalmoscopy*

The study authors stated that no changes considered to be related to test-article exposure were seen during the ophthalmoscopic examinations. However, individual animal data were not provided for verification.

### *Body Weights*

The study authors concluded that no effects on body weights occurred during the course of this study. Based on a review of Table 1 of the study report, our reviewers agree with this conclusion.

### *Food consumption*

The study authors concluded that food consumption was similar for control and treated animals. Based on data presented in Table 2 of the study report, our reviewers agree with the study authors' conclusion.<sup>3</sup>

### *Hematology*

The study authors concluded that no test-article related effects were seen with respect to hematology parameters. Our reviewers note that male and female hematology data were analyzed together. Standard toxicity guidelines recommend that male and female data should be analyzed separately because males may be more or less sensitive to toxic effects of bisphenol A than females. For hematology data, in addition to assessing grouped mean values presented by the study authors (Table 3 of the study report) for toxicological relevance, we also compared normal ranges for both males and females (Hall 1992) to individual animal data presented by the study authors (Tables 4 - 7). Based on this comparison, our reviewers agree that no test-article related effects were observed with respect to hematology because individual animal data were generally within normal ranges and the number of animals outside the normal range were similar among all dose-groups (i.e., the number of animals with values outside of the normal range did not increase from control to high-dose animals). The most obvious exception was the apparent neutrophilia in the single 3000 ppm male at 2 months (absolute neutrophil count of 23,900). However, the 3-month value was normal, suggesting either a transient problem unrelated to treatment, or

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<sup>3</sup> We note that the mean for the 9000 ppm female body weight was incorrectly reported as 10.4 during the control period. The correct value should be 7.8.

a technical error (sampling of marginated leukocyte pool, incomplete dilution). We also note that the authors only reported the leukocyte differential as relative (%) values.

### *Clinical Chemistry*

No test-article related effects were seen with respect to clinical chemistry parameters. Our reviewers note that, as with hematology data, male and female clinical chemistry data were analyzed together as one group. As with hematology data, in addition to assessing grouped mean values presented by the study authors (Table 8 of the study report) for toxicological relevance, we also compared the normal ranges for both males and females (Hall 1992) to individual animal data presented by the study authors (Tables 9 - 12). Based on this review, our reviewers agree that no test-article related effects were observed with respect to clinical chemistry because individual animal data were generally within normal ranges<sup>4</sup> and the number of animals outside the normal range were similar among all dose-groups (i.e., the number of animals with values outside of the normal range did not increase from control to high-dose animals).

### *Urinalysis*

The study authors reported that no test-article related effects were observed with regard to urinalysis parameters. Our reviewers note that means and standard deviations were not reported for appropriate urinalysis parameters. Our reviewers calculated mean values for volume, pH, and specific gravity and note that a dose-related trend was not present for any parameter in males or females (see Table 1 below). Furthermore, dose-related trends were not seen with respect to any urinalysis parameter evaluated. Therefore, our reviewers agree with the study authors' conclusion that no effects were seen with respect to urinalysis parameters based on Tables 13-16 of the study report.

**Table 1. Summary of Urine Volume, Ph, and Specific Gravity at Study Initiation and at 1, 2, and 3 Months after Exposure of Male and Female Beagle Dogs to Bisphenol a in the Diet<sup>a</sup>**

Dose	Urinalysis Parameter					
	Volume (mL)		pH		Specific Gravity	
	<i>Males</i>	<i>Females</i>	<i>Males</i>	<i>Females</i>	<i>Males</i>	<i>Females</i>
<b>Prior to Exposure</b>						
0 ppm	151±94	108±46	6.38±0.25	6.62±0.29	1.022±0.011	1.036±0.013
1,000 ppm	139±20	89±61	6.42±0.42	6.62±0.68	1.026±0.008	1.035±0.010
3,000 ppm	102±45	126±86	6.50±0.32	6.68±0.50	1.042±0.005	1.028±0.007
9,000 ppm	62±29	118±45	6.45±0.17	6.78±0.19	1.042±0.016	1.025±0.008

<sup>4</sup> Our reviewers note that there were multiple bilirubin values that exceeded the normal range (0.6-0.8 mg/dL); however, the excessive values were observed in both control and treated animals.

Dose	Urinalysis Parameter					
	Volume (mL)		pH		Specific Gravity	
	<i>Males</i>	<i>Females</i>	<i>Males</i>	<i>Females</i>	<i>Males</i>	<i>Females</i>
<b>1 Month After Exposure</b>						
0 ppm	99±30	108±54	6.82±0.19	6.82±0.17	1.054±0.013	1.032±0.005
1,000 ppm	138±102	70±28	6.95±0.19	6.72±0.05	1.044±0.018	1.054±0.006
3,000 ppm	104±33	89±52	6.70±0.08	6.72±0.19	1.048±0.016	1.038±0.014
9,000 ppm	94±31	60±13	6.88±0.32	6.68±0.15	1.059±0.009	1.046±0.010
<b>2 Months After Exposure</b>						
0 ppm	86±40	98±9	6.80±0.32	6.68±0.24	1.057±0.015	1.048±0.017
1,000 ppm	142±36	75±20	6.92±0.39	6.68±0.15	1.036±0.017	1.062±0.008
3,000 ppm	121±46	169±80	6.88±0.12	6.72±0.17	1.045±0.017	1.042±0.016
9,000 ppm	88±22	79±29	6.92±0.17	6.68±0.17	1.048±0.012	1.066±0.009
<b>3 Months After Exposure</b>						
0 ppm	51.2±6.3	47.5±12	6.6±0.29	6.8±0.37	1.048±0.013	1.042±0.012
1,000 ppm	77.5±36	62.5±6.4	6.2±0.15	6.6±0.13	1.045±0.014	1.042±0.009
3,000 ppm	60.0±42	96.2±37	6.6±0.22	7.0±0.29	1.052±0.006	1.040±0.010
9,000 ppm	71.2±18	51.2±12	6.4±0.15	6.6±0.17	1.052±0.019	1.054±0.016

a Data are presented as mean ± standard deviation and were calculated by our reviewers

### ***Organ Weights***

The study authors appeared to have evaluated combined male and female data; however, there was no information in the study text indicating what data were analyzed (i.e., absolute and/or relative data of which tissues). Therefore, our reviewers analyzed all combined data (absolute and relative tissue weights) statistically.<sup>5</sup> Our results agreed with the conclusions of the study authors that the relative liver weights were statistically significantly increased ( $p \leq 0.01$ ) in the 9000 ppm exposure group and that no other significant changes were noted. The study authors did attribute the significant increase in relative liver weights in the 9000 ppm exposure group to test-article exposure. Our reviewers note that a dose-dependent trend was not seen; however, because the effect was seen in both males (14% increase)<sup>6</sup> and females (35% increase)<sup>6</sup>, our reviewers feel that this observation may have been test-article related.

<sup>5</sup> Our reviewers note that testis and ovary data were not combined.

<sup>6</sup> Calculated by our reviewers

Our reviewers note that the study authors apparently performed statistical analyses on combined data presumably to increase the number of observations and increase the statistical power. We note that statistical analyses should be performed on male and female data separately because one sex may be more or less sensitive to treatment than the other sex. Therefore, our reviewers also performed statistical analyses on the male and female data separately. These analyses indicated that the relative liver weights were significantly increased ( $p \leq 0.01$ ) in the 9000 ppm female exposure group but not in the 9000 ppm male exposure group. The small sample size precluded meaningful interpretation of the statistical data, but we do note that a clear dose-related trend was not seen. The data do support the conclusion that treatment-related increases in liver weights in the 9000 ppm bisphenol A exposure group may have been seen because 1) the combined male and female did indicate a significant increase in relative liver weights compared to the control group, 2) female data indicated a significant increase in the relative liver weights compared to the control group, and 3) absolute liver weights in the males and females were approximately 14% and 35% greater in the male and female exposure group, respectively, than the corresponding control groups. However, the increases in absolute liver weights were not statistically significant. Taken together, organ weight data indicated a possible treatment-related effect on the liver of both males and females in the 9000 ppm exposure groups.

Our reviewers also note that a significant decrease in the absolute kidney weights was seen in males exposed to 1000 ppm. Our reviewers do not feel that this decrease was indicative of test-article-induced toxicity because the mean values for the 3000 and 9000 ppm exposure groups were not significantly less than the control group's value. Means and standard deviations of the absolute and relative organ weights for the combined male and female data were calculated by our reviewers and are provided in Table 2 below. Means and standard deviations of the separated male and female absolute and relative organ weight data calculated by our reviewers are reported in Table 3 below. Based on these data, our reviewers do not attribute any other observations to treatment.

**Table 2. Mean Absolute and Relative Organ Weights of Male and Female Beagle Dogs (combined data) Exposed to Bisphenol A in the Diet For 90 Days**

Tissue		Dose Group (ppm)			
		0	1,000	3,000	9,000
<b>Males and Females Combined</b>					
Spleen	Absolute <sup>a</sup>	21.9±4.1	23.0±9.4	21.8±5.3	21.8±6.6
	Relative <sup>a</sup>	0.24±0.03	0.26±0.08	0.24±0.03	0.24±0.07
Liver	Absolute	220.1±42.2	226.5±51.0	218.2±45.7	271.0±35.0
	Relative <sup>a</sup>	2.4±0.2	2.6±0.2	2.4±0.2	3.0±0.3*
Adrenals	Absolute	1.2±0.2	1.1±0.2	1.1±0.1	1.3±0.2
	Relative <sup>b</sup>	1.3±0.2	1.2±0.2	1.2±0.2	1.4±0.2
Kidneys	Absolute	43.6±9.7	37.6±7.8	42.2±7.6	44.8±4.8
	Relative <sup>a</sup>	0.48±0.06	0.43±0.03	0.46±0.05	0.49±0.05

Tissue		Dose Group (ppm)			
		0	1,000	3,000	9,000
Heart	Absolute	75.7±12.3	76.7±19.9	72.6±9.6	78.5±7.0
	Relative <sup>a</sup>	0.84±0.10	0.87±0.12	0.79±0.09	0.86±0.06
Thyroid/ parathyroid	Absolute	1.10±0.18	1.11±0.13	1.04±0.11	1.24±0.30
	Relative <sup>b</sup>	1.24±0.28	1.30±0.30	1.14±0.15	1.37±0.40
Brain	Absolute	72.0±8.3	71.7±10.3	70.8±6.8	70.8±8.3
	Relative <sup>a</sup>	0.80±0.10	0.84±0.16	0.78±0.06	0.77±0.05
Pituitary	Absolute	0.060±0.02	0.065±0.02	0.056±0.006	0.060±0.01
	Relative <sup>c</sup>	0.66±0.18	0.74±0.15	0.62±0.10	0.64±0.10

\* Statistically significant at  $p \leq 0.05$  by Dunnett's test

a Absolute data reported in grams, relative data reported as % of body weight

b Relative adrenal and thyroid/parathyroid weights were reported as % of body weight x 100

c Relative pituitary weights reported as % of body weight x 1,000

**Table 3. Mean Absolute and Relative Organ Weights of Beagle Dogs (Male and Female Data Separated) Exposed to Bisphenol A in the diet For 90 Days**

Tissue		Dose Group (ppm)			
		0	1,000	3,000	9,000
<b>Males</b>					
Body Weight (kg)		10.5±1.5	9.8±2.6	9.8±1.2	10.0±0.9
Spleen	Absolute <sup>a</sup>	23.3±2.5	23.2±10.4	24.2±7.0	25.6±7.8
	Relative <sup>a</sup>	0.22±0.03	0.23±0.04	0.24±0.04	0.26±0.10
Liver	Absolute	254±16	245±53	247±51	290±18
	Relative <sup>a</sup>	2.4±0.3	2.5±0.3	2.5±0.2	2.9±0.3
Adrenals	Absolute	1.21±0.12	1.19±0.13	1.12±0.13	1.27±0.1
	Relative <sup>b</sup>	1.17±0.15	1.26±0.23	1.15±0.26	1.27±0.09
Kidneys	Absolute	51.2±2.6	39.6±8.2*	48.5±5.2	47.6±2.6
	Relative <sup>a</sup>	0.498±0.08	0.408±0.03	0.495±0.04	0.480±0.06
Testes	Absolute	13.1±4.1	16.7±5.4	15.3±5.8	15.1±4.5
	Relative <sup>a</sup>	0.125±0.02	0.17±0.03	0.152±0.04	0.150±0.04

Tissue		Dose Group (ppm)			
		0	1,000	3,000	9,000
Heart	Absolute	82.7±6.5	81.3±16	75.2±10	84.3±3.4
	Relative <sup>a</sup>	0.80±0.10	0.85±0.16	0.76±0.07	0.84±0.09
Thyroid/ parathyroid	Absolute	1.12±0.19	1.11±0.19	1.11±0.08	1.21±0.31
	Relative <sup>b</sup>	1.10±0.3	1.19±0.4	1.14±0.2	1.22±0.4
Brain	Absolute	77.2±2.3	77.6±3.6	73.6±7.9	76.2±5.8
	Relative <sup>a</sup>	0.74±0.08	0.84±0.24	0.75±0.04	0.76±0.03
Pituitary	Absolute	0.065±0.02	0.066±0.006	0.056±0.007	0.06±0.01
	Relative <sup>c</sup>	0.62±0.2	0.70±0.15	0.58±0.13	0.63±0.12
<b>Females</b>					
Body weight		7.8±1.3	8.0±1.9	8.5±0.5	8.4±0.5
Spleen	Absolute	20.6±5.3	22.7±9.9	19.3±0.8	17.9±1.4
	Relative <sup>a</sup>	0.26±0.03	0.28±0.1	0.23±0.02	0.22±0.02
Liver	Absolute	187±30.5	208±49	189±6.8	252±40
	Relative <sup>a</sup>	2.4±0.16	2.6±0.14	2.2±0.12	3.0±0.40**
Adrenals	Absolute	1.12±0.3	0.980±0.2	1.12±0.1	1.29±0.3
	Relative <sup>b</sup>	1.44±0.22	1.24±0.25	1.32±0.10	1.53±0.27
Kidneys	Absolute	36.1±7.7	35.7±8.1	36.0±1.6	41.9±5.1
	Relative <sup>a</sup>	0.46±0.02	0.44±0.03	0.42±0.01	0.50±0.04
Ovaries	Absolute	0.71±0.14	0.69±0.08	1.4±1.2	1.3±0.72
	Relative <sup>b</sup>	0.91±0.12	0.88±0.17	1.7±1.5	1.5±0.77
Heart	Absolute	68.8±13.6	72.0±24.6	70.0±9.6	72.7±3.4
	Relative <sup>a</sup>	0.88±0.1	0.88±0.09	0.82±0.1	0.87±0.02
Thyroid/ parathyroid	Absolute	1.07±0.2	1.11±0.07	0.96±0.09	1.28±0.33
	Relative <sup>b</sup>	1.38±0.20	1.42±0.22	1.14±0.14	1.52±0.42
Brain	Absolute	66.8±9.2	65.8±12.0	68.1±5.2	65.4±7.1
	Relative <sup>a</sup>	0.86±0.09	0.83±0.08	0.80±0.08	0.78±0.06

Tissue		Dose Group (ppm)			
		0	1,000	3,000	9,000
Pituitary	Absolute	0.056±0.02	0.063±0.03	0.056±0.006	0.056±0.01
	Relative <sup>c</sup>	0.70±0.1	0.76±0.17	0.66±0.04	0.66±0.08

\* Statistically significant at  $p \leq 0.05$  by Dunnett's test

\*\* Statistically significant at  $p \leq 0.01$  by Dunnett's test

a Absolute data reported in grams, relative data reported as % of body weight

b Relative adrenal, thyroid/parathyroid, and ovary weights were reported as % of body weight x 100

c Relative pituitary weights reported as % of body weight x 1,000

### Gross Pathology

Table 4 below reports the incidence of gross lesions in each exposure group as reported by the study authors in Table 17 of the study report. The study authors concluded that no treatment-related effects were observed with respect to gross pathology. Based on the necropsy observations presented in the study report, our reviewers agree that no test-article related effects were observed in any dose group

**Table 4. Summary of Gross Lesions in Males and Females Exposed to Bisphenol A in the Diet for 90 Days**

Gross Lesion	Male Exposure Group				Female Exposure Group			
	0	1,000	3,000	9,000	0	1,000	3,000	9,000
No gross lesions	2/4 <sup>a</sup>	1/4	1/4	3/4	2/4	2/4	2/4	3/4
<b>Lymphoid Tissues</b>								
<i>Gut-associated Lymphoid Tissues</i>								
Prominent Peyer's patches	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
<i>Spleen</i>								
Capsular hemosiderosis, focal <sup>b</sup>	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
<b>Gastrointestinal Tract</b>								
<i>Stomach</i>								
Focal mucosal congestion	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
<i>Duodenum</i>								
Focal mucosal congestion and edema	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4

Gross Lesion	Male Exposure Group				Female Exposure Group			
	0	1,000	3,000	9,000	0	1,000	3,000	9,000
<b><i>Ileocecal valve</i></b>								
Focal mucosal congestion	1/4	1/4	1/4	0/4	2/4	2/4	1/4	1/4
<b><i>Colon</i></b>								
Contents adhered to mucosa	1/4	1/4	1/4	0/4	0/4	0/4	0/4	1/4
Mucosal congestion	2/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
<b><i>Cecum</i></b>								
Focal mucosal congestion	0/4	0/4	1/4	0/4	0/4	0/4	1/4	0/4
<b><i>Rectum</i></b>								
Focal mucosal congestion	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
<b><i>Lung</i></b>								
Congestion and hemorrhage, right lung, cardiac lobe	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Focal congestion and hemorrhage, left lung, cardiac and diaphragmatic lobe	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
Foci, right lung, diaphragmatic lobe	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
<b><i>Urinary Bladder</i></b>								
Dark red raised foci on mucosa	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4

a Data reported as number of animals with lesion/total number of animals in group

b This diagnosis includes the 2x2 cm yellowish zone covered by yellowish granules observed on the capsular surface of spleen

### ***Histopathology***

Table 5 below summarized the microscopic lesions in each exposure group. The study authors concluded that no test article related effects were seen with respect to histopathology. Based on Table 19 of the study report, our reviewers agree. However, we note that there were no histologic findings in the liver, despite the increased liver weights seen in both males and females. Given the *ad libitum* availability of food, we were surprised not to see occasional diagnosis of hepatic vacuolation and/or fatty change. Thomassen (1992) indicates that microscopic alterations are reported most frequently in the liver in dogs on toxicity studies.

**Table 5. Summary of Microscopic Lesions in Male and Female Dogs Exposed to Bisphenol A in the Diet for 90 Days**

Microscopic Lesion <sup>a</sup>	Male Exposure Group		Female Exposure Group	
	0	9,000	0	9,000
Pituitary, cyst	0/4	0/4	0/4	1/4
Thyroid/parathyroid, multilocular cyst	0/4	0/4	1/4	0/4
Adrenal, inflammatory cell foci -- cortex	0/4	1/4 (3) <sup>b</sup>	0/4	0/4
Lung, perivascular inflammation	0/4	1/4 (2)	1/4 (2)	0/4
Lung, focal interstitial pneumonia	1/4 (4)	0/4	1/4 (2)	0/4
Spleen, hemosiderosis	2/4 (3)	2/4 (3)	1/4 (3)	0/4
Salivary gland, periductal inflammation	0/4	1/4 (3)	0/4	0/4
Salivary gland, interstitial inflammation	0/4	0/4	0/4	1/4 (4)

a The brain, spinal cord, peripheral nerve, eye, heart, aorta, lymph node, thymus, bone marrow, esophagus, stomach, large intestine, rectum, pancreas, gallbladder, kidney, skin<sup>7</sup>, urinary bladder, and skeletal muscle were considered normal in all exposure groups

b Histologic severity grades 2 = very slight, 3 = slight, 4 = moderate, 5 = marked, 6 = extreme. The scale was apparently 0-6, but the attribution for "1" was not provided

#### ASSESSMENT

Our reviewers believe that this study is of limited utility and sensitivity in evaluating the sub-chronic toxicity of bisphenol A to Beagle dogs. The study was compromised by lack of concentration and homogeneity analyses, lack of test article intake analysis, limited clinical chemistry evaluation, and grouping the male and female data for analyses.

#### CONCLUSION

No treatment-related effects were observed with respect to mortality, clinical observations, body weight, food consumption, hematology parameters, clinical chemistry parameters, urinalysis parameters, gross pathology, or histopathology. Liver weights were increased in male and female animals exposed to 9000

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<sup>7</sup> One control male and two high-dose males did not have an "n" next to the skin (indicating normal tissue); therefore, there was not indication that the skin was examined in these animals

ppm bisphenol A. The NOEL was identified as 3000 ppm. However, our reviewers believe this study is of limited utility and sensitivity in evaluating the sub-chronic toxicity of the test article to Beagle dogs.

#### **EXECUTIVE SUMMARY**

Beagle dogs (4/sex/group) were administered bisphenol A in the diet at target concentrations of 0, 1000, 3000, or 9000 ppm. No treatment-related effects were observed in the following endpoints: mortality, clinical observations, body weight, food consumption, hematology parameters, clinical chemistry parameters, urinalysis parameters, gross pathology, or histopathology. Liver weights were statistically significantly increased in dogs exposed to 9000 ppm. This was considered treatment related. Thus, the NOEL was considered to be 3000 ppm. However, this study is of limited utility and sensitivity due to numerous study limitations including lack of concentration analyses, and limited clinical chemistry analysis.

#### **REFERENCES**

Hall RL. 1992. Clinical Pathology of Laboratory Animals in *Animal Models in Toxicology*, SC Gad and CP Chengelis (eds.), Marcel Dekker, Inc, New York p. 805.

Haggerty GC. The dog: Toxicology. In *Animal Models in Toxicology*, SC Gad and CP Chengelis (eds.), Marcel Dekker, Inc, New York p. 567-599

Thomassen RW. The dog. Pathology. In *Animal Models in Toxicology*, SC Gad and CP Chengelis (eds.), Marcel Dekker, Inc, New York p. 600-674

**Bisphenol A: Ninety Day Oral Toxicity Study in Dogs**

**Technical Reviewer**

/s/ [Redacted Signature]

Pitner Anderson, M.E.M.

7/20/01

Date

**QA/QC Reviewer**

/s/ [Redacted Signature]

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

7/20/01

Date

## Appendix A: Data Validation

### Bisphenol A: Ninety Day Oral Toxicity Study in Dogs

#### I. Animals Followed Throughout the Study

Approximately 20% of all animals used in this study were selected for review using the random number generator in Microsoft EXCEL and are listed in the following table. Data for all animals selected for review were checked in all tables containing individual animal data. No abnormalities were noted in examining the individual animal data.

**Males:**

**Females:**

Control	1000 ppm	3000 ppm	9000 ppm	Control	1000 ppm	3000 ppm	9000 ppm
75-717	75-718	75-725	75-732	75-741	75-752	75-743	75-744

#### II. Critical Effects and Spot Checks

A study protocol was not included in the study report; therefore, a comparison of the study protocol to the materials and methods was not performed. However, information included in the summary, methods, results, discussion, and tables were examined. All findings are presented below.

- The study authors stated in the methods that they performed ophthalmoscopic examinations on all dogs. They reported in the results that no changes were considered to be related to the compound, however, no individual data were included in the study report.
- Table 2, (average food consumption, pg. 10), included a footnote that said data were not available for that animal. No further explanation was given.
- Tables 4-7 (individual hematological values for control, 1, 2, and 3 months, pgs. 12-15), Tables 10-12 (individual biochemical values-for 1, 2 and 3 months, pgs. 18-20), and Tables 14-16 (individual urinalysis values for 1, 2, and 3 months, pgs. 22-24) had footnotes stating that some data were repeat determinations. No further explanation was given.

Group mean values were verified through calculation using individual animal data in the following tables. Standard deviations were not calculated because the data were not included in the report.

- Table 1: Body weight (males, control and 3000 ppm exposure group, females, 1000 and 9000 ppm exposure group for control week 1 and administration week 2,5,9,13);
- Table 2: Food consumption (males, 1000 and 3000 ppm exposure group, females, control and 9000 ppm exposure group for control week 2 and administration week 3,7,10,12);
- Table 3: Hematological values (all data for control at control and 1000 ppm, 3000 ppm at 1 month, 9000 ppm at 2 months, and 1000 ppm at 3 months);
- Table 8: Biochemical values (all data for 1000 and 9000 ppm during control, control and 3000

ppm at 1 month, control at 2 months, and 3000 ppm at 3 months);

- Table 13-16: Urinalysis data - Mean urinalysis data were not included in the study report; therefore, data validation of individual data to mean data was not performed; and
- Table 18: Organ weights - Mean absolute and relative organ weight data were not included in the study report; therefore, data validation of individual data to mean data was not performed.

The following deviations were noted:

- In Table 1 (body weight, pg. 9), the mean body weight for females at 9000 ppm for control week 1 was recorded as 10.4 kg, but was calculated by our data validator as 7.9 kg.
- In Table 8 (mean biochemical value, pg. 16), alkaline phosphatase at 9000 ppm for the control was recorded as 115 u/ml, but was calculated by our data validator as 119 u/ml.
- In Table 8 (mean biochemical value, pg. 16), B.U.N. at 3000 ppm for 1 month was recorded as 15.0 mg/100 ml, but was calculated by our data validator as 13.6 mg/100 ml.
- In Table 8 (mean biochemical value, pg. 16), albumin at 3000 ppm for 1 month was recorded as 3.96 gm/100 ml, but was calculated by our data validator as 3.84 gm/100 ml.
- In Table 8 (mean biochemical value, pg. 16), S.G.O.T. at 3000 ppm for 1 month was recorded at 36 u/ml, but was calculated to 37 u/ml, and cholesterol of the control group at 1 month was recorded at 153 mg/100 ml, but was calculated by our data validator as 154 mg/100ml. Our reviewers assumed these to be rounding errors.

### III. Findings

As noted above, discrepancies or inconsistencies revealed in this data validation included unclear footnotes and differences in reported and calculated means of body weight and biochemical data. These issues, however, do not affect the confidence in the study data.

/s/  /s/

Lilia Chen, B.S.

6/28/00  
Date

AUG 16 2001

**Contract Number**

223-96-2302

**Work Assignment Number**

2000-20  
(ICF 020)

**Task Number**

03

**FDA Study Identification Numbers**

HSE-K-001304-011

**Signature of Program Manager**

/s/ [Redacted Signature]

Date

8-7-01

## **STUDY TITLE**

Bisphenol A: 13-Week Aerosol Toxicity Study with Fischer 344 Rats

## **TESTING LABORATORY**

Mammalian and Environmental Toxicology Research Laboratory Health and Environmental Sciences  
The Dow Chemical Company  
Midland, MI

## **COMPLETION DATE OF STUDY**

March 18, 1988

## **SPONSORS OF STUDY**

Dow Chemical U.S.A., Midland, MI

## **STUDY SUMMARY**

### ***Statement of Compliance and Quality Assurance***

A signed and dated compliance statement was included in the study report. The study director indicated that the study was conducted in compliance with U S EPA Good Laboratory Practice Regulations (Title 40, CFR Parts 792, 798.2450, and 799)

A signed and dated quality assurance (QA) statement was also included in the study report with a list of inspection dates. Based on the provided dates, the quality assurance findings were reported promptly to the study director and management. This QA statement also indicated that the study was conducted in accordance with EPA and FDA published Good Laboratory Practice Regulations.

### ***Study Objective***

This study was conducted to determine the toxicity of whole body inhalation exposure to 0, 10, 50, or 150 mg/m<sup>3</sup> polycarbonate grade bisphenol A aerosol to Fischer 344 rats for 13-weeks (6 hr/day, 5 days/week). Animals were also observed over a 4- or 12-week reversibility period following treatment to assess reversibility of any treatment-related effects and to determine any residual effects resulting from bisphenol A treatment.

### ***Test Article***

The test article, polycarbonate grade bisphenol A (lot TB 84071221), was described as a light-colored solid and was supplied by The Dow Chemical Company, Freeport, TX. Only smaller particles (separated using a 1 mm mesh, steel screen) were used to generate the aerosol. Compositional analysis did not reveal any differences between the original sample and the sample that was separated using the steel screen.

Bisphenol A was found to be 99.7% pure as determined by gas chromatography with thermal conductivity detection and 99.9% pure as determined by differential scanning calorimetry. Three impurities reported by the study authors were 0.1% phenol, approximately 0.1% isopropenyl phenol, and 0.1-0.3% 2,4-isopropylidene diphenyl.

After the last exposure, the test article was reanalyzed for stability. The study authors indicated that the test article was stable throughout the study duration.

### ***Test Animals***

Six-week old Fischer 344 rats (30/sex/dose) were obtained from Charles River Breeding Laboratories, Kingston, NY. The study authors indicated that Fischer rats were chosen because of the availability of historical data on these animals and because the same species was used in a previous bisphenol A aerosol study.<sup>1</sup> The laboratory veterinarian evaluated the health status of each animal upon arrival to the testing facility.<sup>2</sup> All animals were acclimatized for at least 7-days prior to study initiation.

All rats were ranked by body weight; animals with outlying body weights were removed until only the number of animals needed for the study remained. Animals chosen for study were then assigned to treatment groups randomly based on body weight. Animals were individually housed in stainless steel mesh cages (including exposure periods) and were identified using ear tags. Temperature and relative humidity were kept at 22° C and 50%, respectively. A 12 hour light/dark cycle was used. Animals not chosen for study were housed in a separate room.

### ***Diet***

Animals were fed ground Purina Certified Rodent Chow #5002<sup>3</sup> and municipal tap water *ad libitum* except during exposure periods. Food was analyzed by the supplier for nutritional value and for selected contaminants. The study authors also indicated that the municipal tap water was analyzed periodically.

### ***Experimental Design***

Thirty rats/sex/group were exposed to aerosols of bisphenol A at target concentrations of 0, 10, 50, or 150 mg/m<sup>3</sup>. Rats were exposed for 6-hours/day, 5-days per week, for 13-weeks. The highest dose selected (150 mg/m<sup>3</sup>) approximated the maximal achievable concentration of polycarbonate grade bisphenol A in air. Ten rats/sex/group were sacrificed one-day after the final bisphenol A exposure (terminal sacrifice group), 10 rats/sex/group were allowed to recover for 4-weeks, and 10 rats/sex/group were allowed to recover for 12-weeks without additional exposure to the test article, and then sacrificed (4-week and 12-week recovery sacrifice groups, respectively).

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<sup>1</sup> Nitschke et. al., 1985a and 1985b

<sup>2</sup> The testing facility was accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC)

<sup>3</sup> Ralston Purina Company, St. Louis, MO

### ***Exposure Chamber and Aerosol Generation System***

Exposure to bisphenol A aerosol was carried out in Rochester-type steel and glass chambers under dynamic airflow conditions. Air flow in the chambers was kept at a rate of approximately 225 liters/min (13.5 air changes/hour).

Bisphenol A particles were aerosolized in a modified Marple dust generator using approximately 60 liters/min of dry, compressed air. The remaining air supplied to the test chambers was filtered and climate-controlled with temperature settings kept at 22°C and relative humidity settings at 50%. Temperature, relative humidity, and airflow were recorded at 30 minute intervals during exposure.

An aerodynamic particle sizer was used to measure the mass concentration and aerodynamic particle size of the test-article in the breathing zone of the animals in the exposure chambers (from 5 points in the chamber). Test-article concentration was determined using a mass monitor or gravimetrically three times per day for each chamber. Particle size was measured weekly using a cascade impactor and aerodynamic particle sizer during the exposure period.

### ***Clinical Observations***

Prior to study initiation, an ophthalmic examination was conducted on all animals using a penlight. The study authors indicated that no significant ocular lesions were found<sup>4</sup>. After each exposure, all animals were observed for changes in appearance. Changes in the fur, eyes, mucous membranes, and respiration were included in the observations. Also, behavior patterns and nervous system activity were evaluated by noting changes in lethargy, tremors, convulsions, salivation, lacrimation, diarrhea, and other signs of altered central nervous system function (not specified by the study authors).

During weekends, daily observations and monitoring was limited to ensuring that food and water were available to the animals.

### ***Body Weights and Food Consumption***

All animals were weighed prior to study initiation and weekly thereafter until sacrifice. Body weight gain was not calculated. Food consumption was determined weekly. Spillage was not recorded.

### ***Clinical Laboratory Determinations***

Prior to necropsy, blood samples were collected from the orbital sinus from methoxyflurane-anesthetized animals. The following parameters were evaluated:

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<sup>4</sup> Our reviewers note that penlight examination reveals information about the lens, anterior chamber, conjunctiva, and sometimes tapetum. No information is provided regarding the retina unless an ophthalmoscope or ophthalmic loop is also used. Additionally, the qualifications of the person(nel) performing the pre-study initiation penlight examinations were not indicated in the study report.

## Hematology

Standard hematology parameters are listed below. A check indicates that the corresponding parameter was evaluated in all groups for this study. In addition to the parameters included below, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and reticulocyte count were determined. Erythrocyte and leukocyte morphology were evaluated on blood smears during the leukocyte differential counting process. Nucleated red blood cells were included in the differential leukocyte counts.

- |   |                                |
|---|--------------------------------|
| ✓ hematocrit/packed cell volume           | ✓ total leukocyte count        |
| ✓ hemoglobin                              | ✓ differential leukocyte count |
| ✓ erythrocyte count                       | ✓ --neutrophils                |
| ✓ clotting potential (partial evaluation) | ✓ --lymphocytes                |
| --clotting time                           | ✓ --eosinophils                |
| --prothrombin time                        | ✓ --basophils                  |
| --activated partial thromboplastin time   | ✓ --monocytes                  |
| ✓ --platelet count                        |                                |

Our reviewers considered the assessment of clotting potential to be incomplete as only platelet numbers (indicator of ability to form primary hemostatic plug) were evaluated. Prothrombin time and activated partial thromboplastin time were not evaluated, hence, there was no determination of clotting factor function (indicator of ability to form stable clot).

## Clinical Chemistry

At terminal sacrifice, blood samples were collected to determine the following clinical chemistry parameters. All samples were refrigerated or kept on ice until clinical chemistry analyses were performed. Standard clinical chemistry parameters are listed below. A check indicates that the corresponding parameter was evaluated in all groups for this study. In addition to the parameters listed below, the study authors determined globulin concentration.

### *Electrolytes*

calcium  
chloride  
phosphorus  
potassium  
sodium

### *Other*

✓ albumin  
bilirubin (total)  
creatinine  
✓ glucose  
✓ protein (total)  
✓ urea nitrogen

### *Enzymes*

- ✓ alanine aminotransferase<sup>5</sup>
- ✓ alkaline phosphatase
- ✓ aspartate aminotransferase<sup>6</sup>
- γ-glutamyl transferase
- ornithine decarboxylase

Our reviewers note that there was no evaluation of serum or plasma electrolytes. Evaluation of electrolytes is a critical indicator of overall health and nutritional plane as well as the integrity of multiple organ systems (e.g., calcium/phosphorus levels are monitors of parathyroid, kidney, bone, and intestine, etc.). Furthermore, there was no evaluation of total bilirubin which is an important monitor of erythrocyte breakdown/clearance as well as hepatic function/obstruction. Additionally, there was no evaluation of creatinine which is an important adjunct to the BUN evaluation to assess renal versus pre-renal changes. For these reasons, we considered the overall clinical chemistry evaluation to be incomplete.

### Urinalysis

Urinalysis was not performed. Our reviewers consider urinalysis important as a critical indicator of urinary tract damage.

### ***Ophthalmology***

Ophthalmic exams were not conducted during the study. Our reviewers do not consider post-mortem macroscopic or microscopic evaluation of the eyes sufficient to elucidate all ophthalmic changes. For example, the globe is generally fixed and hardened off prior to sectioning. The usual histologic sections are taken relatively centrally within the globe. The lens and vitreous body may or may not be included in the typical histologic sections. The retina may be artifactually detached. Peripheral (nasal or temporal) ocular structures (particularly internal ocular structure) may never be viewed by a pathologist and treatment-related alterations may never be diagnosed. For these reasons, we considered the overall ophthalmology evaluation to be incomplete.

### ***Gross Pathology***

Ten animals were necropsied immediately after the final exposure to bisphenol A, ten animals were sacrificed 28 days after the final bisphenol A exposure, and ten animals were sacrificed 12 weeks after the final bisphenol A exposure. The same necropsy method was used for all animals sacrificed. Prior to sacrifice, all animals were fasted overnight. A comprehensive gross pathology examination was conducted at necropsy, eyes were examined in-situ. The study authors indicated that all animals were humanely euthanized, however, the method of euthanasia was not provided in the study text.

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<sup>5</sup> The study authors used the former name of this enzyme (glutamic pyruvic transaminase)

<sup>6</sup> The study authors used the former name of this enzyme (glutamic oxaloacetic transaminase)

All animals were submitted for a complete gross necropsy, however, no details regarding this procedure were provided. The study authors did not indicate any procedure used to minimize gross pathology bias (e.g., randomization of animal order for necropsy) Our reviewers note that multiple personnel may be involved in recording gross lesions and may vary in their thoroughness, threshold for recording (e.g. what is red to one person may be pink to another), and terminology used. Therefore, procedures to minimize bias are necessary, but were not described in the study report

### ***Organ weights***

The following organs were weighed at necropsy. Relative organ weights (per 100 g body weight) were also calculated

brain	lung (wet weight)
liver	testes
kidneys	

### ***Histopathology***

A complete set of tissues was collected from all terminal sacrifice and recovery sacrifice animals as indicated by a check on the standard toxicity guideline tissue list below Additional tissues collected included auditory sebaceous glands, cervix, coagulating glands, lacrimal/hardarian glands, larynx, mediastinal tissues, mesenteric tissues, oviducts, rectum, tongue, and vagina

#### Standard List of Tissues for Histopathology

##### *Cardiovascular/Hemopoietic*

- ✓ aorta
- ✓ bone marrow
- ✓ heart
- ✓ lymph node (mediastinal, mesenteric)
- ✓ spleen
- ✓ thymus

##### *Digestive system*

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

*Urogenital*

- ✓ epididymides
- ✓ kidneys
- ✓ ovaries
- ✓ prostate
- ✓ seminal vesicle
- ✓ testes
- ✓ urinary bladder
- ✓ uterus

*Neurologic/Special Senses*

- ✓ brain (cerebrum, brainstem, cerebellum)
- ✓ eyes
- ✓ pituitary
- ✓ nerve (peripheral)
- ✓ spinal cord (cervical, thoracic, lumbar)

*Glandular*

- ✓ adrenals
- ✓ mammary gland
- ✓ thyroid/parathyroid

*Respiratory*

- ✓ lungs
- ✓ nasal turbinates
- ✓ trachea

*Musculoskeletal*

- ✓ bone
- ✓ skeletal muscle

*Other*

- ✓ all gross lesions
- ✓ skin

Tissues were preserved in 10% neutral buffered formalin. Immersion fixation was used with two exceptions. Lungs were filled with fixative by airway perfusion until the normal inspiratory volume was approximated. Nasal tissues were flushed with a fixative using the pharyngeal duct to facilitate rapid fixation of the mucosal surfaces. Histologic processing and histologic evaluation were performed on complete tissue sets from control and high-dose terminal sacrifice animals.

Restricted tissue sets were examined for other treatment, control, and sacrifice groups as indicated in Table 1 below. Histologic evaluation was by a board-certified veterinary pathologist (L. Lomax, D.V.M., Ph.D., D.A.C.V.P.).

**Table 1. Tissues Evaluated Histologically in Each Sacrifice Group**

<b>Sacrifice Time</b>	<b>Dose Group(s)</b>	<b>Tissues Evaluated Histologically</b>
13-Week Terminal Sacrifice	Controls 150 mg/m <sup>3</sup>	Complete tissue set - HE slides Nasal tissues - PAS/alcian blue
	10 mg/m <sup>3</sup> 50 mg/m <sup>3</sup>	Nasal tissues, lungs, cecum, gross lesions - HE slides
4-Week Recovery Sacrifice	Controls 150 mg/m <sup>3</sup>	Nasal tissues, lungs, mediastinal tissues, mediastinal lymph nodes, trachea, cecum, and gross lesions - HE slides Nasal tissues - PAS/alcian blue
	10 mg/m <sup>3</sup> 50 mg/m <sup>3</sup>	Nasal tissues, lungs, cecum, gross lesions - HE slides
12-Week Recovery Sacrifice	Controls 150 mg/m <sup>3</sup>	Nasal tissues, lungs, mediastinal tissues, mediastinal lymph nodes, trachea, kidneys, cecum, and gross lesions - HE slides
	10 mg/m <sup>3</sup> 50 mg/m <sup>3</sup>	Nasal tissues, lungs, kidneys, gross lesions - HE slides

Our reviewers note that the study protocol and protocol amendments were not available for review, so we could not determine whether the histologic processing and evaluation of the restricted/customized tissue sets was strictly justified. For example, the rationale for examining kidneys in all 4-week recovery sacrifice animals but not in any 13-week sacrifice, low- and intermediate- dose animals was not apparent. The study authors did not explain this inconsistency in the study report narrative. We also assumed that cecum was included in the restricted tissue sets because it had a gross lesion that was potentially related to treatment (discussed further in the Results section). However, examination of “grossly normal” cecal tissues probably should have required a protocol amendment.

Lastly, we also note that the submandibular, tracheobronchial and various cervical lymph nodes were not collected (the sternal lymph node and caudal deep cervical lymph node may have been included as “mediastinal tissues”). Although evaluation of these lymph nodes is not always included in sub-chronic toxicity studies, it can be important in inhalation studies as these lymph nodes serve as drainage/clearance routes for particles collected on various respiratory surfaces.

### ***Statistical Analysis***

Quantitative data (i.e., body weights, absolute and relative organ weights, clinical chemistry data, and appropriate hematology data) were analyzed using Bartlett’s test for equality of variance ( $\alpha = 0.01$ ). A parametric or non-parametric ANOVA ( $\alpha = 0.1$ ) followed by Dunnett’s test ( $\alpha = 0.05$ ) or Wilcoxon Rank-Sum test with Bonferroni correction for multiple comparisons ( $\alpha = 0.05$ ) was used to evaluate the data. Outliers were identified using a sequential test ( $\alpha = 0.02$ ) and excluded from analysis only if scientifically sound reasons were evident. Statistically significant findings were only considered

toxicologically relevant when a dose-dependant trend was evident and when the findings did not contradict other biological findings

### ***Appraisal of Experimental Design***

The study protocol and any protocol amendments were not available for review. Our reviewers considered the study to have several important strengths and multiple critical weaknesses. Strengths included selection of dose and form of the test article (i.e., selection of filtered, respirable particles of polycarbonate bisphenol A, doses based on previous short-term toxicity testing) as well as careful attention to delivery and monitoring of appropriate, stable, relatively uniform, test-article concentrations under realistic exposure conditions in a toxicity testing laboratory. However, our reviewers identified multiple deficiencies in the study design based on recommendations of standard sub-chronic toxicity testing guidelines. Body weight gain was not calculated and food spillage was not measured or addressed. The clinical pathology assessment (hematology, clinical chemistry and urinalysis) was incomplete as several important components were omitted: (1) no evaluation of clotting factor function as an indicator of the ability to form a stable clot, (2) no evaluation of serum or plasma electrolytes (critical indicators of overall health and nutritional plane as well as the integrity of multiple organ systems), (3) no evaluation of total bilirubin (important monitor of erythrocyte turnover and hepatic function), and (4) no evaluation of creatinine (important adjunct to urea nitrogen evaluation). Ophthalmology was incomplete as ophthalmic exams were only performed on animals prior to entry on study and post-mortem macroscopic and microscopic examination of the globe is insufficient to elucidate all ophthalmic changes potentially related to treatment. Urinalysis was not performed. Urinalysis is recommended by standard toxicity guidelines and is an indicator of urinary tract damage. Because of these deficiencies, our reviewers were forced to consider the study marginally sensitive to assess the sub-chronic toxic potential of bisphenol A in rats exposed via whole body inhalation.

## **STUDY RESULTS**

### ***Test-Article Concentration***

Mean daily exposure concentrations were determined to be within 7% of target concentrations. The daily range of exposure concentration varied more significantly (up to approximately 35% variation from target concentration). The time-weighted averages, however, indicated that the exposure concentrations were usually close to target concentrations. Analysis of the diameter of the particles indicated that most of the aerosol particles were respirable in size. Table 1 below presents selected exposure chamber data.

**Table 2. Exposure Concentrations and Mass Median Aerodynamic Diameter of the Test Article**

<b>Dose Group (mg/m<sup>3</sup>)</b>	<b>Exposure Concentration (Time Weighted Average [TWA])</b>	<b>Range of Daily TWA Exposure Concentration</b>	<b>Mass Median Aerodynamic Diameter Determined with laser velocimetry</b>	<b>Mass Median Aerodynamic Diameter Determined Gravimetrically</b>
0	0.2±0.1 mg/m <sup>3</sup>	0.0-0.7 mg/m <sup>3</sup>	--	--
10	10.5±1.0 mg/m <sup>3</sup>	8.5-13.5 mg/m <sup>3</sup>	1.52±6.3 µm	2.18±0.73 µm
50	53.3±3.9 mg/m <sup>3</sup>	45.9-63.5 mg/m <sup>3</sup>	2.89±0.55 µm	5.15±1.06 µm
150	148.6±13.1 mg/m <sup>3</sup>	99.0-176.7 mg/m <sup>3</sup>	2.14±0.38 µm	3.74±1.16 µm

***Mortality***

One male animal exposed to 10 mg/m<sup>3</sup> died from “traumatic causes” on day 16 of exposure. The sacrifice group of this animal was not reported in the study text. Based on data presented in the summary tables, it appears that this animal belonged to the 13-week sacrifice group. Further explanation of the death was not provided. No other animals died during the course of the study.

***Clinical Effects***

13-week Terminal Sacrifice Group. A summary of clinical effects was not included in the study report. Furthermore, our reviewers note that the study authors did not differentiate between terminal sacrifice groups and recovery groups when presenting clinical effects. In the results section of the study report, the study authors reported that reddish material (likely porphyrin) was observed around the nose of male and female animals exposed to 50 or 150 mg/m<sup>3</sup>. The number of animals affected was not reported. The study authors also reported that a very slight amount of porphyrin-like material was seen around the nose of several male animals exposed to 10 mg/m<sup>3</sup>, and a very slight amount of porphyrin was seen around the nose and eyes of several females in the same dose group. Perineal soiling was also seen in several animals (male and female) exposed to 50 or 150 mg/m<sup>3</sup> bisphenol A and in 2 female animals exposed to 10 mg/m<sup>3</sup>. The study authors indicated that these effects did not intensify over the 13-week exposure period.

***Body Weights***

13-Week Terminal Sacrifice Group. Body weight data were reported in Tables 3 and 4 of the study report. Male and female animals exposed to 50 or 150 mg/m<sup>3</sup> exhibited statistically significant decreases in body weights compared to control values throughout the study. Additionally, male and female animals exposed to 10 mg/m<sup>3</sup> had body weights that were statistically significantly lower than control values for most of the exposure duration. The study authors reported that at week 13 the decrease in body weights relative to control animals for males and females in the 10 mg/m<sup>3</sup> exposure group were approximately 4 and 2%, respectively. Our reviewers note that body weights were statistically significantly decreased at almost every measurement between study initiation and week 13 of treatment for males (except at

measurement day 78 and 92) and a dose-dependent trend was evident. Terminal body weights were decreased in male and female, terminal sacrifice animals exposed to 150 mg/m<sup>3</sup> (5 and 11% decrease relative to controls, respectively).

4-Week Recovery Group. After a 4-week recovery period, body weights were still depressed in male animals exposed to 150 mg/m<sup>3</sup>. Body weights for all other animals were similar to control values

12-Week Recovery Group. After a 12-week recovery period, body weights of males exposed to 150 mg/m<sup>3</sup> were statistically significantly decreased compared to control values. Body weights of females of this exposure group and males and females of all other exposure groups were not statistically different from controls

Body weight gain was not measured.

### ***Food consumption***

The study authors concluded that food consumption was similar for control and treated animals throughout the study period and during the recovery period. Based on review of Tables 5 and 6 of the study report, our reviewers agree that there were no statistically significant differences between control and treated groups with respect to food consumption. Our reviewers note consistent, non-statistically significant, approximately 4.8% and 5.7% decreases in total food consumption in the high-dose, terminal sacrifice males and females, respectively. Further, we note that the study authors did not address spillage, decreasing our confidence in the precision of the food consumption measurements and our ability to determine the exact relationship between food consumption and body weight and/or body weight gain. However, we observed that the non-statistically significant decreases in food consumption were greater in females whereas the statistically significant decreases in body weight were greater in males. This suggested males were more sensitive than females with respect to the test article effects on body weight and that the decreased body weights in males were greater than that expected due to decreased food consumption alone.

### ***Hematology***

13-Week Terminal Sacrifice Group. The study authors indicated that a statistically significant increase in hemoglobin concentration was seen in terminal sacrifice males rats exposed to 10 mg/m<sup>3</sup> bisphenol A. The study authors concluded that this increase was not biologically significant. Our reviewers do not attribute this observation to test article exposure because the increase was seen at only one exposure level and the effect was slight. No other statistically significant findings were observed.

4-Week Recovery Group. After a 4-week reversibility period, female animals exposed to 10 or 150 mg/m<sup>3</sup> had statistically significantly decreased white blood cell counts. Our reviewers note that all total leukocyte counts were within normal reference ranges for young adult CD rats (3-14.5 x 10<sup>3</sup>/μL [males]; 2-11.5 x 10<sup>3</sup>/μL [females] - Hall 1992). The study authors indicated that the differential counts were comparable to controls; however, they did not calculate the absolute differential counts and provided the information as relative percentages. Our reviewers calculated the following absolute differential counts:

**Table 3. Absolute Differential Leukocyte Counts Calculated by Our Reviewers**

Dose Group (mg/m <sup>3</sup> )	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
0	1020	4740	180	60	0
10	611	3950	94	47	47
50	850	4000	100	100	0
150	846	3710	94	47	0

Although neutrophil counts were decreased 17-40% compared to control values, all values fell within normal reference ranges for young adult CD rats (0.3-3.0 X 10<sup>3</sup>/μL [males], 0.1-2.0 X 10<sup>3</sup>/μL [females] - Hall 1992) Again, our reviewers note that all total leukocyte counts were within normal reference ranges for young adult CD rats (Hall 1992) and agreed with the study authors that biological variability was the most likely explanation.

12-Week Recovery Group After a 12-week reversibility period, male animals in the low- and high-dose groups both had statistically significantly increased white blood cell counts. The study authors attributed the increase to natural biological variability and not to bisphenol A treatment because the differential counts were comparable for all exposure groups Our reviewers also note that a dose-response relationship was not evident.

### ***Clinical Chemistry***

13-Week Terminal Sacrifice Group. The study authors concluded that clinical chemistry parameters were not affected by test article exposure The study authors noted the presence of several statistically significant changes in clinical chemistry parameters, these are listed in Table 4 below. The study authors reported that these findings were not biologically significant. Our reviewers do not attribute the statistically significant observations to treatment because a dose-response relationship was not evident and the changes were slight We do note that dose-related trends were seen with total protein and albumin concentration in females; however, the values remained within reference ranges for female Fischer rats of similar age (3.5 - 4.3 g/dL).<sup>7</sup>

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<sup>7</sup> Charles River Laboratories, Technical Bulletin, Spring 1998

**Table 4. Statistically Significant Clinical Chemistry Findings**

<i>13-Week Terminal Sacrifice Males</i>			
Dose Group (mg/m <sup>3</sup> )	<i>SGPT (MU/mL)</i>	<i>SGOT (MU/mL)</i>	<i>Gluc (mg/mL)</i>
0	56±23	79±30	120±15
10	42±7	63±5	111±10
50	48±18	66±17	120±23
150	38±6 <sup>#</sup>	61±5 <sup>#</sup>	102±15*
<i>13-Week Terminal Sacrifice Females</i>			
Dose Group (mg/m <sup>3</sup> )	<i>AP (MU/mL)</i>	<i>TP (g/dL)</i>	<i>ALB (g/dL)</i>
0	46±6	6.2±0.3	3.8±0.2
10	48±9	6.2±0.2	3.8±0.1
50	61±8*	6.2±0.2	3.7±0.1
150	59±12*	6.0±0.2*	3.6±0.1*

# Statistically different from control value ( $\alpha=0.05$ ) using Wilcoxon's test

\* Statistically different from control value ( $\alpha=0.05$ ) using Dunnett's test

4-Week Recovery Group After a 4-week recovery period, glucose levels were increased in males exposed to 150 mg/m<sup>3</sup>. Females in the same exposure group had increased alkaline phosphatase and decreased serum glutamic pyruvic transaminase activities. Alkaline phosphatase activity was also increased in females exposed to 10 mg/m<sup>3</sup>. The study authors did not consider these effects to be toxicologically significant. Based on a review of Tables 21 and 22 of the study report, our reviewers agree with the study authors' conclusions.

12-Week Recovery Group. After a 12-week recovery period, urea nitrogen levels were slightly elevated in male rats exposed to 10 or 150 mg/m<sup>3</sup> and total protein and globulin concentration were statistically significantly lower than control values in female rats exposed to 150 mg/m<sup>3</sup> bisphenol A (Tables 31 and 32 of the study report). Our reviewers note that all statistically significant values were within normal ranges for female Fischer rats of similar age and therefore do not attribute these observations to treatment.<sup>8</sup>

### Urinalysis

Urinalysis was not performed.

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<sup>8</sup> Charles River Laboratories, Technical Bulletin, Spring 1998

## Organ Weights

13-Week Terminal Sacrifice Group. The study authors concluded that no treatment-related effects were observed with respect to organ weights. A summary of statistically significant changes in absolute or relative organ weights are presented in Table 5 below for all non-recovery animals.

**Table 5 Statistically Significant Organ Weight Findings (absolute or relative)**

<i>Males</i>	<b>Dose Group</b>			
<i>Parameter</i>	<i>0</i>	<i>10 (mg/m<sup>3</sup>)</i>	<i>50 (mg/m<sup>3</sup>)</i>	<i>150 (mg/m<sup>3</sup>)</i>
Terminal body weights (g)	297.3±13.9	286.3±12.1	290.5±19.7	282.0±11.6
Liver weights (g)	7.296±0.31	6.914±0.34 <sup>#</sup>	7.419±0.92	6.96±0.24 <sup>#</sup>
<i>Females</i>	<b>Dose Group</b>			
<i>Parameter</i>	<i>0</i>	<i>10 (mg/m<sup>3</sup>)</i>	<i>50 (mg/m<sup>3</sup>)</i>	<i>150 (mg/m<sup>3</sup>)</i>
Terminal body weights (g)	174.5±13.5	172.9±5.7	165.2±5.7	156.0±9.0*
Relative Brain weights (g/100)	0.993±0.05	0.987±0.03	1.044±0.04*	1.099±0.05*
Kidney weights (g)	1.147±0.11	1.104±0.06	1.083±0.04	1.034±0.06*
Liver weights (g)	4.448±0.39	4.326±0.24	4.314±0.21	4.112±0.31*
Relative Lung weights (g/100)	0.457±0.02	0.456±0.02	0.464±0.02	0.485±0.02*

<sup>#</sup> Statistically significant ( $\alpha = 0.05$ ) using Wilcoxon's test

\* Statistically significant ( $\alpha = 0.05$ ) using Dunnett's test

The study authors attributed increases in relative organ weights to decreases in whole body weights and not to bisphenol A treatment because associated microscopic changes were not present. The study authors noted previous similar effects (Oishi et Al., 1979)<sup>9</sup> in rats on a feed restriction diet. Our reviewers only partially agree and consider the increases in relative organ weights to be secondary to the primary effect of bisphenol A to decrease body weight independent of decreased food consumption (at least in the more sensitive male sex). Furthermore, our reviewers note that absolute kidney weights were statistically significantly decreased in females in the high dose group. A dose-related trend was evident. This observation was also seen in animals in the 4- and 12-week recovery sacrifice groups (the decrease was not statistically significant in animals of the 4-week recovery group). Therefore, our reviewers assert that the dose-related decreases in absolute kidney weight in high-dose females were likely related to treatment with bisphenol A.

<sup>9</sup> Oishi, S, Oishi, H, and Hivaga, K (1979). The effect of food restriction for 4 weeks on common toxicity parameters in male rats. *Toxicol. Appl. Pharmacol.* 47, 15-22

4-Week Recovery Group. After a 4-week recovery period, final body weights were statistically significantly decreased (approximately 6%) in both males and females in the high-dose group. Males of the same dose group also had increased relative brain weights that were statistically significant. Our reviewers note that this was likely associated with the statistically significant decrease in body weights. Also, males in the mid-dose group had a statistically significant increase in relative liver weights. A dose-dependent trend was not observed, therefore, our reviewers do not attribute this observation to treatment. Females in the high-dose group had a statistically significant decrease in terminal body weights. This observation was attributed to treatment.

12-Week Recovery Group. After a 12-week recovery period, both males and females exposed to 150 mg/m<sup>3</sup> had a statistically significant decrease in absolute kidney weights as previously noted. No other statistically significant observations were noted.

### ***Gross Pathology***

13-Week Terminal Sacrifice Group. Gross observations that were considered test-article related are included in Table 6 below. The study authors noted that increased cecal size (high- and intermediate-dose animals) was not accompanied by alterations in cecal wall morphology. The increased size was attributed to distention of the cecum with food ingesta by the study authors. Gastric erosion or ulceration did not accompany the hemolyzed blood found in the stomach (high- and intermediate-dose males, mid-dose females); however, the study authors did indicate that minimal gastric erosion may have occurred as a secondary response to treatment. Porphyrin staining of the face and urine staining of the perineum were described as secondary responses to stress. Our reviewers were not convinced by the study authors' explanations for the gross pathology findings. Regarding the potential relationship between cecal distention and excess ingesta, we note that the treated animals had slightly lower food consumption than control animals (although statistically significant changes were not seen), therefore, it was unlikely that the distention was secondary to the excess ingesta. Additionally, we note that there are multiple causes of large intestinal dilatation (including neurologic causes) for which no distinctive histologic findings are evident and the excess ingesta is secondary to primary distention.

Regarding the attribution of multiple observations (hemolyzed blood in stomach with or without gastric erosion, porphyrin staining of face, urine staining of perineum) to stress responses, we note the following. We agree that those observations can be ascribed to chronic adrenal corticosteroid release or other prolonged stress responses and that prolonged stress responses can certainly be observed in high-dose animals on sub-chronic or chronic toxicity studies. However, in the present study, there was limited evidence for "so-called stress responses." There were no changes in the peripheral blood picture to indicate chronic adrenal corticosteroid release (i.e., mature neutrophilia and chronic lymphopenia were not evident) and there were no changes histologically (see below) to suggest adrenal cortical alterations or gastric erosion. We consider it was likely that the hemolyzed blood in the stomach was respiratory or oropharyngeal in origin. We also consider it possible that some of the reddish staining on the face was secondary to low-grade, possibly intermittent, blood loss from respiratory or oropharyngeal tissues. We note that low-grade, possibly intermittent, blood loss from respiratory or oropharyngeal tissues, would lead to the coughing up and swallowing of small amounts of blood (hence, deposition in the stomach) and might leave little trace on the pharyngeal or respiratory mucosae. Lastly, we note that the submandibular lymph nodes were not examined histologically (it cannot be determined whether these

tissues were examined grossly), and that traces of low-grade pharyngeal or respiratory blood loss might also be found in these locations.

**Table 6. Treatment-Related Gross Observations**

Gross Observation	Dose Group							
	0		10 mg/m <sup>3</sup>		50 mg/m <sup>3</sup>		150 mg/m <sup>3</sup>	
	Males	Females	Males	Females	Males	Females	Males	Females
Increased Cecum Size	0/10	0/10	0/10	0/10	10/10	10/10	10/10	10/10
Facial Soiling - porphyrin	0/10	0/10	0/10	0/10	10/10	7/10	9/10	2/10
Perineal Soiling	0/10	0/10	0/10	2/10	9/10	10/10	9/10	10/10
Stomach (hemolyzed blood)	0/10	0/10	0/10	0/10	7/10	2/10	3/10	0/10

4-Week Recovery Group After a 4-week recovery period, the enlarged cecum was still evident in males exposed to 150 mg/m<sup>3</sup> bisphenol A

12-Week Recovery Group After a 12-week recovery period, no macroscopic alterations were evident

### ***Histopathology***

13-Week Terminal Sacrifice Group Histologic alterations associated with bisphenol A exposure were restricted to the mucosae and submucosae of the nasal passages and ranged from very slight to moderate in intensity in terminal sacrifice males and females (Table 7 below). Changes included bilaterally symmetrical hyperplasia of the stratified squamous epithelium of the anterior portion of the ventral meatus, hyperplasia of the ciliated respiratory epithelium adjacent to the vomeronasal organ, and inflammation of the underlying submucosae at both sites. Our reviewers noted that these hyperplastic and inflammatory changes are common chronic responses of the respiratory tract to irritation and that the anterior portion of the ventral meatus would be the first site of contact with bisphenol A particles delivered by inhalation and deposited by gravity (perhaps secondary to aggregation *in situ*). We also noted that the particles were “respirable in size” and hence should not have been deposited at this site. The only other explanation is local chronic irritation/reactive inflammation in response to the inhalation process itself, but that there was not evidence of reactive inflammation in the anterior nares of the control animals. The changes in the local respiratory epithelium adjacent to the vomeronasal organ were indicative of further local deposition or irritation. Lastly, the study pathologist also diagnosed goblet cell hyperplasia in respiratory epithelium lining the naso- and maxillo- turbinates and lateral nasal wall. We note that this again is a histologic response to chronic irritation as the irritant spreads further into the nasal passages.

We agree with the study authors that no treatment-related changes were evident lower in the respiratory tract (e.g., histologic changes were not evident in larynx, trachea and/or lung, or limited set of lymph nodes examined which drain the respiratory tract). Evaluation of a wider set of cervical, submandibular, and tracheobronchial lymph nodes was not performed.

**Table 7. Treatment-Related Histopathologic Observations**

<i>Histopathology Endpoint</i>	0 mg/m <sup>3</sup>		10 mg/m <sup>3</sup>		50 mg/m <sup>3</sup>		150 mg/m <sup>3</sup>	
	<i>Males</i>	<i>Females</i>	<i>Males</i>	<i>Females</i>	<i>Males</i>	<i>Females</i>	<i>Males</i>	<i>Females</i>
Hyperplasia, stratified squamous epithelium, bilateral, multifocal (slight to very slight)	0/10	0/10	0/10	0/10	10/10	10/10	10/10	10/10
Hyperplasia, epithelial, respiratory epithelium, bilateral, multifocal (slight to very slight)	0/10	0/10	0/10	0/10	10/10	10/10	10/10	10/10
Inflammation, chronic, submucosa, bilateral, multifocal (slight to very slight)	0/10	0/10	0/10	0/10	10/10	10/10	10/10	10/10
Hyperplasia, goblet cell, respiratory epithelium, bilateral, multifocal (slight to moderate)	0/10	0/10	0/10	0/10	10/10	10/10	10/10	10/10

4-Week Recovery Sacrifice Group. After a 4-week recovery period, very slight inflammation of the submucosa was still evident in the anterior nasal cavity in high-dose males and females. Goblet cell hyperplasia (very slight) was also evident. The study authors interpreted these observations to indicate partial resolution of the treatment-related, histologic changes in the nasal cavity during the 4-week recovery period. Our reviewers agree.

12-Week Recovery Sacrifice Group. After a 12-week recovery period, no treatment-related histologic alterations were observed in the nasal cavity, indicative of full resolution of the treatment-related effect.

## ASSESSMENT

Our reviewers feel that this study was only marginally sensitive to detect toxic effects to Fischer rats after whole body aerosol exposure to bisphenol A because of multiple study deficiencies. First, urinalysis was not performed. Standard toxicity guidelines recommend urinalysis to assess kidney damage. Second, the clinical pathology assessment (hematology, clinical chemistry and urinalysis) was incomplete as several important components were omitted (1) no evaluation of clotting factor function as an indicator of the ability to form a stable clot, (2) no evaluation of serum or plasma electrolytes (critical indicators of overall health and nutritional plane as well as the integrity of multiple organ systems); (3) no evaluation of total bilirubin (important monitor of erythrocyte turnover and hepatic function); and (4) no evaluation of creatinine (important adjunct to urea nitrogen evaluation). Third, body weight gain was not calculated and food spillage was not measured or addressed. Last, ophthalmology was incomplete as ophthalmic exams were only performed on animals prior to entry on study and post-mortem macroscopic and microscopic examination of the globe is insufficient to elucidate all ophthalmic changes potentially related to treatment. Because of these deficiencies, our reviewers consider the study only marginally sensitive to assess the sub-chronic potential of bisphenol A in rats exposed via whole body inhalation.

## CONCLUSION

13-Week Terminal Sacrifice Group. All treatment related effects are listed in the following table. No effects were seen with respect to clinical chemistry or hematology parameters. Body weights were statistically significantly decreased in all exposure groups throughout the exposure period, but recovered somewhat by the end of treatment. Gross pathology observations considered to be related to treatment in male and female animals exposed to 50 or 150 mg/m<sup>3</sup> included enlarged cecum, facial staining with porphyrin, perineal soiling, and hemolyzed blood in the stomach. Two females exposed to 10 mg/m<sup>3</sup> also had perineal soiling and several male and female animals had facial staining (most likely porphyrin). Histologic findings were seen in both males and females exposed to 50 or 150 mg/m<sup>3</sup> and included very slight goblet cell hyperplasia in the respiratory epithelium adjacent to the ventral meatus and on nasal turbinates. There was also a very slight inflammation of the submucosa beneath the epithelium adjacent to the vomeronasal organ.

4-Week Recovery Group. After a 4-week recovery period, enlarged cecum was still evident in males exposed to 150 mg/m<sup>3</sup>. Also, very slight inflammation of the submucosa was still evident in the anterior nasal cavity in high-dose males and females and goblet cell hyperplasia (very slight) was also evident in male and female animals exposed to 150 mg/m<sup>3</sup>.

12-Week Recovery Group. After a 12-week recovery period, all treatment-related effects were no longer evident.

**Table 8. Summary of Treatment-Related Effects**

<b>Parameter</b>	<b>Observation</b>	<b>Groups Affected</b>
<i>Gross pathology</i>	Increased cecum size	50 and 150 mg/m <sup>3</sup> , males and females
	Facial soiling	50 and 150 mg/m <sup>3</sup> , males and females
	Perineal soiling	50 and 150 mg/m <sup>3</sup> , males and females and 10 mg/m <sup>3</sup> females
	Hemolyzed blood in stomach	50 and 150 mg/m <sup>3</sup> , males and females
<i>Histopathology</i>	Squamous and respiratory epithelium hyperplasia	50 and 150 mg/m <sup>3</sup> , males and females
	Goblet cell Hyperplasia	50 and 150 mg/m <sup>3</sup> , males and females
	Chronic inflammation of the submucosa	50 and 150 mg/m <sup>3</sup> , males and females
<i>Clinical observations</i>	Reddish material around the nose	50 and 150 mg/m <sup>3</sup> , males and females
		10 mg/m <sup>3</sup> , males and females
	Perineal soiling	50 and 150 mg/m <sup>3</sup> , males and females
		10 mg/m <sup>3</sup> , 2 females
<i>Body Weights</i>	Decrease in body weights	All groups, both sexes (body weights did recover somewhat by the end of treatment)
<i>Organ Weights</i>	No effects related to treatment	
<i>Clinical Chemistry</i>	No effects related to treatment	
<i>Hematology</i>	No effects related to treatment	
<i>Food consumption</i>	No effects related to treatment	
<i>Mortality</i>	No effects related to treatment	

Based on a 5.1% decrease in body weights (males) and on clinical observations (porphyrin staining around the nose (males and females), and perineal staining (females) in animals exposed to 10 mg/m<sup>3</sup>, our reviewers do not agree with the study authors' conclusion that the NOEL was 10 mg/m<sup>3</sup>. Based on effects seen in animals exposed to 10 mg/m<sup>3</sup>, our reviewers do not feel that a NOEL was established for this study. However, this study was of limited sensitivity to detect toxic effects of bisphenol A when administered to Fischer rats via whole body inhalation exposure because of numerous study deficiencies including incomplete clinical chemistry, ophthalmic, and histopathologic evaluations

## EXECUTIVE SUMMARY

Fischer 344 rats (30/sex/dose) were administered bisphenol A via inhalation in whole body exposure chambers at target concentrations of 0, 10, 50, or 150 mg/m<sup>3</sup> for 13-weeks. One-third of the animals were sacrificed immediately after the 13-week exposure period, one-third of the animals were sacrificed after a 4-week recovery period, and one-third of the animals were sacrificed after a 12-week recovery period. Treatment related effects included, statistically significantly decreased body weights in all exposure groups that recovered somewhat by the end of treatment; gross pathology observations in males and females exposed to 50 or 150 mg/m<sup>3</sup> including enlarged cecum, presence of hemolyzed blood in the stomach, and perineal and facial soiling; and histopathologic findings in both males and females exposed to 50 or 150 mg/m<sup>3</sup> including very slight goblet cell hyperplasia in the respiratory epithelium adjacent to the ventral meatus and on nasal turbinates. There was also a very slight inflammation of the submucosa beneath the epithelium adjacent to the vomeronasal organ.

After a 4-week recovery period, enlarged cecum was still evident in males exposed to 150 mg/m<sup>3</sup>. Also, very slight inflammation of the submucosa was still evident in the anterior nasal cavity in high-dose males and females and goblet cell hyperplasia (very slight) was also evident. All treatment related effects seen during treatment were no longer present after a 12-week recovery period.

No treatment-related effects were observed in the following endpoints: mortality, hematology parameters, organ weights, or clinical chemistry parameters. No systemic, treatment related effects were seen in any dose-group. No treatment-related effects were seen after a 12-week recovery period. The NOEL was considered to be 10 mg/m<sup>3</sup> by the study authors, however, based on a 5.1% decrease in body weights of males and clinical observations (porphyrin staining of the nose [males and females] and perineal staining [2 females]) seen in animals exposed to 10 mg/m<sup>3</sup>, our reviewers do not feel that a NOEL was established. Additionally, this study was considered to be of limited sensitivity in evaluating the sub-chronic toxicity of bisphenol A for numerous reasons including inadequate clinical chemistry evaluations and incomplete ophthalmic evaluation.

## REFERENCES

Nitschke, K.D., Quast, J.F., and Wolfe, E.L. (1985a). Bisphenol A: Acute aerosol toxicity study in Fischer 344 rats. Dow unpublished report, mammalian and environmental toxicology research laboratory, Dow Chemical Company.

Nitschke, K.D., Quast, J.F., and Wolfe, E.L. (1985b). Bisphenol A: 2-Week aerosol toxicity study in Fischer 344 rats. Dow unpublished report, mammalian and environmental toxicology research laboratory, Dow Chemical Company.

**Bisphenol A: 13-Week Aerosol Toxicity Study with Fischer 344 Rats**

**Technical Reviewer**

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7-20-01

Date

**QA/QC Reviewer**

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7-20-01

Date

## Appendix A: Data Validation

### Bisphenol A: 13-Week Aerosol Toxicity Study with Fischer 344 Rats

#### I. Animals Followed Throughout the Study

Individual animal data were not included in the study; therefore, individual animal data were not followed.

#### II. Critical Effects and Spot Checks

A study protocol was not included in the study report; therefore, a comparison of the study protocol to the materials and methods was not performed. Also, no spot checks were performed because individual animal data were not included in the study report. However, information included in the summary, methods, results, and discussion were compared with available data. All findings are presented below.

- The study authors noted in the results that individual 87A0633 died from traumatic causes on test day 16. No further explanation was included.

#### III. Findings

A data validation was not performed due to the lack of individual animal data and a study protocol; however, information included in the summary, methods, results, and discussion appeared consistent.

  
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Lihua Chen, B.S.

6/28/00  
Date