

National Toxicology Program  
U.S. Department of Health and Human Services



NTP

## Center for the Evaluation of Risks to Human Reproduction

# **NTP-CERHR EXPERT PANEL REPORT on the REPRODUCTIVE and DEVELOPMENTAL TOXICITY of BISPHENOL A**

November 26, 2007

## PREFACE

The National Toxicology Program (NTP)<sup>1</sup> established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of the CERHR is to provide timely, unbiased, scientifically sound evaluations of the potential for adverse effects on reproduction or development resulting from human exposures to substances in the environment. The NTP-CERHR is headquartered at NIEHS, Research Triangle Park, NC and is staffed and administered by scientists and support personnel at NIEHS.

Bisphenol A is a high-production volume chemical used in the production of epoxy resins, polyester resins, polysulfone resins, polyacrylate resins, polycarbonate plastics, and flame retardants. Polycarbonate plastics are used in food and drink packaging; resins are used as lacquers to coat metal products such as food cans, bottle tops, and water supply pipes. Some polymers used in dental sealants and tooth coatings contain bisphenol A. Exposure to the general population can occur through direct contact with bisphenol A or by exposure to food or drink that has been in contact with a material containing bisphenol A. CERHR selected bisphenol A for evaluation because of (1) high production volume, (2) widespread human exposure, (3) evidence of reproductive toxicity in laboratory animal studies, and (4) public concern for possible health effects from human exposures.

Relevant literature on bisphenol A was identified from searches of the PubMed (Medline) and Toxline databases through February 2007 using the term “bisphenol” and the bisphenol A CAS RN (80-05-7). References were also identified from databases such as REPROTOX®, HSDB, IRIS, and DART, from the bibliographies of the literature reviewed, by members of the expert panel, and in public comments.

CERHR convened a 12-member, independent panel of government and non-government scientists to evaluate the scientific studies on the potential reproductive and developmental hazards of bisphenol A. The expert panel met publicly on March 5 - 7, 2007 and August 6 - 8, 2007. The Expert Panel Report on Bisphenol A is intended to (1) interpret the strength of scientific evidence that bisphenol A is a reproductive or developmental toxicant based on data from *in vitro*, animal, or human studies, (2) assess the extent of human exposures to include the general public, occupational groups, and other sub-populations, (3) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive and developmental health effects may be associated with such exposures, and (4) identify knowledge gaps to help establish research and testing priorities to reduce uncertainties and increase confidence in future evaluations. This report has been reviewed by members of the expert panel and by CERHR staff scientists. Copies of this report have been provided to the CERHR Core Committee<sup>2</sup> and will be made available to the public for comment.

Following the public comment period, CERHR will prepare the NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A. This monograph will include the NTP Brief, the Expert Panel Report, and all public comments received on the Expert Panel Report. The NTP-CERHR Monograph will be made publicly available and transmitted to appropriate health and regulatory agencies. **Reports can be obtained from the web site (<http://cerhr.niehs.nih.gov>) or from:**

Michael D. Shelby, Ph.D.  
NIEHS EC-32  
PO Box 12233  
Research Triangle Park, NC 27709  
919-541-3455  
[shelby@niehs.nih.gov](mailto:shelby@niehs.nih.gov)

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<sup>1</sup> NTP is an interagency program headquartered in Research Triangle Park, NC at the National Institute of Environmental Health Sciences, a component of the National Institutes of Health.

<sup>2</sup> The Core Committee is an advisory body consisting of scientists from government agencies. Agencies currently represented are: Environmental Protection Agency, Centers for Disease Control and Prevention, Food and Drug Administration, Consumer Product Safety Commission, National Institute for Occupational Safety and Health, and National Institute for Environmental Health Sciences.

## **A Report of the CERHR Expert Panel:**

Robert E. Chapin, Ph.D., Chair	Pfizer, Inc., Groton CT
Jane Adams, Ph.D.	University of Massachusetts, Boston MA
Kim Boekelheide, M.D., Ph.D.	Brown University, Providence RI
L. Earl Gray, Jr., Ph.D.	US Environmental Protection Agency, Research Triangle Park NC
Simon W. Hayward, Ph.D.	Vanderbilt University Medical Center, Nashville TN
Peter S.J. Lees, Ph.D.	Johns Hopkins University, Baltimore MD
Barry S. McIntyre, Ph.D.	Schering Plough Research Institute, Summit NJ
Kenneth M. Portier, Ph.D.	American Cancer Society, Atlanta GA
Teresa M. Schnorr, Ph.D.	National Institute for Occupational Safety and Health, Cincinnati OH
Sherry G. Selevan, Ph.D.	US Public Health Service (Ret), Silver Spring MD
John G. Vandenberg, Ph.D.	North Carolina State University, Raleigh NC
Susan R. Woskie, Ph.D.	University of Massachusetts, Lowell MA

Members of this panel participated in the evaluation of bisphenol A as independent scientists. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of their employers.

## **With the Support:**

### **NTP/NIEHS**

Michael D. Shelby, Ph.D.	Director, CERHR
Paul M.D. Foster, Ph.D.	Deputy Director, CERHR
Kristina Thayer, Ph.D.	CERHR
Diane Spencer, M.S.	CERHR
John Bucher, Ph.D.	Associate Director, NTP
Allen Dearry, Ph.D.	Interim Associate Director, NTP
Mary Wolfe, Ph.D.	Director, NTP Office of Liaison, Policy & Review
Denise Lasko	NTP Office of Liaison, Policy & Review

### **Sciences International, Inc.**

Anthony Scialli, M.D.	Principal Scientist
Annette Iannucci, M.S.	Toxicologist
Gloria Jahnke, D.V.M.	Toxicologist
Vera Jurgenson, M.S.	Research Assistant

## **Note to Reader:**

This report is prepared according to the Guidelines for CERHR Panel Members established by NTP/NIEHS. The guidelines are available from the CERHR web site (<http://cerhr.niehs.nih.gov/>). The format for this report follows that of CERHR Expert Panel Reports including synopses of studies reviewed, and an evaluation of the Strengths/Weaknesses and Utility (Adequacy) of the study for a CERHR evaluation. Statements and conclusions made under Strengths/Weaknesses and Utility evaluations are those of the CERHR Scientists and are prepared according to the NTP/NIEHS guidelines. In addition, the report includes comments or notes limitations of the study in the synopses. Bold, square brackets are used to enclose such statements. As discussed in the guidelines, square brackets are used to enclose key items of information not provided in a publication, limitations noted in the study, conclusions that differ from authors, and conversions or analyses of data conducted by CERHR. **The findings and conclusions of this report are those of the Expert Panel and should not be construed to represent the views of the National Toxicology Program.**

## ABBREVIATIONS

µg	microgram(s)
µM	micromolar
ADA	American Dental Association
ANCOVA	analysis of covariance
ANOVA	analysis of variance
atm	atmosphere
AUC	area under the time-concentration curve
AUC <sub>BPA</sub>	area under the time-concentration curve for bisphenol A
BMD <sub>1SD</sub>	benchmark dose, 1 control standard deviation
BMD <sub>10</sub>	benchmark dose, 10% effect level
BMDL	benchmark dose 95 <sup>th</sup> percentile lower confidence limit
BrdU	bromodeoxyuridine
bw	body weight
cAMP	cyclic adenosine monophosphate
CAS RN	Chemical Abstracts Service registry number
CERHR	NTP Center for the Evaluation of Risks to Human Reproduction
CFR	Code of Federal Regulations
CHO	Chinese hamster ovary
CI	confidence interval
C <sub>max</sub>	maximum plasma concentration
CNS	central nervous system
CYP	cytochrome P
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EC <sub>10</sub>	10% effective concentration
EC <sub>50</sub>	median effective concentration
ECD	electrochemical detection
ELISA	enzyme-linked immunosorbent assay
eq	equivalent(s)
ER	estrogen receptor
EROD	7-ethoxyresorufin-O-deethylase
ERK	extracellular signal-regulated kinase
FDA	Food and Drug Administration
Fl	fluorescence
fM	femtomolar
fmol	femtomole
FSH	follicle stimulating hormone
GABA	γ-aminobutyric acid
GC/MS	gas chromatography/mass spectrometry
GD	gestation day(s)
GLP	Good Laboratory Practices
GST	glutathione-S-transferase
hCG	human chorionic gonadotropin
HPLC	high performance liquid chromatography
hprt	hypoxanthine phosphoribosyl transferase
IC <sub>50</sub>	median inhibitory concentration
IgG	immunoglobulin G
ip	intraperitoneal(ly)
IU	international unit

im	intramuscular
iv, IV	intravenous(ly)
kg	kilogram(s)
$K_m$	rate constant
L	liter(s)
LC	liquid chromatography
LD <sub>50</sub>	median lethal dose
LH	luteinizing hormone
LOD	limits of detection
LOQ	limits of quantification
m	meter(s)
M	molar
mm	millimeter
MAPK	mitogen activated protein kinase
mCi	millicurie(s)
MDL	minimum detection limit
mg	milligram(s)
mL	milliliter(s)
mM	millimolar
mmol	millimole
mol	mole(s)
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MURST	Italian Ministry for Universities and Scientific and Technological Research
ng	nanogram(s)
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NCTR	National Center for Toxicological Research
ND	not detected
NHANES	National Health and Nutrition Examination Survey
NICHHD	National Institute of Child Health and Human Development
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no observed adverse effect level
NOEL	no observed effect level
nM	nanomolar
Nmol	nanomole
NTP	National Toxicology Program
OECD	Organisation for Economic Cooperation and Development
PBPK	physiologically based pharmacokinetic model
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction
pM	picomolar
PND	postnatal day(s)
ppb	parts per billion
pg	picogram
ppm	parts per million
RIA	radioimmunoassay
RNA	ribonucleic acid
RT	reverse transcriptase

PVC	polyvinylchloride
sc	subcutaneous(ly)
SD	standard deviation
SDN-POA	sexually dimorphic nucleus in the preoptic area of the hypothalamus
SEM	standard error of the mean
sst <sub>3</sub>	somatostatin subtype 3
T <sub>1/2</sub>	half-life
tk	thymidine kinase
T <sub>max</sub>	time to maximum plasma concentration
TUNEL	terminal deoxynucleotidal transferase-mediated dUTP nick-end labeling
TWA	time-weighted average
UDPGT	uridine diphosphate glucuronosyltransferase
US	United States
US EPA	United States Environmental Protection Agency
V <sub>max</sub>	maximum velocity
WEEL	workplace environmental exposure level

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## 1.0 CHEMISTRY, USE, AND HUMAN EXPOSURE

### 1.1 Chemistry

Section 1 is initially based on secondary review sources. Primary study reports are addressed by the Expert Panel if they contain information that is highly relevant for determining the effect of exposure on developmental or reproductive toxicity or if the studies were released subsequent to the reviews.

#### 1.1.1 Nomenclature

The CAS RN for bisphenol A is 80-05-7. Synonyms for bisphenol A listed in Chem IDplus (1) include: 2-(4,4'-Dihydroxydiphenyl)propane; 2,2-Bis(4-hydroxyphenyl)propane; 2,2-Bis(hydroxyphenyl)propane; 2,2-Bis(p-hydroxyphenyl)propane; 2,2-Bis-4'-hydroxyfenylpropan [Czech]; 2,2-Di(4-hydroxyphenyl)propane; 2,2-Di(4-phenylol)propane; 4,4'-(1-Methylethylidene)bisphenol; 4,4'-Bisphenol A; 4,4'-Dihydroxydiphenyl-2,2-propane; 4,4'-Dihydroxydiphenyldimethylmethane; 4,4'-Dihydroxydiphenylpropane; 4,4'-Isopropylidene diphenol; 4,4'-Isopropylidenebisphenol; 4,4'-Isopropylidene diphenol; Biphenol A; Bis(4-hydroxyphenyl) dimethylmethane; Bis(4-hydroxyphenyl)dimethylmethane; Bis(4-hydroxyphenyl)propane; Bisferol A [Czech]; Bisphenol. Bisphenol A; DIAN; Diano; Dimethyl bis(p-hydroxyphenyl)methane; Dimethylbis(p-hydroxyphenyl)methane; Dimethylmethylene-p,p'-diphenol; Diphenylolpropane; Ipognox 88; Isopropylidenebis(4-hydroxybenzene); Parabis A, Phenol; (1-methylethylidene)bis-, Phenol; 4,4'-(1-methylethylidene)bis-, Phenol, 4,4'-dimethylmethylenedi-, Phenol, 4,4'-isopropylidenedi-, Pluracol 245, Propane; 2,2-bis(p-hydroxyphenyl)-; Rikabanol; Ucar bisphenol A; Ucar bisphenol HP; beta,beta'-Bis(p-hydroxyphenyl)propane; beta-Di-p-hydroxyphenylpropane; p,p'-Bisphenol A; p,p'-Dihydroxydiphenyldimethylmethane; p,p'-Dihydroxydiphenylpropane; p,p'-Isopropylidenebisphenol; and p,p'-Isopropylidenediphenol.

#### 1.1.2 Formula and molecular mass

Bisphenol A has a molecular mass of 228.29 g/mol and a molecular formula of  $C_{15}H_{16}O_2$  (2). The structure for bisphenol A is shown in [Figure 1](#).

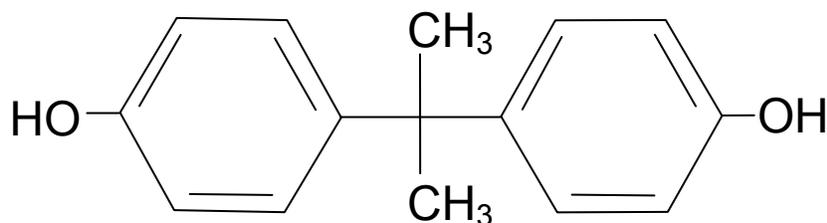


Figure 1. Structure for Bisphenol A.

#### 1.1.3 Chemical and physical properties

Bisphenol A is a white solid with a mild phenolic odor (2). Physicochemical properties are listed in [Table 1](#).

1 **Table 1. Physicochemical Properties of Bisphenol A**

Property	Value
Odor threshold	No data found
Boiling point	220°C at 4 mm Hg; 398 C at 760 mm Hg
Melting point	150–157°C
Specific gravity	1.060–1.195 g/mL at 20–25°C
Solubility in water	120–300 mg/L at 20–25°C
Vapor pressure	$8.7 \times 10^{-10}$ – $3.96 \times 10^{-7}$ mm Hg at 20–25°C
Stability/reactivity	No data found
Log $K_{ow}$	2.20–3.82
Henry constant	$1.0 \times 10^{-10}$ atm·m <sup>3</sup> /mol

From Staples et al. (3).

#### 2 3 *1.1.4 Technical products and impurities*

4 Purity of bisphenol A was reported at 99–99.8%, and common impurities observed were phenol and ortho  
5 and para isomers of bisphenol A [reviewed in (2)]. Terasaki et al. (4) used reversed phase  
6 chromatography and nuclear magnetic resonance spectroscopy to characterize the composition of 5  
7 commercial bisphenol A samples. The nominal purity of the samples was 97 or 98%. Actual purities were  
8 95.3 to > 99%. Up to 15 contaminants were identified among which were: 4-hydroxyacetophenone; 4,42-  
9 (1,3-dimethylbutylidene) bisphenol; *p*-cumylphenol; 4-hydroxyphenyl isobutyl methyl ketone; 2,4\*-  
10 dibhydroxy-2,2-diphenylpropane; 2,42-dibhydroxy-2,2-diphenylpropane; 2,4-bis(4-  
11 hydroxycumyl)phenol; 2,3-dihydro-3-(42-hydroxyphenyl)-1,1,3-trimethyl-1*H*-inden-5-ol; 2-(42-  
12 hydroxyphenol)-2,2,4-trimethylchroman; and 4-(42-hydroxyphenol)-2,2,4-trimethylchroman (5).

13  
14 No information on trade names for bisphenol A was located.

#### 15 16 *1.1.5 Analytical considerations*

17 Measurement of bisphenol A in environmental and biologic samples can be affected by contamination  
18 with bisphenol A in plastic laboratory ware and in reagents (6, 7). Accuracy is also affected by  
19 measurement technique, particularly at the very low concentrations that can now be measured. Enzyme-  
20 linked immunosorbent assay (ELISA) has poor correlation with the LC-ECD method and also the  
21 different ELISA kits correlate poorly with each other. ELISA methods may over-estimate bisphenol A in  
22 biologic samples due to lack of specificity of the antibody and effects of the biologic matrix (8, 9).  
23 Although high performance liquid chromatography (HPLC) with ultraviolet, fluorescence, or  
24 electrochemical detection can be sensitive to concentrations < 0.5 ng/ml (10-13), these methods are  
25 unable to make definitive identification of bisphenol A or bisphenol A glucuronides, because similar  
26 retention times may occur for the metabolites of other endogenous and exogenous compounds (7). Use of  
27 LC- mass spectrometry (MS) with and without hydrolysis of bisphenol A glucuronide permits  
28 determination of free and total bisphenol A with a limit of quantification of 0.1 for MS (10) and 1  
29 µg/L for MS/MS (7). Gas chromatography (GC)/MS has been used with solid phase extraction after  
30 treatment with glucuronidase and derivitization to measure total bisphenol A with a limit of detection of  
31 0.05 µg/L for MS (14), 0.1 µg/L for MS/MS (15). Some of the variability in studies cited in this and  
32 subsequent sections may be due to differences in measurement techniques and to contamination.  
33 Bisphenol A glucuronidate can be an unstable product that can be degraded in acidic and basic pH  
34 solutions and can be hydrolyzed to free bisphenol A at neutral pH and room temperature in diluted rodent  
35 urine, placental and fetal tissue homogenates at room temperature. However, conjugates in urine are  
36 stable for at least 7 days when stored at -4°C and at least 180 days when stored at -70°C (16, 17).

37

1  
2 **1.2 Use and Human Exposure**

3  
4 *1.2.1 Production information*

5 Bisphenol A is manufactured by the acid catalyzed condensation of phenol and acetone (18).

6  
7 In 1998, members of the Society of the Plastics Industry Bisphenol A Task Group [**assumed**  
8 **manufacturers of bisphenol A**] included Aristech Chemical Corporation, Bayer Corporation, Dow  
9 Chemical Company, and Shell Chemical Company (3). Current manufacturers of bisphenol A in the US  
10 are Bayer MaterialScience, Dow Chemical Company, General Electric, Hexion Specialty Chemicals, and  
11 Sunoco Chemicals ((18), S. Hentges, public comments, February 2, 2007). There are currently 6  
12 bisphenol A and 4 polycarbonate plants in the US (S. Hentges, personal communication, October 30,  
13 2006); 3 of the 4 polycarbonate plants are located within bisphenol A plants. In 2000, there were 13  
14 epoxy plants in the US, but was not clear if all of the plants manufactured bisphenol A-containing epoxy  
15 resins.

16  
17 In mid-2004, US bisphenol A production volume was reported at 1.024 million metric tons [**~2.3 billion**  
18 **pounds**] (18). A production volume of 7.26 billion g [**16 million pounds**] was reported for bisphenol A  
19 in 1991 (reviewed in (19). US bisphenol A consumption was reported at 856,000 metric tons [**~1.9 billion**  
20 **pounds**] in 2003 (18); 2003 consumption patterns included 619,000 metric tons [**~1.4 billion pounds**]  
21 used in polycarbonate resins, 184,000 metric tons [**~406 million pounds**] used in epoxy resins, and  
22 53,000 metric tons [**~117 million pounds**] used in other applications.

23  
24 *1.2.2 Use*

25 In 1999 and 2003, it was reported that most bisphenol A produced in the US was used in the manufacture  
26 of polycarbonate and epoxy resins and other products [reviewed in (3, 18)]. Polycarbonate plastics may  
27 be used in the manufacture of compact discs, “solid and multi wall sheet in glazing applications and  
28 film,” food containers (e.g., milk, water, and infant bottles), and medical devices [reviewed in (2)].  
29 Bisphenol A may have been used at one time in Europe in polyvinyl chloride cling film and plastic bags,  
30 but that use is believed to have been discontinued (20). Contact with drinking water may occur through  
31 the use of polycarbonate for water pipes and epoxy-phenolic resins in surface coatings of drinking water  
32 storage tanks [reviewed by the European Food Safety Authority (20)].

33  
34 Polycarbonate blends have been used to manufacture injected molded parts utilized in alarms, mobile  
35 phone housings, coil cores, displays, computer parts, household electrical equipment, lamp fittings, and  
36 power plugs. Automotive and related uses for polycarbonate blends include light reflectors and coverings,  
37 bumpers, radiator and ventilation grills, safety glazing, inside lights, and motorcycle shields and helmets.  
38 Epoxy resins are used in protective coatings, structural composites, electrical laminates, electrical  
39 applications, and adhesives. The European Union (2) reported that smaller volumes of bisphenol A are  
40 used in production of phenoplast, phenolic, and unsaturated polyester resins, epoxy can coatings,  
41 polyvinylchloride (PVC) plastic, alkoxyated bisphenol A, thermal paper, and polyols/polyurethane.  
42 Other uses reported for products manufactured from bisphenol A included protective window glazing,  
43 building materials, optical lenses, and development of dyes [reviewed in (3)]. A search of the National  
44 Library of Medicine Household Products Database (21) revealed that bisphenol A-based polymers are  
45 used in coatings, adhesives, and putties available to the general public for use in automobiles, home  
46 maintenance and repair, and hobbies, but only 3 epoxy products, used for crafts and hobbies, contain  
47 bisphenol A itself.

48  
49 Some polymers manufactured with bisphenol A are Food and Drug Administration (FDA)-approved for  
50 use in direct and indirect food additives and in dental materials, as reported in the Code of Federal  
51 Regulations (CFR) (22). In the CFR, bisphenol A is often referred to as 4,4'-isopropylidnediphenol.

## 1.0 Chemistry, Use, and Human Exposure

1 Polymers manufactured with bisphenol A are FDA-approved for use as anoxomers and in coatings,  
2 adhesives, single and repeated food contact surfaces, and tooth shade resin materials.

3  
4 The European Union (2) noted that resins, polycarbonate plastics, and other products manufactured from  
5 bisphenol A can contain trace amounts of residual monomer and additional monomer may be generated  
6 during breakdown of polymer. The American Plastics Council reports that residual bisphenol A  
7 concentrations in polycarbonate plastics and epoxy resins are generally <50 ppm (S. Hentges, personal  
8 communication, October 30, 2006). Polymer hydrolysis can occur at elevated temperature or extreme pH.  
9 An example of potential human exposure is migration of bisphenol A from a food container into the food.  
10 Exposure to bisphenol A through food is discussed in detail in Section 1.2.3.2.

### 11 *1.2.3 Occurrence*

#### 12 *1.2.3.1 Environmental fate and bisphenol A levels in environment*

13  
14 Bisphenol A may be present in the environment as a result of direct releases from manufacturing or  
15 processing facilities, fugitive emission during processing and handling, or release of unreacted monomer  
16 from products (2). According to the Toxics Release Inventory database, total environmental release of  
17 bisphenol A in 2004 was 181,768 pounds, with releases of 132,256 pounds to air, 3533 pounds to water,  
18 172 pounds to underground injection, and 45,807 pounds to land (23).

19  
20  
21 Bisphenol A released to the atmosphere is likely degraded by hydroxy radicals (2). Half-life for the  
22 reaction between bisphenol A and hydroxy radicals was estimated at 0.2 days. It was also noted that  
23 photolysis and photodegradation of bisphenol A in the atmosphere is possible and photooxidation half-  
24 lives of 0.74–7.4 hours were estimated [reviewed in (2, 3)]. The European Union (2) noted that because of  
25 its low volatility and relatively short half-life in the atmosphere, bisphenol A is not likely to enter the  
26 atmosphere in large amounts. Removal by precipitation and occurrence in rain water were thought likely  
27 to be negligible. Because of its short half-life in the atmosphere, bisphenol A is unlikely to be transported  
28 far from emission points.

29  
30 Based on vapor pressure and Henry constant (Table 1), the European Union (2) and Staples et al. (3)  
31 concluded that bisphenol A is of low volatility and not likely to be removed from water through  
32 volatilization. Both groups concluded that hydrolysis of bisphenol A in water is unlikely. However, there  
33 was disagreement on potential for photooxidation of bisphenol A in water. Based on physical and  
34 chemical properties, the European Union concluded that photolysis of bisphenol A in water is unlikely.  
35 Staples et al. noted that bisphenol A is able to absorb ultraviolet light, especially in a basic solution.  
36 Therefore, it was concluded that photolysis from surface water is possible, depending on conditions such  
37 as pH, turbidity, turbulence, and sunlight. Photooxidation half-life of bisphenol A in water was estimated  
38 at 66 hours to 160 days [reviewed in (3)]. Rapid biodegradation of bisphenol A from water was reported  
39 in the majority of studies reviewed by the European Union (2) and Staples et al. (3). A biodegradation  
40 half-life of 2.5–4 days was reported in a study measuring bisphenol A concentrations in surface waters  
41 near the receiving stream of a bisphenol A manufacturer [reviewed in (3)].

1  
2**Table 2. Concentrations of Bisphenol A Detected in Water**

Sample Type	Detection Method	Detection Rate (%)	Concentration ( $\mu\text{g/L}$ ) Range [median]	Reference
<b>Surface Water</b>				
German Rivers	GC-MS	100	0.005-0.014[3.8]	Kuch et al. (24)
Louisiana, U.S.	GC-MS	0	< MDL 0.1	Boyd et al. (25)
U.S Streams	GC-MS	41.2	[0.14] max 12	Kolpin et al. (26)
Netherlands	GC-MS	78-93	Max marine 0.33; Max fresh 21	Belfroid et al. (27)
<b>Drinking Water</b>				
Louisiana, U.S.	GC-MS	0	< MDL 0.1	Boyd et al. (25)
Ontario, Canada	GC-MS	0	< MDL 0.1	Boyd et al. (25)
Germany	GC-MS	100	0.005-0.002 [1.1]	Kuch et al. (24)
<b>Landfill Leachate</b>				
Japan	GC-MS	100	740	Kawagoshi et al. (28)
Japan	GC-MS	70% sites	1.3-17, 200 [269]	Yamamoto et al. (29)
<b>Sewage Treatment Works</b>				
Germany	GC-MS	94	0.005-0.047[10]	Kuch et al. (24)
Louisiana	GC-MS	0	< MDL 0.1	Boyd et al. (25)

3  
4 When the Staples et al. (3) review was published, soil sorption constants had not been measured but were  
5 estimated at 314–1524. Based on such data, the European Union (2) and Staples et al. (3) concluded that  
6 bisphenol A adsorption to soils or sediments would be “modest” or “moderate.” Based on data for  
7 degradation of bisphenol A in water, the European Union (2) predicted that bisphenol A would be  
8 degraded in soil and estimated a half-life of 30 days for degradation of bisphenol A in soil. Subsequent to  
9 the Staples et al. and European Union reviews, a study examining fate of  $^{14}\text{C}$ -bisphenol A in soils through  
10 laboratory soil degradation and batch adsorption tests was released by Fent et al. (30). In that study,  $^{14}\text{C}$ -  
11 bisphenol A was rapidly dissipated and not detectable in 4 different soil types within 3 days. Soil  
12 distribution coefficients were determined at 636–931, and based on those values, the study authors  
13 concluded that bisphenol A has low mobility in soil. The study authors concluded that bisphenol A is not  
14 expected to be stable, mobile, or bioavailable from soils.

15  
16 In studies reviewed by the European Union (2) and Staples et al. (3), bioconcentration factors for fish  
17 were measured at 3.5–68 and were found to be lower than values estimated from the  $K_{ow}$ . Both groups  
18 concluded that potential for bioconcentration of bisphenol A is low in fish. Higher bioconcentration  
19 factors (134–144) were determined for clams [reviewed in (2)].

20  
21 Two studies examining aggregate exposures in preschool age children in the US used GC/MS to measure  
22 bisphenol A concentrations in environmental media (31, 32). In the first study (31), bisphenol A  
23 concentrations were measured in air outside 2 day care centers and the homes of 9 children. Bisphenol A  
24 was detected in 9 of 13 outdoor air samples at <0.100–4.72  $\text{ng/m}^3$  (mean concentration 2.53  $\text{ng/m}^3$  at day  
25 care centers and 1.26  $\text{ng/m}^3$  at home). In indoor air from day care centers and homes, bisphenol A was  
26 detected in 12 of 13 samples at <0.100–29  $\text{ng/m}^3$  (mean concentration 6.38  $\text{ng/m}^3$  at day care centers and  
27 11.8  $\text{ng/m}^3$  at home). At those same locations, bisphenol A was detected in all of 13 samples of floor dust  
28 at means (range) of 1.52–1.95 (0.567–3.26) ppm ( $\mu\text{g/g}$ ) and play area soils at means (range) of 0.006–  
29 0.007 (0.004–0.014) ppm ( $\mu\text{g/g}$ ). In the second study (32), bisphenol A concentrations were measured  
30 inside and outside at least 222 homes and 29 daycare centers. Bisphenol A was detected in 31–44% of  
31 outdoor air samples from each location; concentrations ranged from <LOD (0.9) to 51.5  $\text{ng/m}^3$ . Medians  
32 were < LOD. Forty-five to 73% of indoor air samples contained detectable concentrations of bisphenol A;  
33 concentrations were reported at <LOD (0.9)–193  $\text{ng/m}^3$ . Median values were <LOD–1.82  $\text{ng/m}^3$ .

## 1.0 Chemistry, Use, and Human Exposure

1 Bisphenol A was detected in 25–70% of dust samples; concentrations were reported at <LOD (20)–707  
2 ng/g. Median values were <LOD–30.8 ng/g.

3  
4 A second US study used a GC/MS method to measure bisphenol A concentrations in dust from 1 office  
5 building and 3 homes and in air from an office building and 1 home (33). Bisphenol A was detected in 3  
6 of 6 dust samples (reporting limit > 0.01 µg/extract) at concentrations of 0.25–0.48 µg/g dust. In indoor  
7 air samples collected from offices and residences, bisphenol A was detected in 3 of 6 samples (detection  
8 limit ~0.5 ng/m<sup>3</sup>) at concentrations of 0.002–0.003 µg/m<sup>3</sup>. In another study using a GC/MS technique,  
9 bisphenol A concentrations in indoor air from 120 US homes were below reporting limits (0.018 µg/m<sup>3</sup>)  
10 (34). Median (range) bisphenol A concentration in dust in this study was 0.821 (<0.2–17.6) µg/g, with  
11 86% of samples above the reporting limit.

12  
13 Limited information is available for bisphenol A concentrations in US water (Table 2). In 1996 and/or  
14 1997, mean bisphenol A concentrations were reported at 4–8 µg/L in surface water samples near 1  
15 bisphenol A production site but bisphenol A was not detected (<1 µg/L) in surface water near 6 of 7  
16 bisphenol A production sites in the US (35). Bisphenol A was detected at a median concentration (in  
17 samples with detectable bisphenol A above the reporting limit of 0.09 µg/L) of 0.14 µg/L and a maximum  
18 concentration of 12 µg/L in 41.2% of 85 samples collected from US streams in 1999 and 2000 (26). In  
19 2001 and 2002, bisphenol A was not detected (< 0.001 µg/L) in effluent from a wastewater treatment  
20 plant in Louisiana, and concentrations were not quantifiable [quantification limit not defined] in  
21 samples collected from surface waters in Louisiana and in drinking water at various stages of treatment at  
22 plants in Louisiana and Ontario, Canada (25). In water samples collected in Europe and Japan from the  
23 1970s through 1989, bisphenol A concentrations were ≤1.9 µg/L and in most cases were ≤0.12 µg/L  
24 [reviewed in (2)].

### 25 1.2.3.2 Potential exposures from food and water

26 The European Union (2) noted that the highest potential for human exposure to bisphenol A is through  
27 products that directly contact food. Examples of food contact materials that can contain bisphenol A  
28 include food and beverage containers with internal epoxy resin coatings and polycarbonate tableware and  
29 bottles, such as those used to feed infants.

30  
31 In addition to commercial food sources, infants consume breast milk. Calafat et al. (36) reported a median  
32 bisphenol A concentration of ~1.4 µg/L [as estimated from a graph] in milk from 32 women (Table 3).  
33 Bisphenol A was measured after enzymatic hydrolysis of conjugates. Ye et al. (37) found measurable  
34 concentrations of bisphenol A in milk samples from 18 of 20 lactating women. Free bisphenol A was  
35 found in samples from 12 women. The median total bisphenol concentration in milk was 1.1 µg/L (range:  
36 undetectable to 7.3 µg/L). The median free bisphenol A concentration was 0.4 µg/L (range: undetectable  
37 to 6.3 µg/L). Sun et al. (12) used an HPLC method to measure bisphenol A concentrations in milk from  
38 23 healthy lactating Japanese women. Bisphenol A concentrations ranged from 0.28 to 0.97 µg/L, and the  
39 mean ± SD concentration was reported at 0.61 ± 0.20 µg/L. No correlations were observed between  
40 bisphenol A and triglyceride concentrations in milk. Values from 6 milk samples were compared to  
41 maternal and umbilical blood samples previously reported in a study by Kuroda et al. (11). Bisphenol A  
42 values were higher in milk, and the milk/serum ratio was reported at 1.3. Bisphenol A values in milk were  
43 comparable to those in umbilical cord serum. [It was not clear whether milk and serum samples were  
44 obtained from the same volunteers in the two studies.]  
45

1 **Table 3. Bisphenol A Concentrations in Human Breast Milk**

Source (n)	Method	LOD	Free (ng/ml) mean +/- SD (range)	Total (ng/ml) mean +/- SD (range)	Detection Rate (%)	Reference
Japanese (23)	HPLC-FI	0.11 ng/ml	0.61 +/- 0.20 (0.28- 0.97)		100%	Sun et al. (12)
Japanese (101) (colostrum 3 days after delivery)	ELISA	N.A.		3.41 +/- 0.13 (1-7)	100%	Kuruto et al. (38)
U.S. (20)	HPLC- MS/MS	0.3 ng/ml	1.3 (<0.3-6.3)	1.9 (<0.3-7.3)	60% Free 90% Total	Ye et al. (37)
Japanese (3)	GC-MS	0.09 ng/g		0.46 (<0.09-0.65)	67%	Otaka et al. (39)
U.S. (32)	N.A	N.A	N.A	1.4 *	N.A	Calafat et al. (36)

2 \*estimated from a graph

3  
4 Studies have measured migration of bisphenol A from polycarbonate infant bottles or containers into  
5 foods or food simulants. Results of those studies are summarized in Table 4. Analyses for bisphenol A  
6 were conducted by GC/MS or HPLC. The European Union (2) group noted that in many cases bisphenol  
7 A concentrations were below the detection limit in food simulants. When bisphenol A was detected,  
8 concentrations were typically  $\leq 50$   $\mu\text{g/L}$  in simulants exposed to infant bottles and  $\leq 5$   $\mu\text{g/kg}$  in simulants  
9 exposed to polycarbonate tableware. An exception is 1 study that reported bisphenol A concentrations at  
10 up to  $\sim 192$   $\mu\text{g/L}$  in a 10% ethanol food simulant and 654  $\mu\text{g/L}$  in a corn oil simulant (40). In the study,  
11 cut pieces of bottles were incubated, and the study authors acknowledged that bisphenol A could have  
12 migrated from the cut edges. **[The Expert Panel notes that incubations were at 70 or 100 °C for 240**  
13 **hours, representing conditions not anticipated for normal use of baby bottles.]** One study conducted  
14 with actual infant food (formula and fruit juice) reported no detectable bisphenol A (41). Some studies  
15 examining the effects of repeated use of polycarbonate items noted increased leaching of bisphenol A  
16 with repeated use (42-44). It was suggested that the increase in bisphenol A migration was caused by  
17 damage to the polymer during use. Results from other reports suggested that leaching of bisphenol A  
18 decreased with repeated use, and it was speculated that available bisphenol A was present at the surface of  
19 the product and therefore removed by washing (45) and (46), reviewed by the European Union (2) and  
20 Haighton et al. (47). One study (46) demonstrated higher concentrations of bisphenol A in simulants  
21 exposed to products that had been recalled because of unacceptable residual concentrations of bisphenol  
22 A and other compounds. The study by Biles et al. (45) demonstrated that infant bottles exposed to 50 or  
23 95% ethanol at 65°C for 240 hours leached bisphenol A at concentrations exceeding residual monomer  
24 concentrations, and it was suggested that hydrolysis of the polymer had occurred.

1 Table 4. Examination of Bisphenol A in Polycarbonate Food Contact Surfaces

Sample (Location)	Procedure	Bisphenol A concentration in simulant	Reference
Commercially available infant bottles containing residual bisphenol A concentrations of 7–46 ppm (US)	Common use: Bottles were boiled for 5 minutes, filled with water or 10% ethanol, and stored at room temperature for up to 72 hours. Worst case use: Bottles were boiled for 5 minutes, filled with water or 10% ethanol, heated to 100°C for 0.5 hour, cooled to room temperature, and refrigerated for 72 hours.	Not detected (ND) (LOD 5 ppb [ $\mu\text{g/L}$ ]; corresponding to a food concentration of 1.7 ppb) following either procedure.	FDA (48)
21 new and 12 used (1–2-year-old) infant bottles (UK)	Bottles were pre-washed, steam sterilized, filled with boiling water or 3% glacial acetic acid, refrigerated at 1–5°C for 24 hours, and heated to 40°C prior to sampling.	ND (LOD 10 $\mu\text{g/L}$ ) [ <b>ppb</b> ] from new bottles; ND (<10 $\mu\text{g/L}$ ) to 50 $\mu\text{g/L}$ from used bottles exposed to either simulant [ <b>mean not given</b> ].	Earls et al. (42)
Infant bottles with residual bisphenol A concentrations of 26 mg/kg [ <b>number tested not indicated</b> ]. (UK)	Bottles were sterilized with hypochlorite, in dishwasher, or by steam; filled with infant formula, fruit juice, or distilled water; microwaved for 30 seconds; and left to stand for 20 minutes (1 cycle). Samples were analyzed after 3, 10, 20, or 50 cycles. Other bottles were filled with distilled water and left to stand for 10 days at 40°C.	ND (LOD 0.03 mg/kg) [ <b>&lt; 30 <math>\mu\text{g/kg}</math> or ppb</b> ] under any condition.	Mountfort et al. (41)
6 infant feeding bottles (country of purchase not known)	Bottles were filled with water at 26°C and left to stand for 5 hours or filled with water at 95°C and left to stand overnight.	ND (LOD 2 ppb [ $\mu\text{g/L}$ ]) in bottles filled with water at 26°C and 3.1–55 ppb [ $\mu\text{g/L}$ ] in bottles filled with water at 95°C.	Hanai(49) <sup>a</sup>
14 samples of new infant feeding bottles and tableware including a bowl, mug, cup, and dish recalled because residual bisphenol A and other phenol concentrations exceeded 500 ppm [ <b>mg/kg</b> ] (Japan)	Products were exposed to n-heptane, water, 4% acetic acid, or 20% ethanol; in some cases simulant was heated to 60 or 95°C; in other cases, the object was boiled for 5 minutes; analyses were usually conducted after a 30-minute contact period.	Up to 40 ppb [ <b><math>\mu\text{g/kg}</math></b> ] from recalled products and ND (LOD 0.2) to 5 $\mu\text{g/kg}$ from commercially available products.	Kawamura et al. (46) <sup>a,b</sup>
Discs prepared from commercial food-grade polycarbonate resins (residual bisphenol A at 8800 to 11,200 $\mu\text{g/kg}$ ) from US manufacturers	Materials exposed to water, 10% ethanol, or Miglyol® (fractionated coconut oil) at 100°C for 6 hours or water, 3% acetic acid, 10% ethanol, or Miglyol at 49°C for 6–240 hours.	ND (LOD 5 ppb [ $\mu\text{g/L}$ ]) under all conditions.	Howe and Borodinsky (50)

## 1.0 Chemistry, Use, and Human Exposure

Sample (Location)	Procedure	Bisphenol A concentration in simulant	Reference
2 infant bottles from Japan	In 3 repeated tests, boiling water was added to bottles; bottles were incubated at 95°C for 30 minutes and cooled to room temperature. Prior to repeating the test a 4 <sup>th</sup> time, the bottles were scrubbed with a brush.	Below quantification limit (LOD 0.57 ppb [ <b>µg/L</b> ]) to mean concentrations of 0.75 ppb prior to brushing and <0.57 to 0.18 ppb after brushing.	Sun et al. (51)
4 new different brands of infant bottles (Argentina)	Bottles were exposed to distilled water, 3% acetic acid, or 15% ethanol at 80°C for 2 minutes or distilled water at 100°C for 0.5 minutes.	1.1–2.5 ppb [ <b>µg/L</b> ].	D’Antuono et al. (52)
12 infant bottles (Norway)	Bottles were tested prior to washing and following 51 and 169 dish washings; bottles were occasionally brushed (13 times by 2 <sup>nd</sup> test and 23 times by 3 <sup>rd</sup> test) and boiled (12 times by 2 <sup>nd</sup> testing and 25 times by 3 <sup>rd</sup> testing). Unwashed bottles were rinsed with boiling water before testing. For testing, bottles were filled with hot water and incubated at 100°C for 1 hour.	Mean (range) µg/L [ <b>ppb</b> ]: 0 washes: 0.23 (0.11–0.43) 51 washes: 8.4 (3.7–17) 169 washes: 6.7 (2.5–15)	Brede et al. (43)
18 infant bottles (12 tested) (UK)	Bottles were tested prior to and after 20 and 50 dish washings; bottles were brushed after every 2 wash cycles. Bottles were sterilized with boiling water, filled with 3% acetic acid, or 10% ethanol, and incubated at 70°C for 1 hour.	Prior to washing: ND (LOD 1.1 ppb or µg/L) in 10% ethanol and ND (LOD 0.34 ppb or µg/L) in 3% acetic acid; 20 washes: ND to 4.5 ppb in 10% ethanol and ND to 0.51 ppb in 3% acetic acid; 50 washes: ND to 3.1 ppb in 10% ethanol and ND to 0.7 ppb in 3% acetic acid.	CSL (44)
28 brands of new infant bottles (residual bisphenol A concentrations of <3 to 141 mg/kg) manufactured in Europe or Asia (Singapore)	Bottles were cut, and the pieces were exposed to 10% ethanol at 70°C or corn oil at 100°C for 8–240 hours.	ND (LOD 0.05) to 1.92 µg/in <sup>2</sup> [ <b>&lt;5–192 µg/L or ppb</b> ] in 10% ethanol and ND (LOD 0.05) to 6.54 µg/in <sup>2</sup> [ <b>&lt;5–654 µg/L</b> ] in corn oil over the 240-hour exposure period.	Onn Wong et al. (40)
22 new infant bottles and 20 used (3–36 months) bottles (Netherlands)	Bottles were immersed in boiling water for 10 minutes prior to testing and filled with distilled water or 3% acetic acid and incubated at 40 °C for 24 hours.	ND in new bottles (< 2.5 µg/L (LOD) [ <b>ppb</b> ] in distilled water and < 3.9 µg/L (LOD) in 3% acetic acid) or in used bottles exposed to 3% acetic acid; not detected to non-quantifiable (<5 µg/L) in distilled water from used bottles.	FCPSA (53)

## 1.0 Chemistry, Use, and Human Exposure

Sample (Location)	Procedure	Bisphenol A concentration in simulant	Reference
New unwashed infant bottles (number not indicated) (Japan)	Bottles were exposed to water at 95°C for 30 minutes.	ND (LOD 0.05 µg/L [ <b>ppb</b> ]) to 3.9 µg/L.	Japanese studies reviewed in Miyamoto and Kotake (54)
5-gallon water carboys	Water was stored in the carboys for 3, 12, or 39 weeks, temperature not indicated.	0.1–0.5 µg/L [ <b>ppb</b> ] at 3 and 12 weeks and 4.6–4.7 µg/L at 39 weeks. <sup>c</sup>	Biles et al. (45)

<sup>a</sup>Reviewed by European Union (2).

<sup>b</sup>Reviewed by Haighton et al. (47).

<sup>c</sup>The authors of this study identified an error in the units reported in their study and that the correct concentrations are 1000-fold higher than indicated in the paper, the correct values are indicated in table above (T. Begley, email communication, August 6, 2007).

1 High molecular weight, heat-cured bisphenol A-based epoxy resins are used as protective linings in cans  
 2 for food and beverages and may be used in wine storage vats (2). Residual bisphenol A monomer can  
 3 migrate from the coatings to foods or beverages contained within cans. Studies were conducted to  
 4 measure actual concentrations of bisphenol A in commercially available foods or to measure  
 5 concentrations of bisphenol A leaching from can linings into food simulants. Because the actual  
 6 measurement of bisphenol A concentrations in canned foods represents the most realistic situation, the  
 7 CERHR review will focus on those data. Studies conducted with simulants will not be reviewed, with the  
 8 exception of one study by Howe et al. (55) that was considered by the FDA (48) in their estimates of  
 9 bisphenol A intake.

10  
 11 Bisphenol A concentrations detected in infant foods are summarized in Table 5, and bisphenol A  
 12 concentrations detected in non-infant foods are summarized in Table 6. With the exception of isolated  
 13 cases in which bisphenol A concentrations were measured at up to ~0.8 mg/kg food, most measurements  
 14 were below 0.1 mg/kg. The European Union also noted an extraction study conducted with an epoxy resin  
 15 that is occasionally used to line wine vats. Based on that study, a worst-case scenario of 0.65 mg/L  
 16 bisphenol A in wine was used. The European Union noted that the value represents a very worst-case  
 17 exposure scenario but decided to use that number in risk estimates because no other value was available.  
 18 **[The Expert Panel notes that a study of bisphenol A in wine (56) identified a maximum**  
 19 **concentration of 2.1 µg/L (Table 6).]**

20  
 21 In one study, empty cans were filled with soup, beef, evaporated milk, carrots, or 10% ethanol (57). The  
 22 cans were then sealed, processed at 5, 20, or 40°C, and sampled at 1 or 10 days or 1, 3, or 9 months. Half  
 23 the cans processed according to each condition were dented. It was determined that 80–100% of the  
 24 bisphenol A migrated to food immediately after processing, and that bisphenol A concentrations did not  
 25 change during storage or as a result of denting. The study authors concluded that most migration occurred  
 26 during can processing. Boiling the cans or heating to 230°C did not increase migration of bisphenol A, but  
 27 that finding appears to contrast with findings of others. Kang et al. (58) examined the effects of  
 28 temperature, duration of heating, glucose, sodium, and oil on migration of bisphenol A from cans. In cans  
 29 filled with water, heating to 121°C compared to 105°C increased migration of bisphenol A but the  
 30 duration of heating had no significant effect. Compared to cans filled with water, increased amounts of  
 31 bisphenol A migrated from cans filled with 1–10% sodium chloride, 5–20% glucose, or vegetable oils and  
 32 heated to 121°C. Takao et al. (59) reported increased leaching of bisphenol A from cans into water when  
 33 the cans were heated to ≥80°C.

34  
 35 **Table 5. Surveys of Bisphenol A Concentrations in Canned Infant Formulas or Food**

Food (no. sampled)	Bisphenol A concentration, µg/kg or µg/L	Country	Reference
Infant formula (14)	Mean 5 (0.1–13.2ppb [ <b>µg/L</b> ]); when diluted with water to make prepared formula, mean concentrations would be 2.5 (0.05–6.6).	US	Biles et al. (60) and FDA (48)
Infant formula (4)	Not detected (LOD 2 ug/kg)	UK	Goodson et al. (61) and UKFSA (62)
Infant formula (5)	44–113 ug/kg	Taiwan	Kuo and Ding (63)
Infant dessert (3)	18.9–77.3 ug/kg	UK	Goodson et al. (61)
Infant vegetable food (4)	< LOQ (LOQ 10 ug/kg)	New Zealand	Thomson and Grounds (64)
Infant dessert (3)	< LOQ (LOQ 10 ug/kg)		

<sup>a</sup> Values prior to and following heating in can and from non-dented and dented cans; values did not differ under the various conditions and were presented together.

1 **Table 6. Surveys of Bisphenol A Concentrations in Canned or Bottled Foods or Food Simulants**

Food (no. sampled)	Bisphenol A concentration, range in $\mu\text{g}/\text{kg}$ unless specified	Country of purchase <sup>a</sup>	Reference
Vegetables with liquid (6)	Mean (range) 16 (4–39)	US	FDA (48)
Liquids from canned vegetables or mushrooms (10)	$4.2 \pm 4.1$ (SD) to $22.9 \pm 8.8$ $\mu\text{g}/\text{can}$ [ $12 \pm 12$ to $76 \pm 29$ $\mu\text{g}/\text{kg}$ ]	Spain and US	Brotons et al. (65)
Coffee (13)	ND–213 [median 11] (LOD 2)	Japan	Kawamura et al. (46) (reviewed in (2); English abstract available)
Black tea (9)	ND–90 [median <2] (LOD 2)		
Other tea (8)	ND–22 [median 5.7] (LOD 2)		
Alcoholic beverages (10)	ND except for 1 sample with 13 (LOD 2)		
Soft drinks (7)	Not detected (LOD 2)		
Vegetables (10)	9–48 [median 21]	UK	Goodson et al. (61) and UKFSA (62)
Desserts (5)	ND (LOD 2) to 14 [median 10]		
Fruits (2)	19 and 38		
Pastas (5)	ND to 41 [median 11] (LOD 7)		
Meats (5)	16–422 <sup>b</sup> [median 52]		
Fish (10)	ND to 44 [median 16.8] (LOD 2)		
Non-alcoholic or alcoholic beverages (11)	ND except for 1 sample above LOD (LOD 2) but below LOQ (7)		
Soups (10)	ND to 21 [median <2] (LOD 2)		
Vegetables, fruits, or mushrooms (14)	ND (LOD 10) to 95.3 in solid portion; ND (LOD 0.005 $\mu\text{g}/\text{mL}$ ) to 0.004 $\mu\text{g}/\text{mL}$ in liquid portion; ND to 11.1 $\mu\text{g}/\text{can}$ [85 $\mu\text{g}/\text{kg}$ ] total		Yoshida et al. (66)
Meat products <sup>d</sup> (2)	8.6–25.7	UK	Goodson et al. (57)
Pasta <sup>d</sup> (1)	67.3–129.5		
Vegetables or beans <sup>c</sup> (2)	11.3–14.4		
Soup <sup>c</sup> (1)	18.5–39.1		
Pudding <sup>c</sup> (3)	3.8–53.2		
Pudding <sup>d</sup> (1)	18.5–28.1		
Grains and potatoes <sup>e</sup>	0 <sup>f</sup> –75 [mean not given]	Japan	Reviewed in Miyamoto and Kotake (54)
Sugar, sweets, snacks <sup>e</sup>	0 <sup>f</sup> –4 [mean not given]		
Fats <sup>e</sup>	0 <sup>f</sup>		
Fruits (including canned drinks), vegetables, mushrooms, seaweeds <sup>c</sup>	0 <sup>f</sup> –450 [mean not given]		
Seasoning and beverages <sup>e</sup>	0 <sup>f</sup> –213 [mean not given]		
Fish	9–480 [mean not given]		
Meat and eggs <sup>e</sup>	12.5–602 [mean not given]		
Milk and dairy products <sup>e</sup>	0 <sup>e</sup> –6 [mean not given]		
“Other” [not specified further] <sup>e</sup>	36–310 [mean not given]		
Canned fish (7)	1–23 [median 6]	Japan	Sajiki et al. (67)
Canned meat (5)	4–20 [median 10]		
Canned fruit (3)	Not detected (LOD 0.2)		
Canned vegetables (13)	3–78 [median 15]		
Canned soup (12)	1–156 [median 15]		
Canned sauce (6)	Not detected (LOD 0.2)–842 [median 220]		
Canned coconut milk	56–247		
Drinks in plastic containers (3)	Not detected (LOD 0.2) to 1 [median 0.3]	Japan	
Cookies in plastic containers (4)	1–14 [median 3.5]		
Soup in plastic containers (2)	Not detected (LOD 0.2) and 3		
Fast food sandwiches (3)	3 (all values)		

## 1.0 Chemistry, Use, and Human Exposure

Food (no. sampled)	Bisphenol A concentration, range in µg/kg unless specified	Country of purchase <sup>a</sup>	Reference
Food in paper containers (16)	Not detected (LOD 0.2) to 1 [median < 0.2]		
Fruits and vegetables (38)	ND (LOQ 10 ) to 24 [median <10]	New Zealand	Thomson and Grounds (64)
Fish (8)	ND (LOQ 20 ) to 109 [median <20–24]		
Soup (4)	ND (LOQ 10 ) to 16 [median <20]		
Sauces (4)	ND (LOQ 10 ) to 21 [median 16]		
Meat (6)	ND (LOQ 20 ) to 98 [median <20]		
Pasta (4)	ND (LOQ 10 )		
Dessert (2)	ND (LOQ 20 )		
Coconut cream (3)	ND (LOQ 20 ) to 192 [median 29]		
Soft drinks (4)	ND (LOQ 10 )		
Beverages (7)	Not detected (LOD 0.9) to 3.4 [median 0.4]	Austria	Braunrath et al. (68)
Vegetables (6) (only solid portion was analyzed, with the exception of tomatoes)	8.5–35 [median 26]		
Fruits (4)	5–24 [median 6.6]		
Canned fat-containing products such as soups, meats, and cream (9)	2.1–37.6 [median 20.7]		
Tuna (9)	< ND (LOQ 7.1) to 102.7 [median 11.2]	Mexico	Munguía-López et al. (69)
Beverage/beer cans exposed to 10% ethanol at 150°F [65.6°C] for 30 minutes and then 120°F [48.9 °C] for 10 days.	ND (LOD 5)	US	Howe et al. (55) and FDA (48)
Food cans exposed to 10 or 95% ethanol at 250°F [121°C] for 2 hours and then 120°F [48.9 °C] for 10 days or at 212°F [100 °C] for 30 minutes and then 120°F [48.9 °C] for 10 days.	ND (LOD 5) to 95 (mean 37) <sup>e</sup>		
Honey (107 samples; ~90% imported in epoxy-lined drums)	ND(LOD 2) to 33.3 [median <2]	Japan	Inoue et al. (70)
Wine stored in steel, wood, or plastic vats, filled into glass bottles, or purchased in local markets (59)	< LOQ (0.2 ng/mL) to 2.1 µg/L; mean 0.58 in samples above the LOQ	Austria	Brenn-Struckhofova and Chichna-Markl (56)
Solid food (309)	ND (<~0.8 ) – 192 [3.52-4.32]	U.S	Wilson et al.(32)
Liquid food (287)	ND (<~ 0.3) – 17.0[0.45-0.79]	U.S	Wilson et al. (32)

<sup>a</sup>Although cans were purchased in 1 or 2 countries for each study, most studies reported that cans were packaged in various locations throughout North America, Europe, and/or Asia.

<sup>b</sup>The UKFSA noted that the higher concentrations of bisphenol A detected in 1 meat product likely resulted from the use of bisphenol A as a cross-linking agent in the resin at that time.

<sup>c</sup>Values were obtained from heated and non-heated cans but presented together because it could not be determined if heating resulted in differing extraction rates.

<sup>d</sup>Values were determined before and after heating in can and from non-dented and dented cans; because the values did not differ under the various conditions, they were presented together.

<sup>e</sup>Total number of samples analyzed was not reported.

<sup>f</sup>As reported by study authors; detection limits not specified.

<sup>g</sup>A maximum concentration of 121 ppb reported in the first phase of the study was determined to have resulted from analytical interference.

1 A study examining aggregate exposures of US preschool age children measured bisphenol A  
2 concentrations in liquid food and solid food served to the children at home and at child care centers (31).  
3 Duplicate plates of food served to 9 children were collected over a 48-hour period. GC/MS analyses were  
4 conducted on 4 liquid food samples and 4 solid food samples from the child care center and 9 liquid food  
5 samples and 9 solid food samples from home. Bisphenol A was detected in all solid food samples, 3  
6 liquid food samples from the child care center, and 2 liquid food samples from the home. Concentrations  
7 of bisphenol A ranged from <0.100 to 1.16 ng/g [**µg/kg**] in liquid foods and from 0.172 to 4.19 ng/g  
8 [**µg/kg**] in solid food.  
9

10 The study examining aggregate exposures of US preschool age children was repeated with a larger  
11 sample and again measured bisphenol A concentrations in liquid food and solid food served to the  
12 children at home and at child care centers (32). Bisphenol A concentrations were measured by GC/MS in  
13 food served over a 48 hour period to at least 238 children at home and 49 children at daycare centers.  
14 Bisphenol A was detected in 83–100% of solid food samples; concentrations were reported at <LOD (0.8)  
15 to 192 ng/g [**µg/kg**]. Sixty-nine to 80% of liquid food contained detectable concentrations of bisphenol A;  
16 concentrations were reported at <LOD (0.3)–17.0 ng/mL in liquid food. Data were also collected for hand  
17 wipes of 193 children at daycare centers and 60 children at home. Bisphenol A was detected in 94–100%  
18 of handwipe samples; concentrations ranged from <LOD [**not defined**] to 46.6 ng/cm<sup>2</sup>. and food  
19 preparation surface wipes. Bisphenol A was detected in 85–89% of food preparation surface wipes from  
20 homes; concentrations were reported at < LOD [**not defined**] to 0.357 ng/cm<sup>2</sup>.  
21

22 A review by Miyamoto and Kotake (54) reported bisphenol A concentrations of 0.011–0.086 mg/kg in  
23 non-canned foods such as fats, fruits, fish, meat, and eggs. However, one study used GC-MS to examine  
24 bisphenol A in 14 types of produce purchased in southern Italy (71). Bisphenol A concentrations were  
25 below the detection limit [**not reported**] in 5 produce samples. In the remaining samples, bisphenol A  
26 was detected at concentrations of  $0.25 \pm 0.02$  (SD) to  $1.11 \pm 0.09$  mg/kg. [**These concentrations are**  
27 **equal to or higher than those found in canned foods, where the presumption is that the source is the**  
28 **epoxy liner of the container.**]  
29

30 Bisphenol A has been found in recycled paper products used for food processing at 10 or more times the  
31 concentrations found in non-recycled paper products [reviewed by the European Food Safety Authority  
32 (20)]. Bisphenol A concentrations were up to 26 µg/g paper. Migration to food was not discussed.  
33

34 Epoxy paints are used to coat the insides of residential drinking water storage tanks. Bisphenol A has  
35 been shown to migrate from painted concrete and stainless metallic plates; however, a water sample from  
36 a recently painted reservoir showed no detectable bisphenol A (72). When exposed to chlorine  
37 disinfectant, bisphenol A disappears within 4 hours, but the chlorinated bisphenol A congeners that are  
38 formed can remain in solution up to 20 hours when low chlorine doses are used (73). The toxicity of these  
39 chlorinated bisphenol A congeners is unknown; however, there is some evidence that estrogenic activity  
40 and receptor binding remains after chlorination (74).  
41

#### 42 *1.2.3.3 Potential migration from dental material*

43 Bisphenol A is used in the manufacture of materials found in dental sealants or composites (i.e., fillings)  
44 (2). Examples of bisphenol A-derived materials used in dental sealants include bis-glycidylmethacrylate  
45 and bisphenol A-dimethyl acrylate. Bisphenol A could potentially be present as an impurity or be released  
46 during degradation of the dental materials. Sealants are comprised of an organic matrix, while composites  
47 contain inorganic filler in addition to the organic matrix. According to the British Dental Association,  
48 filled composites would possibly produce lower exposure to bisphenol A than sealants, because they  
49 contain proportionately less resin than sealants, [reviewed in (2)]. During dental procedures, resin  
50 mixtures are applied as fluid monomers and polymerized in situ by ultraviolet or visible light. According  
51 to the European Union (2), patients can be exposed to bisphenol A during the polymerization stage.

## 1.0 Chemistry, Use, and Human Exposure

1 In a review of in vitro studies examining bisphenol A migration from dental sealants, the European Union  
2 (2) concluded that release of bisphenol A is likely to occur only with degradation of the parent monomer.  
3 The data suggested that bis-glycidylmethacrylate does not degrade; therefore, release of bisphenol A is  
4 only likely to occur with bisphenol A-dimethyl acrylate use. In vivo studies measuring bisphenol A in  
5 saliva following sealant application were reviewed in detail by CERHR because they provide the most  
6 relevant human exposure information.

7  
8 Olea et al. (75) measured saliva concentrations of bisphenol A for 1 hour before and 1 hour after  
9 application of 50 mg bis-glycidylmethacrylate- and bisphenol A-dimethyl acrylate-based sealant across  
10 12 molars of 18 patients. Concentrations of bisphenol A in saliva were measured by GC/MS and HPLC.  
11 Following treatment, saliva contained ~90–931 µg bisphenol A. Based on an assumed saliva production  
12 rate of 0.5 mL/minute, a saliva concentration of 3–30 µg/mL was estimated by the study authors. With the  
13 exception of 1 patient who was excluded from the study, bisphenol A was not detected in saliva prior to  
14 sealant application.

15  
16 Arenholt-Binslev (76) measured bisphenol A in saliva of 8 adult patients who each had 4 molars treated  
17 with 38 mg of 1 of 2 sealants, Delton LC or Visio-seal. Saliva was collected prior to, immediately after,  
18 and at 1 or 24 hours following treatment for measurement of bisphenol A concentrations by HPLC.  
19 Bisphenol A was detected at 0.3–2.8 ppm immediately after application of Delton SC sealant [bisphenol  
20 A-dimethyl acrylate sealant according to the European Union (2)] but was not detected 24 hours later  
21 (detection limit = 0.1 ppm [mg/L]). Bisphenol A was not detected in saliva of patients who received the  
22 Visio-seal sealant (bis-glycidylmethacrylate sealant, according to the European Union). It was noted  
23 that saliva bisphenol A concentrations were much lower than those reported by Olea et al. (75). Possible  
24 reasons for the inconsistencies in results between the 2 studies were stated to be differences in the amount  
25 of sealant used and co-elution of compounds that could have confounded bisphenol A analysis.

26  
27 Fung et al. (77), measured salivary bisphenol A concentrations in 40 patients treated with a dental sealant  
28 (Delton Opaque Light-cure Pit and Fissure Sealant) that was understood to contain bisphenol A-dimethyl  
29 acrylate, according to the European Union (2). Eighteen patients in the low-dose group received 8 mg  
30 dental sealant on 1 tooth, and 22 patients in the high-dose group received 32 mg sealant on 4 teeth. Saliva  
31 and blood were collected for HPLC analysis before the procedure and at 1 and 3 hours and 1, 3, and 5  
32 days after the procedure. More details of this study are included in Section 2.1.1.1. Analysis of the dental  
33 sealant revealed that bisphenol A concentrations were below the detection limit of 5 ppb. At 1 hour  
34 following treatment, Bisphenol A was detected only in saliva samples from 3 of the 18 volunteers in the  
35 low-dose group and 13 of 22 samples from volunteers in the high-dose group. At 3 hours post-treatment,  
36 bisphenol A was detected in samples from 1 of 18 volunteers in the low-dose group and 7 of 22  
37 volunteers from the high-dose group. Concentrations of bisphenol A in saliva at 1 and 3 hours following  
38 exposure were reported at 5.8–105.6 ppb [µg/L]. No bisphenol A was detected in saliva samples at 24  
39 hours after treatment or in serum samples at any time point. Differences in bisphenol A concentrations  
40 and the presence of bisphenol A in saliva of the low-dose compared to the high-dose group at 1 and 3  
41 hours achieved statistical significance. The European Union (2) noted that the concentrations of saliva  
42 bisphenol A reported by Fung et al. (77) were more than 250 times lower than those reported by Olea et  
43 al. (75).

44  
45 Sasaki et al. (78) used ELISA to examine salivary bisphenol A concentrations in 21 patients before and  
46 after 1 cavity was filled with 0.1 g of composite resin. The resins consisted of bisphenol A diglycidylether  
47 methacrylate (i.e., bis-glycidylmethacrylate), triethylene glycol dimethacrylate, and/or urethane  
48 dimethacrylate. Saliva was collected prior to treatment, during the 5 minutes following treatment, and  
49 then immediately after gargling with water. Following treatment, saliva bisphenol A increased [from ≤2  
50 to ~15–100 µg/L]. Gargling reduced bisphenol A to near pretreatment concentrations [≤5 µg/L] in most  
51 patients, with the exception of 1 patient with the highest bisphenol A concentration [reduced from ~100

1 **to 18 µg/L]. [An increase in saliva bisphenol A concentrations was noted in 1 of 2 patients receiving**  
2 **a composite consisting solely of urethane dimethacrylate.]** The study authors noted that cross-  
3 reactivity is possible with the ELISA technique, but that cross reactivity between bisphenol A  
4 diglycidylether methacrylate and triethylene glycol dimethacrylate is low. Therefore, the study authors  
5 thought it possible that they were measuring only bisphenol A. **[As discussed in Section 1.1.5, ELISA**  
6 **may over-estimate bisphenol A.]**  
7

8 Joskow et al. (79) examined bisphenol A in urine and saliva of 14 adults treated with dental sealants. The  
9 volunteers received either Helioclear F (n = 5) or Delton LC (n = 9) sealant. Only the Helioclear F sealant  
10 was noted to carry the American Dental Association (ADA) Seal of Acceptance. Sealant was weighed  
11 before and after application to determine the amount applied, and the numbers of treated teeth were  
12 recorded. The mean number of teeth treated was 6/person and the mean total weight of sealant applied  
13 was 40.35 mg/person. In a comparison of the 2 different sealants, no differences were reported for the  
14 number of teeth treated or amount of sealant applied. Saliva samples were collected before, immediately  
15 after, and 1 hour after sealant application. Urine samples were collected before and at 1 and 24 hours after  
16 sealant placement. A total of 14–15 saliva samples and 12–14 urine samples were collected at each time  
17 point. Samples were treated with β-glucuronidase and analyzed for bisphenol A concentrations using  
18 selective and sensitive isotope-dilution-MS-based methods. Saliva concentrations were highest  
19 immediately following treatment; mean concentrations were reported at 42.8 ng/mL in patients treated  
20 with Delton LC and 0.54 ng/mL in patients treated with Helioclear F. The highest mean urinary  
21 concentrations of bisphenol A were measured at 1 hour following exposure and were reported at 27.3  
22 ng/mL in patients treated with Delton LC and 7.26 ng/mL in patients receiving the Helioclear F sealant.  
23 The study authors noted that saliva and urine bisphenol A concentrations following application of  
24 Helioclear F were comparable to baseline concentrations. More information on bisphenol A concentrations  
25 in saliva and urine is included in Section 2, and exposure estimates are provided in Section 1.2.4.1.2. The  
26 study authors noted that saliva concentrations detected in their study were ~1000 times lower than those  
27 reported by Olea et al. (75) but were within the ranges reported by Fung et al. (77) and Sasaki et al. (78).  
28 Analytical procedures and use of a large amount of sealant were noted as possible reasons for the higher  
29 values reported by Olea et al. (75).  
30

31 The European Union noted a study by Lewis et al. (80) that characterized materials in 28 commercial  
32 resin-based composites and sealants, including those examined by Olea et al. (75). HPLC and infrared  
33 analysis could not verify the presence of bisphenol A in any sealant product. Lewis et al. noted that in the  
34 study by Olea et al. another component in the resin may have been misidentified as bisphenol A because  
35 of difficulties with resolution.  
36

37 In their review of studies examining bisphenol A concentrations in saliva of patients treated with dental  
38 sealants, the European Union (2) noted that the higher concentrations reported may have resulted from  
39 interference during analysis and thus may overestimate bisphenol A exposures from dental treatments. It  
40 was concluded that dental treatment would likely result in saliva bisphenol A concentrations of 0.3–3  
41 ppm. Because bisphenol A was generally not detected in saliva at time points beyond 1 hour after  
42 treatment, it was concluded that bisphenol A exposure resulting from dental treatments is likely to be an  
43 acute event. In their 2002 position statement, the ADA stated that none of the 12 dental sealants that carry  
44 the ADA Seal release bisphenol A (81). Upon initial analysis, one of the sealants was found to leach trace  
45 concentrations of bisphenol A, but following implementation of quality controls by the manufacturer,  
46 bisphenol A could no longer be detected in the final product.  
47

48 A study on orthodontic adhesives found no bisphenol A release from these materials after simulated aging  
49 (82). Another study found plastic orthodontic brackets in water to release bisphenol A at 0.01–0.40 mg/kg  
50 material and denture base resin in water to release bisphenol A at 0.01–0.09 mg/kg material (83).  
51

1 *1.2.3.4 Bisphenol A concentrations measured in biological samples*

2 Bisphenol A concentrations detected in human blood are summarized in [Table 7](#). Goodman et al. (84)  
 3 noted that although blood concentration may provide information on internal dose, it does not allow for  
 4 estimates of daily intake. It was also noted that in many studies in which blood concentration of bisphenol  
 5 A was measured, sample preparation and analysis methods were poorly reported. Many study groups used  
 6 an ELISA method to measure blood bisphenol A concentration. As discussed in Section 1.1.5, the ELISA  
 7 technique is likely to overestimate bisphenol A concentrations as a result of cross-reactivity with other  
 8 substances and due to effects of biologic matrices (8, 9, 84).

9  
 10 Several studies reported concentrations of bisphenol A in human urine; those studies are summarized in  
 11 [Table 8](#). As discussed in greater detail in Section 2, the majority of ingested bisphenol A is excreted in  
 12 urine as bisphenol A glucuronide after acute exposure. Smaller amounts of bisphenol A are metabolized  
 13 to and excreted as bisphenol A sulfate. Some of the studies determined concentrations of parent bisphenol  
 14 A before and after digestion with glucuronidases. With the exception of Fujimaki et al. (85) who used an  
 15 ELISA technique to measure urinary bisphenol A, other study authors used HPLC, GC/MS, or LC/MS.  
 16 Results from 394 participants of the National Health and Nutrition Examination Survey (NHANES) III  
 17 survey are included in [Table 8](#) (15). Bisphenol A was detected in 95% of the participants, which indicated  
 18 widespread exposure to bisphenol A in the US. Consistent with those findings, bisphenol A was detected  
 19 in urine from 85 of 90 (94.4%) 6–8-year-old girls from the US (86). In a review of urinary bisphenol A  
 20 data, Goodman et al. (84) noted that in most cases, median total urinary bisphenol A concentration (the  
 21 sum of parent and conjugated bisphenol A) were ~1–2 µg/L. Two studies (87, 88) reported urinary  
 22 bisphenol A concentrations that were orders of magnitude higher than commonly observed  
 23 concentrations, despite the use of apparently reliable analytical techniques. Goodman et al. (84) has  
 24 suggested that reported hormone concentrations for the study volunteers were also higher than expected,  
 25 indicating the possibility of laboratory or reporting error. The use of urinary bisphenol A concentration to  
 26 estimate daily exposures appears in Section 1.2.4.1.2.

28 **Table 7. Blood Concentrations of Bisphenol A in Adults**

Population	Bisphenol A, µg/L <sup>a,c</sup>	Method	Reference
<b>Germany</b>			
Men (n=7)	<0.5	HPLC-MS/MS	Völkel et al. (7)
Women (n=12)	<0.5	HPLC-MS/MS	Völkel et al. (7)
Pregnant Caucasian women (n=37; 32-41 weeks gestation)	4.4 ± 3.9	GC-MS	Schönfelder et al. (89)
<b>Japan</b>			
Men (n=21; age 22-51)	“almost all” < 0.2 ng/ml	HPLC-ECD	Fukata et al. (9)
Men (n=9; age 30-50)	0.59± 0.21 (0.38–1.0)	HPLC-MS	Sajiki et al.(10)
Men (n=11)	1.49 ± 0.11 (SEM)	ELISA <sup>b</sup>	Takeuchi et al. (90)
Women (n=31; age 22-51)	“almost all” < 0.2 ng/ml	HPLC-ECD	Fukata et al. (9)
Women (n=12; age 30-50)	0.33± 0.54 (0–1.6)	HPLC-MS	Sajiki et al.(10)
Women (n=14)	0.64 ± 0.10 (SEM)	ELISA <sup>b</sup>	Takeuchi et al. (90)
Pregnant women (n=37; late pregnancy )	1.4 ± 0.9	ELISA <sup>b</sup>	Ikezuki et al.(91)
Pregnant women with normal karyotype, early 2 <sup>nd</sup> trimester (n=200)	2.24 (0.63-14.36)	ELISA <sup>b</sup>	Yamada et al. (92)
Pregnant women with abnormal karyotype, early 2 <sup>nd</sup> trimester (n=48)	2.97 (~0.0.7-18.5) <sup>d</sup>	ELISA <sup>b</sup>	Yamada et al. (92)
Population	Bisphenol A, µg/L <sup>a,c</sup>	Method	Reference

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Pregnant women (n=9)	0.43 (0.21–0.79)	HPLC-FI	Kuroda et al. (11)
Infertile women (n=21)	0.46 (0.22–0.87)	HPLC-FI	Kuroda et al. (11)
Women with multiple miscarriages (n=45; mean age 31.6 years)	2.59 ± 5.23	ELISA <sup>b</sup>	Sugiura-Ogasawara et al. (93)
Healthy woman (n=32; mean age 32 years)	0.77 ± 0.38	ELISA <sup>b</sup>	Sugiura-Ogasawara et al. (93)
Women with polycystic ovary syndrome (n=16)	1.04 ± 0.10 (SEM)	ELISA <sup>b</sup>	Takeuchi et al. (90)
Non-obese women with polycystic ovarian syndrome (n = 13; average age 26.5 years)	1.05 ± 0.10 (SEM)	ELISA <sup>b</sup>	Takeuchi et al. (94)
Obese women with polycystic ovarian syndrome (n=6; average age 24.7 years)	1.17 ± 0.16 (SEM)	ELISA <sup>b</sup>	Takeuchi et al. (94)
Non-obese women (n=19; average age 27.5 years)	0.71 ± 0.09 (SEM)	ELISA <sup>b</sup>	Takeuchi et al. (90)
Obese women (n=7; average age 28.8 years)	1.04 ± 0.09 (SEM)	ELISA <sup>b</sup>	Takeuchi et al. (94)
Hyperprolactinemic women (n=7; average age 27.7 years)	0.83 ± 0.12 (SEM)	ELISA <sup>b</sup>	Takeuchi et al. (94)
Amenorrheic women (n=7; average age 25.1 years)	0.84 ± 0.10 (SEM)	ELISA <sup>b</sup>	Takeuchi et al. (94)
Women with normal uterine endometrium (n=11; mean age 48.9 years)	2.5 ± 1.5	ELISA <sup>b</sup>	Hiroi et al. (95)
Women with simple endometrium hyperplasia (n=10; mean age 48.4 years)	2.9 ± 2.0	ELISA <sup>b</sup>	Hiroi et al. (95)
Women with complex endometrium hyperplasia (n=9; mean age 48.4 years)	1.4 ± 0.4	ELISA <sup>b</sup>	Hiroi et al. (95)
Women with endometrial carcinoma (n=7; mean age 63.1 years)	1.4 ± 0.5	ELISA <sup>b</sup>	Hiroi et al. (95)

<sup>a</sup>Mean ± SD or median (range)

<sup>b</sup>As discussed in Section 1.1.5, ELISA may over-estimate bisphenol A.

<sup>c</sup>It is uncertain whether parent, conjugated, or total bisphenol A was measured.

<sup>d</sup>Estimated from a graph

1  
2**Table 8. Urinary Concentrations of Bisphenol A and Metabolites in Adults or Children**

Country	Study population	LOD ( $\mu\text{g/L}$ )	Urinary bisphenol A or metabolite concentrations as median (range) or mean $\pm$ SEM, $\mu\text{g/L}^a$ [detectable fraction, % >LOD]				Reference
			Free	Total	Glucuronide	Sulfate	
US	30 urine samples from demographically diverse, anonymous adult volunteers	0.3	< 0.3 (<0.3–0.6) [10%]	2.12 (<LOD <sup>b</sup> –19.8) [97%]	1.4 (<LOD <sup>b</sup> –19.0) [90%]	0.3 (<LOD <sup>b</sup> –1.8) [47%]	Ye et al. (96)
US	394 adult volunteers (males and females; 20–59 years old) from the NHANES III survey	0.1		1.28 (10 <sup>th</sup> to 95 <sup>th</sup> percentile: 0.22–5.18) <sup>c</sup> [95%]			Calafat et al. (15)
US	23 adults	0.5		0.47 (<1–2.24) [52%]			Liu et al. (97)
US	Nine 9-year-old girls	0.5		2.4 (0.04–16) [89%]			Liu et al. (97)
US	90 girls (6–8-years-old; White, Black, Asian, or Hispanic ethnicity)	8.36		1.8 (<0.3–54.3) [85%]			Wolff et al. (86)
Germany	7 males and 12 females	1.14 (BPA) 10.1 (BPA monoglucuronide)	<1.14 [0%]		<26.26 [LOQ]		Völkel et al. (7)
Korea	15 men (age $42.6 \pm 2.4^d$ years)	1	0.28–2.36; 0.58 $\pm$ 0.14	0.85–9.83; 2.82 $\pm$ 0.73	0.16–11.67; 2.34 $\pm$ 0.85	<MDL <sup>e</sup> –1.03; 0.49 $\pm$ 0.27	Kim et al. (98)
Korea	15 women (age $43.0 \pm 2.7^d$ years)	0.28	0.068–1.65; 0.56 $\pm$ 0.10	1.00–7.64; 2.76 $\pm$ 0.54	<MDL <sup>c</sup> –4.34; 1.00 $\pm$ 0.34	<MDL <sup>e</sup> –3.40; 1.20 $\pm$ 0.32	Kim et al. (98)
Korea	34 males and 39 females (mean age 48.5 years)	0.012		Geometric mean: 9.54 (<0.012–586.14) <sup>b</sup> [75%]			Yang et al. (88)

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Country	Study population	LOD ( $\mu\text{g/L}$ )	Urinary bisphenol A or metabolite concentrations as median (range) or mean $\pm$ SEM, $\mu\text{g/L}^a$ [detectable fraction, % >LOD]				Reference
			Free	Total	Glucuronide	Sulfate	
Korea	81 men not occupationally exposed to bisphenol A			Geometric mean $\pm$ SD: $6.88 \pm 3.72$			Yang et al. (99)
Korea	79 women not occupationally exposed to bisphenol A	0.026		Geometric mean $\pm$ SD: $5.01 \pm 3.16$ [97.5%]			Yang et al. (99)
Japan	48 female college students	0.2	<0.2 [2%]		1.2 (0.2–19.1) [100%]		Ouchi and Watanabe (100)
Japan	Pooled urine samples from at least 5 people	0.12	<0.12	0.11–0.51			Brock et al. (101)
Japan	23 females and 46 males; in each volunteer, 2 samples per volunteer were combined		0.01–0.27	Mean: 0.81 (range: 0.14–5.47)			Tsukioka et al. (6)
Japan	Whole-day urine samples collected from 11 males and 11 females			Mean: 0.81 (range 0.24–2.03)			Tsukioka et al. (6)
Japan	Urine collected from 3 volunteers	0.02	<0.1	0.22, 0.41, and 0.45 [100% after deconjugation]			Kawaguchi et al. (102)
Japan	Spot urine samples collected from 56 women who were 1–9 months pregnant; 21–43 years of age	1.1		<1.1 (<1.1–5.4) <sup>c</sup> (ELISA) [30%]			Fujimaki et al. (85)

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Country	Study population	LOD (µg/L)	Urinary bisphenol A or metabolite concentrations as median (range) or mean ± SEM, µg/L <sup>a</sup> [detectable fraction, % >LOD]				Reference
			Free	Total	Glucuronide	Sulfate	
Japan	21 men and 31 women age 22-51 years of age	0.2	49/51 had <0.2 mean 0.34 (n=2) [4%]	1.92 ± 0.27 [98%]			Fukata et al.(9)
China	10 healthy male volunteers age 21–29 years	2.8		<2.7 to 3950; 1220 ±1380 <sup>d</sup> [60%]			Mao et al. (87)
China	10 healthy female volunteers age 21–29 years	2.8		30–3740; 1290 ± 1220 <sup>d</sup> [100%]			Mao et al. (87)

<sup>a</sup>With the exception of the study by Fujimaki et al. (85), which used the potentially unreliable ELISA, the studies used analytical techniques based on HPLC, GC/MS, and LC/MS.

<sup>b</sup>Limit of detection (LOD) for bisphenol A following digestion of conjugate was 0.3 µg/L.

<sup>c</sup>Samples were only digested with β-glucuronidase and do not account for bisphenol A conjugated to sulfate.

<sup>d</sup>Variance not indicated.

<sup>e</sup>Minimum detection limit based upon free bisphenol A.

1  
2  
3

1 In humans, bisphenol A was measured in cord blood and amniotic fluid, demonstrating distribution to the  
 2 embryo or fetus. Detailed descriptions of those studies are also presented below.

3  
 4 **Table 9. Concentrations of Bisphenol A in Maternal and Fetal Samples**

Study description; analytical method	Bisphenol A concentrations, µg/L, median (range) or mean ± SD		Amniotic fluid	Reference
	Serum or plasma			
	Maternal	Fetal		
21 samples collected in women in the US before 20 weeks gestation; LC with electrochemical detection			0.5 (Non-detectable <0.5 -1.96) 10% of samples detectable	Engel et al. (103)
37 German women, 32–41 weeks gestation; GC/MS	3.1 (0.3 - 18.9); 4.4 ± 3.9	2.3 (0.2–9.2); 2.9 ± 2.5		Schönfelder et al. (104)
37 Japanese women in early pregnancy; ELISA <sup>a</sup>	1.5 ± 1.2			Ikezuki et al. (91)
37 Japanese women in late pregnancy; ELISA <sup>a</sup>	1.4 ± 0.9			Ikezuki et al. (91)
32 Japanese infants at delivery; ELISA <sup>a</sup>		2.2 ± 1.8		Ikezuki et al. (91)
32 Japanese amniocentesis samples at 15–18 weeks gestation; ELISA <sup>a</sup>			8.3 ± 8.9	Ikezuki et al. (91)
38 samples obtained at full-term cesarean section; ELISA <sup>a</sup>			1.1 ± 1.0	Ikezuki et al. (91)
200 Japanese women carrying fetuses with normal karyotype at 16 weeks mean gestation; ELISA	2.24 (0.63 - 14.36)		0.26 (0 - 5.62)	Yamada et al. (92)
48 Japanese women carrying fetuses with abnormal karyotypes at a 16 weeks mean gestation; ELISA	2.97 [~0.7 - 18.5] <sup>b</sup>		0 [~0 - 7.5] <sup>b</sup>	Yamada et al. (92)
9 sets of maternal and umbilical cord blood samples obtained at birth in Japanese patients; HPLC	0.43 (0.21 - 0.79) 0.46 ± 0.2	0.64 (0.45 - 0.76) 0.62 ± 0.13		Kuroda et al. (11)
180 Malaysian newborns; GC/MS		Non-detectable (<0.05) to 4.05 88% of samples detectable		Tan and Mohd (14)

<sup>a</sup>As discussed in Section 1.1.5, ELISA may over-estimate bisphenol A. Some samples were verified by HPLC.

<sup>b</sup>Estimated from a graph.

5  
 6 Engel et al. (103) reported concentrations of bisphenol A in human amniotic fluid. Twenty-one samples  
 7 were obtained during amniocentesis conducted before 20 weeks gestation in women who were referred to  
 8 a US medical center for advanced maternal age. Bisphenol A concentrations in amniotic fluid were

1 measured using LC with electrochemical detection. Bisphenol A was detected in 10% of samples at  
2 concentrations exceeding the LOD (0.5 µg/L). Bisphenol A concentration ranges of 0.5–1.96 µg/L were  
3 reported.

4  
5 Schönfelder et al. (104) examined bisphenol A concentrations in maternal and fetal blood and compared  
6 bisphenol A concentrations in blood of male and female fetuses. In a study conducted at a German  
7 medical center, blood samples were obtained from 37 Caucasian women between 32 and 41 weeks  
8 gestation. At parturition, blood was collected from the umbilical vein after expulsion of the placenta.  
9 Bisphenol A concentrations in plasma were measured by GC/MS. Control experiments were conducted to  
10 verify that bisphenol A did not leach from collection, storage, or testing equipment. Bisphenol A was  
11 detected in all samples tested, and concentrations measured in maternal and fetal blood are summarized in  
12 Table 9. Mean bisphenol A concentrations were higher in maternal ( $4.4 \pm 3.9$  [SD] µg/L) than fetal blood  
13 ( $2.9 \pm 2.5$  µg/L). Study authors noted that in 14 cases fetal bisphenol A plasma concentrations exceeded  
14 those detected in maternal plasma. Among those 14 cases, 12 fetuses were male. Analysis by paired *t*-test  
15 revealed significantly higher mean bisphenol A concentrations in the blood of male than female fetuses  
16 ( $3.5 \pm 2.7$  versus  $1.7 \pm 1.5$  ng/mL,  $P = 0.016$ ). Bisphenol A concentrations were measured in placental  
17 samples at 1.0–104.9 µg/kg.

18  
19 Ikezuki et al. (91) measured concentrations of bisphenol A in serum from 30 healthy premenopausal  
20 women, 37 women in early pregnancy, 37 women in late pregnancy, and 32 umbilical cord blood  
21 samples. Concentrations of bisphenol A were also measured in 32 samples of amniotic fluid obtained  
22 during weeks 15–18 of gestation, 38 samples of amniotic fluid obtained at full-term cesarean section, and  
23 36 samples of ovarian follicular fluid collected during in vitro fertilization procedures. **[It was not stated  
24 if different sample types were obtained from the same subjects.]** An ELISA method was used to  
25 measure bisphenol A concentrations and results were verified by HPLC. The mean  $\pm$  SD concentration of  
26 bisphenol A in follicular fluid was reported at  $2.4 \pm 0.8$  µg/L. As summarized in Table 9 for maternal and  
27 fetal samples, concentrations of bisphenol A in follicular fluid were similar to those detected in the serum  
28 of fetuses and pregnant and non-pregnant women and in amniotic fluid collected in late pregnancy (~1–2  
29 µg/L). Bisphenol A concentrations in amniotic fluid samples collected in early pregnancy were ~5-fold  
30 higher than in other samples, and the difference achieved statistical significance ( $P < 0.0001$ ). Study  
31 authors postulated that the higher concentrations of bisphenol A in amniotic fluid collected during  
32 gestation weeks 15–18 may have resulted from immature fetal liver function. They noted that according  
33 to unpublished data from their laboratory, the percentage of glucuronidated bisphenol A in mid-term  
34 amniotic fluid was ~34%, which is much lower than reported values for other human fluids (>90%).

35  
36 Yamada et al. (92) measured bisphenol A concentrations in maternal serum and amniotic fluid from  
37 Japanese women. Samples were collected between 1989 and 1998 in women undergoing amniocentesis  
38 around gestation week 16. One group of samples was obtained from 200 women carrying fetuses with  
39 normal karyotypes, and a second group of samples was obtained from 48 women carrying fetuses with  
40 abnormal karyotypes. An ELISA method was used to measure bisphenol A concentrations. **[As discussed  
41 in Section 1.1.5, ELISA may over-estimate bisphenol A.]** Concentrations of bisphenol A measured in  
42 maternal plasma and amniotic fluid are summarized in Table 9. Median concentrations of bisphenol A in  
43 maternal serum (~2–3 µg/L) were significantly higher [**~10-fold**] than concentrations in amniotic fluid  
44 (~0–0.26 µg/L) in the groups carrying fetuses with normal and abnormal karyotypes. However, in 8  
45 samples from women carrying fetuses with normal karyotypes, high concentrations (2.80–5.62 µg/L) of  
46 bisphenol A were measured in amniotic fluid. The study authors interpreted the data as indicating that  
47 bisphenol A does not accumulate in amniotic fluid in most cases but that accumulation is possible in some  
48 individuals. Bisphenol A concentrations in maternal blood were significantly higher [**by ~33%**] in  
49 woman carrying fetuses with abnormal versus normal karyotypes. However, the study authors noted that  
50 the effect may not be related to bisphenol A exposure because there was no adjustment for maternal age,  
51 and concentrations in amniotic fluid did not differ between groups. In the group carrying fetuses with

1 normal karyotypes, data obtained from 1989 to 1998 were summarized by year. Median bisphenol A  
2 concentrations in serum significantly decreased over that time from a concentration of 5.62 µg/L detected  
3 in 1989 to 0.99 µg/L in 1998.

4  
5 Kuroda et al. (11) used an HPLC method to measure bisphenol A concentrations in 9 sets of maternal and  
6 cord blood samples obtained from Japanese patients at the time of delivery. Bisphenol A concentrations  
7 were also measured in 21 sets of serum and ascitic fluid samples collected from sterile Japanese patients  
8 of unspecified sexes and ages. Results for pregnant women are summarized in Table 9. Mean ± SD  
9 concentrations of bisphenol A were lower in maternal (0.46 ± 0.20 ppb [µg/L]) than cord blood  
10 (0.62±0.13 ppb [µg/L]). There was a weak positive correlation ( $r = 0.626$ ) between bisphenol A  
11 concentrations in maternal and cord blood. There were no differences between pregnant and non-pregnant  
12 blood levels (11). Mean ± SD concentrations of bisphenol A were higher in ascitic fluid (0.56 ± 0.19 ppb  
13 [µg/L]) than in serum (0.46 ± 0.20 ppb [µg/L]). The correlation between bisphenol A concentration in  
14 serum and ascitic fluid was relatively strong ( $r = 0.785$ ).

15  
16 Tan and Mohd (14) used a GC/MS method to measure bisphenol A concentrations in cord blood at  
17 delivery in 180 patients at a Malaysian medical center. Bisphenol A was detected in 88% of samples. As  
18 noted in Table 9, concentrations ranged from <0.10 to 4.05 µg/L.

19  
20 Schaefer et al. (105) measured concentrations of bisphenol A and other compounds in uterine  
21 endometrium of women undergoing hysterectomy for uterine myoma at a German medical center.  
22 Endometrial and fat samples were obtained between 1995 and 1998 from 23 women (34–51 years old)  
23 with no occupational exposure to bisphenol A. Samples were handled with plastic-free materials and  
24 stored in glass containers. Concentrations of environmental chemicals were measured in samples by  
25 GC/MS. None of 21 fat samples had detectable concentrations of bisphenol A. Bisphenol A was detected  
26 in 1 of 23 endometrial samples; the median concentration was reported at <1 µg/kg wet weight, and the  
27 range was reported at 0–13 µg/kg. **[It is not known why a median value and range were reported  
28 when bisphenol A was only detected in 1 sample.]**

29  
30 As part of a study to compare an ELISA and an LC/MS method for biological monitoring of bisphenol A,  
31 Inoue et al. (8) measured concentrations of bisphenol A in semen samples obtained from 41 healthy  
32 Japanese volunteers (18–38 years old). Analysis by the ELISA method indicated bisphenol A  
33 concentrations ranging from concentrations below the detection limit (2.0 µg/L) to 12.0 µg/L. The  
34 LC/MS method indicated that the bisphenol A concentration in all samples was <0.5 µg/L, the LOQ. The  
35 study authors concluded that the LC/MS method was more accurate and sensitive and that the ELISA  
36 method overestimated bisphenol A concentrations, possibly due in part to nonspecific antibody  
37 interactions.

### 38 39 1.2.4 Human exposure

#### 40 41 1.2.4.1 General population exposure

##### 42 1.2.4.1.1 Estimates based on bisphenol A concentrations in food or environment

43 Wilson et al. (31) estimated aggregate exposures to bisphenol A in preschool aged children (2–5 years)  
44 from the US. In 1997, numerous chemicals were surveyed, but only bisphenol A results are reported here.  
45 Ten child care centers were surveyed and the 2 centers with the highest and lowest overall concentrations  
46 of target pollutants were selected for the study. Both centers were located in North Carolina. Nine  
47 children who attended one of the child care centers participated in the study. Over a 48-hour period,  
48 bisphenol A concentrations were measured in indoor and outdoor air, dust, soil, and food; the ranges  
49 detected are summarized in Sections 1.2.3.1 and 1.2.3.2. In estimating exposures, absorption was  
50 considered to be 100%. Calculations considered ventilation rates, time spent indoors and outdoors, time

1 spent at home and in day care, the measured weight of each child, assumed ingestion of dust and soil, and  
 2 total weight of foods consumed. Mean (range) bisphenol A intake was estimated at 0.042981 (0.018466–  
 3 0.071124) µg/kg bw/day.

4  
 5 Wilson et al. (32) conducted a second study to estimate aggregate exposures in 257 US children aged 1.5–  
 6 5 years. Bisphenol A was one of the compounds assessed in this study of homes and daycare centers in 6  
 7 North Carolina and 6 Ohio counties in 2000–2001. Over a 48-hour period, bisphenol A concentrations  
 8 were measured in indoor and outdoor air, dust, soil, food, and surface and hand wipes; the ranges detected  
 9 are summarized in Sections 1.2.3.1 and 1.2.3.2. In estimating exposures, absorption was considered to be  
 10 50%. Calculations considered ventilation rates, time spent indoors and outdoors, time spent at home and  
 11 in day care, the measured weight of each child, assumed ingestion of dust and soil, and total weight of  
 12 foods consumed. Median (25<sup>th</sup> percentile to maximum) bisphenol A aggregate exposures were estimated  
 13 at 2.56 (1.5–57.2) µg/day for children from North Carolina and 1.88 (1.27–48.6) µg/day in children from  
 14 Ohio. Median (25<sup>th</sup> percentile to maximum) potential aggregate dose, assuming 50% absorption, was  
 15 estimated at 0.0714 (0.0424–1.57) µg/kg bw/day in children from North Carolina and 0.0608 (0.0341–  
 16 0.775) µg/kg bw/day in children from Ohio. The study authors noted that 99% of exposure occurred  
 17 through dietary ingestion.

18  
 19 The European Union (2) conducted a comprehensive exposure estimate that considered exposures  
 20 resulting from food and environmental sources. Oral exposure estimates for children and adults were  
 21 reported and are summarized in Table 10. Estimates were based on migration studies conducted with  
 22 polycarbonate and concentrations of bisphenol A measured in foods packaged in epoxy-lined cans.  
 23 Assumptions used in exposure estimates included 100% oral absorption and body weights of 70 kg for  
 24 adults, 14.5 kg for 1.5–4.5-year-old children, 4.5 kg for 1–2-month-old infants, 7 kg for 4–6-month-old  
 25 infants, and 8.7 kg for 6–12-month-old infants. Estimated exposures for children were said to represent  
 26 realistic worst-case scenarios for food and drink intake relative to body weight.

27  
 28 **Table 10. Bisphenol A Oral Exposure Estimates by the European Union**

Exposure source (exposed population)	Daily food intake	Bisphenol A concentration in food	Bisphenol A intake	
			µg/day	µg/kg bw/day
Infant bottles (1–2 month-old infant)	0.699 L/day milk	50 µg/L	35	8
Infant bottles (4–6-month-old infant)	0.983 L/day milk	50 µg/L	50	7
Polycarbonate tableware (1.5–4.5-year old child)	2 kg food/day	5 µg/kg	10	0.7
Canned food (6–12- month-old infant)	0.375 kg canned food/day	100 µg/kg	40	5
Canned food (1.5–4.5- year-old child)	2 kg canned food/day	100 µg/kg	200	14
Canned food (adult)	1.0 kg canned food/day	100 µg/kg	100	1.4
Wine (adult)	0.75 L/day	650 µg/L	500	7 <sup>a</sup>
Canned food and wine (adult)	0.75 L/day wine and 1.0 kg canned food/day	650 µg/L in wine and 100 µg/kg food	600	9 <sup>a</sup>

<sup>a</sup>The European Union acknowledged that exposure through wine represents a very worst-case scenario.  
 From the European Union (2).

29  
 30 The European Union (2) also estimated human environmental exposure to bisphenol A from sources such  
 31 as drinking water, fish, plants, milk, meat, and air. The values were apparently obtained using the  
 32 “EUSES” model. Total regional exposure to bisphenol A was estimated at 0.0178 µg/kg bw/day. The

highest local exposure was thought to occur in the vicinity of PVC-producing plants and was estimated at 59 µg/kg bw/day. Aggregate exposures in adults involving food, wine, and environmental sources were estimated at 9 µg/kg bw/day for regional scenarios and 69 µg/kg bw/day for worst-case local scenarios occurring near a PVC-manufacturing plant. However, it was noted in the European Union report that use of bisphenol A in PVC manufacture was being phased out.

The European Union (2) noted that exposures to bisphenol A through dental sealant are single and rare events and do not lead to repeated exposure. Therefore, the issue was not considered further.

Exposures to bisphenol A from some consumer products were identified and characterized by the European Union (2). Products included: marine antifouling agents used on boats, wood varnish, wood fillers, and adhesives. With the exception of adhesives for which frequent use was thought possible, exposure to the other products was considered to be relatively rare. Exposures were estimated based on factors such as epoxy and residual bisphenol A concentrations, exposure time, area of skin exposed, and possible generation of mists during processes such as brushing. Inhalation exposures by product were estimated at  $3 \times 10^{-4}$  µg for antifouling agents and 0.02 µg for wood varnish. Dermal exposure by product without protective clothing was estimated at 29 µg for antifouling agents, 3.6 µg for wood varnish, 9 µg for wood filler, and 14 µg for adhesives. **[Dermal exposure to adhesives appears to be incorrectly reported as 1 µg in Table 4.20 of the European Union review.]** Exposure was estimated to be 1–2 orders of magnitude lower when protective clothing such as gloves was used. Assuming an absorption rate of 10%, dermal exposure to bisphenol A through adhesives was estimated at 0.02 µg/kg bw/day.

The European Commission (106) reviewed the report by the European Union (2) in draft and suggested alternate exposure estimates. Those estimates and the assumptions used to support those estimates are summarized in Table 11.

**Table 11. Bisphenol A Exposure Estimates by the European Commission**

Age and body weight	Type of food and amount consumed	Concentration of bisphenol A in food, µg/kg	Exposure estimate, µg/kg bw/day
0–4-month old infant, 4.5 kg	0.7 L of formula/day	10	1.6
6–12-month old infant, 8.8 kg	0.7 L of formula/day	10	0.8
6–12-month old infant, 8.8 kg	0.38 kg canned food/day	20	0.85
4–6-year-old child, 18 kg	1.05 kg canned food/day	20	1.2
Adult, 60 kg	1.05 kg canned food/day	20	0.37
Adult, 60 kg	0.75 L wine/day	9	0.11

From the European Commission (106).

Miyamoto and Kotake (54) estimated aggregate oral and inhalation exposure to bisphenol A in Japanese male children and adults. The estimates were based on unpublished Japanese data. This report is the only known study investigating potential exposure to children through mouthing of toys. Mouthing times were estimated by surveying the mothers of 50 infants and recording 25 infants on video camera. Mean ± SD daily mouthing times were reported at  $41.7 \pm 13.7$  minutes for infants 0–5 months of age and  $73.9 \pm 32.9$  minutes for infants 6–11 months of age. Migration rates were estimated from 0 µg/cm<sup>2</sup>/minute for toys that do not contain bisphenol A to 0.0162 µg/cm<sup>2</sup>/minute, the highest value reported in the Japanese literature. It was assumed that most toys were not manufactured with polycarbonate, epoxy resins, or grades of PVC that contain bisphenol A. Surface area of toys was assumed to be 10 cm<sup>2</sup>. In estimating

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1 oral exposures to bisphenol A, intake from food was also considered. Bisphenol A concentrations  
 2 measured in migration testing of polycarbonate bottles and food surveys are summarized in Section  
 3 1.2.3.2. Volume of food consumption and frequency of article use were considered in estimates of  
 4 bisphenol intake through food. Bisphenol A concentrations in drinking water were considered to be 0–  
 5 0.17 µg/L, and water intake was assumed to be 2 L/day. In estimating inhalation exposures,  
 6 concentrations of bisphenol A were considered to range from 0 to 8.1 ng/m<sup>3</sup> in indoor air and 0 to 28  
 7 ng/m<sup>3</sup> in outdoor air. Time spent indoors and outdoors and breathing rates were considered. Absorption  
 8 from lungs was assumed at 100%. Estimated exposures from mouthing of toys, food and water intake,  
 9 and inhaled air are summarized in [Table 12](#).

11 **Table 12. Average Estimated Exposure to Bisphenol A in Japanese Male Adults and Children**

Exposure source	Bisphenol A concentration (other assumptions)	Average estimated exposures (µg/kg bw/day) in each age group <sup>a</sup>					
		0–5 months	6–11 months	1–6 years	7–14 years	15–19 years	19 years
Human milk	Negligible	0	0				
Formula (water)	0–0.17 µg/L	0.012	0.0096				
Feeding bottle	0–3.9 µg/L	0.015	0.014				
Infant food	0–5.0 µg/kg		0.085				
Toys	0–0.0162 µg/cm <sup>2</sup> /minute (mean mouthing times of 41.7 minutes in 0–5 month olds and 73.9 minutes in 6–11 month olds)	0.026	0.069				
Air	0–8.1 ng/m <sup>3</sup> in indoor air and 0–28 ng/m <sup>3</sup> in outdoor air (90% indoors and 10% outdoors)	0.0026	0.0024	0.0021	0.0017	0.0015	0.0015
Water	0–0.17 µg/L (intake of 2 L/day)			0.012	0.0053	0.0029	0.0027
Food and drink							
Canned	0–602 µg/kg			0.38	0.21	0.20	0.29
Non-canned	0–3 µg/kg			0.38	0.21	0.13	0.12
Tableware	0–39.4 µg/meal/utensil (3 meals/day; 1–5 types of utensils used/meal)			0.40	0.12	0.024	0.022
Total		breast-fed: 0.028 formula-fed: 0.055	breast-fed: 0.16 formula-fed: 0.18	1.2	0.55	0.36	0.43

<sup>a</sup>Assumptions for bodyweights and most media intake levels were not provided.

Source: Miyamoto and Kotake (54).

12  
 13 Additional estimates of bisphenol A exposure through food are summarized in [Table 5](#) and [Table 6](#).  
 14 Details of studies conducted by Earls et al. (42) and Onn Wong et al. (40) are presented in Section 1.2.3.2.  
 15 Exposure estimates conducted by the FDA are described below. Limited details were available from the  
 16 other studies that were presented in reviews.

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1 The FDA (48) estimated bisphenol A intake in infants and adults resulting from exposures to epoxy food-  
 2 can linings and polycarbonate plastics. Exposure estimates occurring through contact of formula with  
 3 polycarbonate bottles were based on results of a study conducted by the Chemistry Methods Branch of the  
 4 FDA. The Chemistry Methods Branch also measured concentrations of bisphenol A in 5 brands of infant  
 5 formula (14 samples total); the study is also published as Biles et al. (60). In estimating adult bisphenol A  
 6 exposure through the consumption of canned foods, the FDA considered surveys conducted by the  
 7 Chemistry Methods Branch, Brotons et al. (65), and the Society of Plastics Industry Group. It appears that  
 8 the study by the Society of Plastics Industry Group was later published by Howe et al. (55) and included a  
 9 re-analysis to correct some interferences observed in analytical methods. Exposure estimates and  
 10 assumptions used to make the estimates are summarized in Table 13.

12 **Table 13. Summaries of Studies Estimating Bisphenol A Exposures Solely from Foods**

Population	Exposure source	Basis and assumptions for estimates	Exposure estimate, µg/kg bw/day	Reference
Infants	Polycarbonate bottles	Bisphenol A migration concentration of 15–20 µg/L; milk consumption of up to 550 mL/day; mean body weight of 11 kg.	0.75–1	Earls et al. (42)
Infants (0–3 months old)	Polycarbonate bottles	Mean upper-bound concentration of bisphenol A migration in 10% ethanol (0.64 µg/in <sup>2</sup> ) and in corn oil (0.43 µg/in <sup>2</sup> ); body weights reported by National Center for Health Statistics, and FDA Dietary Exposure Guidelines with modifications for properties of infant formula.	15–24 <sup>a</sup>	Onn Wong et al. (40)
Not reported	Food from epoxy-lined cans	Bisphenol A concentrations of 5 ppb [µg/L] in beverages and 37 ppb [µg/kg] in other foods; FDA Dietary Exposure Guidelines: dietary intake of 3 kg/day, body weight of 60 kg.	0.105	Howe et al. (55), Haighton et al. (47), and NAS (107)
Adults	Cumulative exposures from food contacting cans and polycarbonate plastics	22 ppb [µg/kg] bisphenol A in vegetables, consumption factor of 0.17 for food contacting polymer-coated metal, intake of 3 kg food/bw/day, 60 kg bw, and insignificant contribution from polycarbonate	0.183	FDA (48)
Infants	Cumulative exposures from food contacting cans and polycarbonate plastics	Bisphenol A concentration of 6.6 µg/kg in prepared infant formula, < 1.7 ppb [µg/L] in infant formula from polycarbonate bottles, consumption of 820 g food/day, and 4 kg infant weight	1.75	
Adults	Canned foods	Data from survey of canned foods and food intake patterns determined from surveys	Mean 0.0083 (0–0.29)	Thomson and Grounds (64)

Population	Exposure source	Basis and assumptions for estimates	Exposure estimate, $\mu\text{g}/\text{kg bw}/\text{day}$	Reference
Adults	Canned foods and canned fish	Data from survey of canned foods and food intake patterns determined from surveys	0.0044 for males $\geq 25$ , 0.0041 for females $\geq 25$ , and 0.0048 for males age 19–24	Thomson et al. (108)
Adults	Wine	Maximum bisphenol A concentration of 2.1 ng/mL in wine, consumption of 0.75 L/day, and 60 kg body weight.	<0.026	Brenn-Struckhofova and Cichna-Markel (56)
Hospital patients	Meals served at 2 hospitals	Mean intake from hospital diets was estimated at 1.3 (0.19–3.7) $\mu\text{g}/\text{day}$ ; [60 kg body weight was assumed]	[0.02 (0.003–0.06)]	Imanaka (2001) as cited in Miyamoto and Kotake (54) and Fujimaki et al. (85)
Japanese adults and children	~200 food items were collected in a total diet study	No details	0.00475 for children age 2–6 years and 0.00195 for adults	Tokyo Metropolitan Government (2003) as cited in Miyamoto and Kotake (54)

<sup>a</sup>The study authors acknowledged the use of aggressive migration testing conditions and conservative assumptions in calculations, thus leading to overestimated infant exposures.

1  
2 [Table 14](#) summarizes exposure estimates for aggregate or food exposures. Studies suggest that the  
3 majority of bisphenol A exposure occurs through food and that environmental exposures do not appear to  
4 substantially affect total exposure, with the possible exception of exposure near point sources. [Table 14](#)  
5 includes estimates that CERHR believes to represent potentially realistic exposure scenarios and does not  
6 include data from extreme worst-case scenarios such as possible point-source exposures.

7  
8 **Table 14. Summary of Food and/or Aggregate Exposures to Bisphenol A**

Population	Basis of Estimates	Exposure estimate, $\mu\text{g}/\text{kg bw}/\text{day}^a$	Reference
1–2-month old infant	Food exposure (data from migration studies of polycarbonate bottles)	8	European Union (2)
0–4-month old infant	Food exposure (data from migration studies of polycarbonate bottles)	1.6	European Commission (106)
0–5-month old infant (formula-fed)	Aggregate exposure (based on formula, environmental, and toy exposures)	0.055	Miyamoto and Kotake (54)
0–5-month old infant (breast fed)	Aggregate exposure (based on human milk, environmental, and toy exposures)	0.028	Miyamoto and Kotake (54)
4–6-month old infant	Food exposure (data from migration studies of polycarbonate bottles)	7	European Union (2)
6–11-month-old infant (formula-fed)	Aggregate exposure (based on formula, food, environmental, and toy exposures)	0.18	Miyamoto and Kotake (54)
6–11-month-old infant (breast-fed)	Aggregate exposure (based on human milk, food, environmental, and toy exposures)	0.16	Miyamoto and Kotake (54)

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Population	Basis of Estimates	Exposure estimate, µg/kg bw/day <sup>a</sup>	Reference
6–12-month-old infant	Food exposure (data from survey of canned foods)	5	European Union (2)
6–12-month-old infant	Food exposure (data from migration studies with infant bottles and canned foods)	1.65	European Commission (106)
Infant	Food exposure (data from polycarbonate bottle leaching studies)	0.75–1	Earls et al. (42)
Infant	Food exposures (contact with cans and polycarbonate plastics)	1.75	FDA (48)
1.5–4.5-year-old child	Food exposure (data from survey of canned foods and migration studies with polycarbonate tableware)	14.7	European Union (2)
1–6-year-old child	Aggregate exposure (based on food, environmental, and tableware exposures)	1.2	Miyamoto and Kotake (54)
1.5–5 year old child	Aggregate exposure (surveys of bisphenol in food, air, dust, soil and hand and surface wipes)	0.06-0.07 (0.03–1.57)	Wilson et al. (32)
3–5-year-old child	Aggregate exposure (surveys of bisphenol in food, air, dust, and soil)	0.04 (0.018–0.07)	Wilson et al. (31)
2–6 year-old child	Food exposure (collection of 200 food items)	0.004	Tokyo Metropolitan Government (2003) as cited in Miyamoto and Kotake (54)
4–6 year-old child	Food exposure (data from survey of canned foods)	1.2	European Commission (106)
7–14 year-old child	Aggregate exposure (based on food, environmental, and tableware exposures)	0.55	Miyamoto and Kotake (54)
15–19 year-old individual	Aggregate exposure (based on food, environmental, and tableware exposures)	0.36	Miyamoto and Kotake (54)
Adult, ≥19 years	Aggregate exposure (based on food, environmental, and tableware exposures)	0.43	Miyamoto and Kotake (54)
Adult	Food exposure (data from survey of canned foods not including wine)	1.4	European Union (2)
Adult	Food exposure (data from surveys of canned food)	0.37	European Commission (106)
Adult	Wine exposure (data from study of epoxy-lined wine drums)	0.11	European Commission (106)
Adult	Wine exposure (data from wine samples)	<0.026	Brenn-Struckhfova and Cichna-Markel (56)
Adult	Food exposure (from contact with epoxy-lined cans and polycarbonate)	0.183	FDA (48)
Adults	Food exposure (survey of canned foods)	0.008	Thomson and Grounds (64)

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Population	Basis of Estimates	Exposure estimate, µg/kg bw/day <sup>a</sup>	Reference
Adult	Food exposure (collection of 200 food items)	0.002	Tokyo Metropolitan Government (2003) as cited in Miyamoto and Kotake (54)

<sup>a</sup>Estimates involving extreme worst case scenarios and Japanese data with very limited information were not included in this table.

1  
2 *1.2.4.1.2 Estimates based on biological monitoring*  
3 Goodman et al. (84) noted that total urinary bisphenol A concentrations were useful for estimating  
4 bisphenol A intake. Because of extensive first-pass metabolism, little parent compound is systemically  
5 circulated, as discussed in more detail in Section 2. Because nearly 100% of an acute exposure to  
6 bisphenol A is excreted in urine within 24 hours (6, 109), bisphenol A intake can be estimated by  
7 measuring bisphenol A in urine over a specified time interval. Arakawa et al, 2004 (110) measured  
8 bisphenol A excretion over a 5-day period and reported intra- and inter-individual variability. As a result,  
9 caution was urged in using single time-point values to estimate long-term exposure. Typical daily intakes  
10 of bisphenol A estimated from urinary levels are <0.01-2.17µg/kg bw/day (Table 15). A Monte Carlo  
11 simulation using the urine data of Tsukioka et al. (6) and Arakawa et al. (110) estimated mean exposures  
12 of 0.028-0.049 ug/kg bw/day for males and 0.034-0.059 ug/kg bw/day for females (54). Using the U.S.  
13 NHANES data and assumptions on excretion rates and body weight a median intake of 0.026 ug/kg  
14 bw/day is estimated. An estimated median exposure based on urinary bisphenol A concentrations in 6–8-  
15 year-old girls was 0.07 µg/kg bw/day; (86)  
16  
17 Joskow et al. (79) used values for total bisphenol A in urine to estimate exposure to bisphenol A  
18 following dental sealant application. Urinary concentrations of bisphenol A are reported in Table 8.  
19 Factors or assumptions used in the exposure estimates were recovery of bisphenol A in urine as its  
20 glucuronide conjugate within 24–34 hours following exposure, a 5.4 hour half-life of elimination for  
21 bisphenol A glucuronide, and a 1.5 L/day urinary excretion volume. Estimated doses of bisphenol A  
22 [based on a 60-kg bw] were 49–239 µg [0.82–4.0 µg/kg bw] following application of Delton LC and 0–  
23 9.5 µg [0–0.16 µg/kg bw] following application of HeliOSEAL F. The study authors stated that the  
24 estimates were likely low because a substantial amount of bisphenol A was potentially eliminated by  
25 collection of saliva samples immediately following treatment.  
26

1 Table 15. Estimates of Bisphenol A Intakes Based on Urinary Excretion

Population	Basis for estimates	Mean or median (range) of estimated intake, $\mu\text{g}/\text{kg bw}/\text{day}^{\text{a}}$	Reference
22 Japanese adults	Mean excretion of 1.68 $\mu\text{g}/\text{day}$ (0.48–4.5 $\mu\text{g}/\text{day}$ )	0.028 (0.008–0.075)	Tsukioka et al. (6)
36 Japanese male students	Median excretion of 1.2 $\mu\text{g}/\text{day}$ (<0.21–14 $\mu\text{g}/\text{day}$ )	0.02 (<0.0035–0.23)	Arakawa et al. (110)
5 Japanese males	Median excretion of 1.3 $\mu\text{g}/\text{day}$ (<0.58–13 $\mu\text{g}/\text{day}$ ) over a 5-day period	0.022 (<0.01–0.22)	Arakawa et al. (110)
Data from Tsukioka et al. (6) and Arakawa et al. (110)	Monte Carlo simulations	Mean exposure: 0.028–0.049 in males and 0.034–0.059 in females; low exposures (5 <sup>th</sup> percentile) 0.021–0.037 in males and 0.025–0.044 in females; high exposures (95 <sup>th</sup> percentile): 0.037–0.064 in males and 0.043–0.075 in females	Miyamoto and Kotake (54)
56 pregnant Japanese women	Bisphenol A concentration in 1 spot sample was normalized to creatinine and exposure was estimated using average creatinine and urine volume excretion rates, which resulted in a median intake of <2 $\mu\text{g}/\text{day}$ (<0.3–7.9 $\mu\text{g}/\text{day}$ ).	<0.04 (<0.006–0.16) <sup>b</sup>	Fujimaki et al. (85)
48 Japanese female college students	Authors estimated bisphenol A intake of 0.6–71.4 $\mu\text{g}/\text{day}$ , based on a median bisphenol A concentration of 0.77 ng/mg (0.1–11.9 ng/mg) creatinine in a spot urine sample, assumed creatinine excretion of 1200 mg/day and that 20% of the dose is excreted in urine. <b>[CERHR recalculated values using a 100% urinary excretion rate which is consistent with human data]</b>	0.01–1.2 based on study author assumptions <b>[0.015 (0.002–0.24) based on a 100% urinary excretion rate]</b>	Ouchi and Watanabe (100)
7 male and 12 female without intentional exposure	All measurements < LOD of 1.14 $\mu\text{g}/\text{L}$	Based on 2 Liter urine excreted and 60 kg adult exposure < 0.038	Völkel et al(7)

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Population	Basis for estimates	Mean or median (range) of estimated intake, $\mu\text{g}/\text{kg bw}/\text{day}^{\text{a}}$	Reference
394 participants in the NHANES III survey (US)	Median (10 <sup>th</sup> –95 <sup>th</sup> percentile) 1.32 (0.23-7.95) $\mu\text{g}$ bisphenol A/g creatinine in a spot urine sample; <b>[assumed 100% urinary excretion of bisphenol A in 24 hours and creatinine excretion of 1200 mg/day]</b>	<b>[median: 0.026; 10<sup>th</sup>–95<sup>th</sup> percentile: 0.005-0.159]</b>	Calafat et al. (15)
90 girls, 6–8 years-old (US)	Median (range) 1.8 $\mu\text{g}/\text{L}$ (<0.3-54.3) <b>[assumed 100% urinary excretion of bisphenol A in 24 hours; 1 L per day; 25kg body weight ]</b>	<b>[0.07 (&lt;0.012-2.17)]</b>	Wolff et al. (86)

<sup>a</sup>Consistent with estimates conducted by Goodman et al. (84), body weights of 60 kg were assumed, unless otherwise indicated.

<sup>b</sup>A 50 kg body weight was assumed.

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### 1.2.4.2 Occupational exposure

Occupational exposure to bisphenol A could potentially occur during its manufacture, in the production of polycarbonate plastics, and during the manufacture or use of epoxy resins, powder coatings paints, or lacquers (2). Possible exposure to bisphenol A during PVC manufacture has been considered, but the European Union (2) stated that the application was being phased out. According to the European Union, bisphenol A is generally available as granules, flakes, or pellets, thus reducing exposure potential. Bisphenol A is manufactured in closed systems, but exposure is possible during sampling, container filling, and plant maintenance. In the manufacture of polycarbonate, bisphenol A enters the plant and remains in a closed system prior to extrusion. Sampling is conducted by a closed loop system. Following extrusion, the polycarbonate is chopped into granules and bagged, and it is during that stage that exposure to residual bisphenol A (reported at  $\leq 100$  ppm) through dust is possible. However, it is noted that polycarbonate is stable and that residual bisphenol A is contained within the polymer matrix. The European Union stated that exposure to bisphenol A during the manufacture of polycarbonate items is not likely to exceed values observed during the manufacture of polycarbonate. In the production of epoxy resin, bisphenol A exposure is most likely during reactor charging, but exposure during maintenance is also possible. A residual bisphenol A concentration of 300 ppm was reported for epoxy resins, but it was noted that most bisphenol A was trapped within the resin matrix. Exposure to bisphenol A during production of epoxy paints is reported to be negligible. In the manufacture of powder epoxy coatings, exposure is thought possible during weighing and milling. Exposure to bisphenol A during the use of powder paints has been documented.

There are no known regulatory limits for occupational exposure to bisphenol A in the US. In 2004, the American Industrial Hygiene Association proposed a workplace environmental exposure level (WEEL) of  $5 \text{ mg/m}^3$  for bisphenol A. The draft WEEL was based upon irritation observed in an inhalation toxicity study (111). The value is consistent with the time weighted average (TWA) exposure limits established in Germany and the Netherlands (2).

The European Union (2) summarized occupational exposure data for bisphenol A in Europe and the US. Only measured data for bisphenol A are summarized in this report. The European Union stated that the values reported did not account for the effects of personal protective equipment in order to avoid difficulties in attempting to quantify protection provided. TWA bisphenol A concentrations measured in occupational settings are summarized in Table 16. The limited number of values reported indicated that bisphenol A concentrations were below  $5 \text{ mg/m}^3$ . Bisphenol A exposures ( $>1 \text{ mg/m}^3$ ) were observed in spraying of powdered bisphenol A-containing coatings, bisphenol A manufacture and manufacture of epoxy resins. The highest daily average exposures were observed in the manufacture of bisphenol A. There is limited information on short-term exposure to bisphenol A. In manufacture of bisphenol A one facility reported short term task exposures from  $0.13 - 9.5 \text{ mg/m}^3$ (2).

Data for powder paint use summarized in Table 16 were obtained from a NIOSH Health Hazard Evaluation conducted at a company that manufactured fan and ventilation equipment (112). In plant 1 of the company, parts were coated with an epoxy-based powder paint by dipping. At plant 2, an epoxy-based powder was applied to parts via electrostatic spraying. As evident in the data in Table 16, exposures were higher at the plant utilizing electrostatic spraying. Monitoring for bisphenol A was discussed in 2 other NIOSH Health Hazard Evaluation reports. In those reports, bisphenol A was not detected in a plant where an epoxy resin coating was used in the manufacture of electronic resistors (113) or in a plant where an epoxy resin coating was applied to steam turbine generators (114). Rudel et al. (33) used a GC/MS technique to measure bisphenol A concentrations at one US workplace where plastics were melted and glued; a concentration of  $0.208 \text{ } \mu\text{g/m}^3$  was reported.

**[Bisphenol A exposures in US powder paint workers were estimated at  $\sim 0.1$ – $100 \text{ } \mu\text{g/kg bw/day}$  based on TWA exposures of  $0.001$ – $1.063 \text{ mg/m}^3$ , an inhalation factor of  $0.29 \text{ m}^3/\text{kg day}$  (115), 100% absorption from the respiratory system, and 8 hours worked per day.]**

1  
2 No information was located for dermal exposure to bisphenol A in occupational settings. Using their  
3 Estimation and Assessment of Substance Exposure model, the European Union (2) estimated that dermal  
4 exposure of workers to bisphenol A was unlikely to exceed 5 mg/cm<sup>2</sup>/day. It was noted that the highest  
5 potential exposure to bisphenol A would occur during bag filling and maintenance work.  
6

7 One study provided information on biological monitoring of bisphenol A in workers exposed to an epoxy  
8 compound. In 3 Japanese plants, exposed workers included 42 men who sprayed an epoxy hardening  
9 agent consisting of a mixture of bisphenol A diglycidyl ether (10–30%), toluene (0–30%), xylene (0–  
10 20%), 2-ethoxyethanol (0–20%), 2-butoxyethanol (0–20%), and methyl isobutyl ketone (0–30%) (116).  
11 The workers wore “protection devices” during spraying. Controls consisted of 42 male assembly workers  
12 from the same plants who did not use bisphenol A diglycidyl ether. In 1999, urine samples were  
13 periodically collected, treated with β-glucuronidase, and examined for bisphenol A by HPLC. Urinary  
14 bisphenol A concentrations were significantly higher in exposed workers (median: 1.06 μmol/mol  
15 creatinine [**2.14 μg/g creatinine**]; range: <0.05 pmol to 11.2 μmol/mol creatinine [**<0.1 pg to 22.6 μg/g**  
16 **creatinine**]) compared to controls (median: 0.52 μmol/mol creatinine [**1.05 μg/g creatinine**]; range:  
17 <0.05 pmol to 11.0 μmol/mol creatinine [**<0.1 pg to 22.2 μg/g creatinine**]). The difference of the  
18 averages was reported as 2.5 μmol/mol creatinine [**5.05 μg/g creatinine**] (95% CI 1.4–4.7 μmol/mol  
19 creatinine [**2.8–9.5**]). Bisphenol A was not detected in 3 exposed workers and 1 control. [**Assuming**  
20 **excretion of 1200 mg/day creatinine (100), mean (ranges) of bisphenol excretion in urine were 2.57**  
21 **μg/day (<0.12 pg to 27.1 μg/day) in exposed workers and 1.26 μg/day (<0.12 pg to 26.6 μg/day) in**  
22 **unexposed workers. With an assumed body weight of 60 kg, bisphenol A occupational intake was**  
23 **estimated at 0.043 μg/kg bw/day (<0.002 pg to 0.45 μg/kg bw/day) in exposed workers and 0.021**  
24 **μg/kg bw/day (<0.002 pg to 0.44 μg/kg bw/day) in unexposed workers.]**  
25

### 26 1.3 Utility of Data

27  
28 Numerous studies reported bisphenol A concentrations in canned foods and infant formula. Experiments  
29 examined potential concentrations of bisphenol A resulting from leaching of bisphenol A from  
30 polycarbonate bottles under a variety of conditions. There minimal data available for bisphenol A  
31 concentrations in drinking water but these show concentrations below the limit of detection. Bisphenol A  
32 has been detected in surface waters and solid waste landfill leachates. Bisphenol A has been detected in  
33 indoor dust samples and indoor and outdoor air samples. Data for occupational exposure to bisphenol A  
34 in the US are very limited. Only 2 studies reported TWA exposures to bisphenol A in US workers.  
35 Several estimates of human bisphenol A exposure were developed using bisphenol A concentrations  
36 measured in food and the environment. Although very limited for US populations, there are data reporting  
37 bisphenol A concentrations in urine, breast milk and amniotic fluid, but none for blood or fetal blood.  
38 Exposure estimates have been derived from urinary bisphenol A concentrations in multiple studies..  
39

### 40 1.4 Summary of Human Exposure

41  
42 In 1999 and 2003, it was reported that most bisphenol A produced in the US was used in the manufacture  
43 of polycarbonate and epoxy resins and other products [reviewed in (3, 18)]. Polycarbonate plastics are  
44 used in various consumer products and the products most likely to contribute to human exposure are  
45 polycarbonate food containers (e.g., milk, water, and infant bottles). Epoxy resins are used in protective  
46 coatings. Food cans lined with epoxy resin are a potential source of human exposure. Some polymers  
47 manufactured with bisphenol A are FDA-approved for use in direct and indirect food additives and in  
48 dental materials (22). Resins, polycarbonate plastics, and other products manufactured from bisphenol A  
49 can contain trace amounts of residual monomer and additional monomer may be generated during  
50 breakdown of the polymer (2).  
51

1 **Table 16. TWA Measurements of Bisphenol A in the Workplace**

Industry or activity	Location/year	Number of samples	Sample type	8-hour TWA (mg/m <sup>3</sup> ) mean (range) <sup>b</sup>
<b>Bisphenol A manufacture</b>				
Various	US/not specified	Not specified	Bisphenol A	N.S. (Not detected (not specified) to 2.6)
Filling big bags	Europe/1998	3	Inhalable bisphenol A	0.81 (0.21-1.79)
Filling silo tankers	Europe/1998	3	Inhalable bisphenol A	0.89 (<0.5-1.61)
Various tasks	Europe/1998	8	Inhalable bisphenol A	0.3 (0.13-0.62)
Plant operator	Europe/not specified	7	Inhalable bisphenol A	N.S. (0.021-1.04)
Maintenance	Europe/not specified	3	Inhalable bisphenol A	N.S. (0.52-1.35)
Maintenance	Europe/1998–2000	8	Bisphenol A	N.S. (<0.05–0.62)
Charging big bags	Europe/1996–1997	5	Inhalable bisphenol A	0.35 (0.02–0.93)
Plant operator	Europe/not specified	13	Bisphenol A	0.61 (0.02–2.13)
Maintenance operator	Europe/not specified	2	Bisphenol A	1.06 (0.4–2.08)
<b>Epoxy Resin Manufacture</b>				
Loading/unloading	US/1970-mid 1990's	26	Bisphenol A	0.18 (<0.1-0.99)
Bagging/palletizing	US/1970-mid 1990's	37	Bisphenol A	0.25 (<0.1-2.8)
Process operators	US/1970-mid 1990's	25	Bisphenol A	0.26 (<0.1-1.1)
Equipment technician	US/1970-mid 1990's	6	Bisphenol A	<0.1
Maintenance	US/1970-mid 1990's	2	Bisphenol A	0.8 (0.37-1.2)
<b>Bisphenol A Use</b>				
Powder paint use <sup>a</sup>	US/~1979	7 (3 personal and 4 area samples)	Bisphenol A (plant 1)	0.005 (0.004–0.006)
		21 (15 personal and 6 area samples)	Bisphenol A (plant 2)	0.175 (0.001–1.063)

<sup>a</sup>From NIOSH (112). Other data are from the European Union (2).

<sup>b</sup>Range given representing different occupational activities

2  
3 Bisphenol A may be present in the environment as a result of direct releases from manufacturing or  
4 processing facilities, fugitive emissions during processing and handling, or release of unreacted monomer  
5 from products (2). Because of its low volatility and relatively short half-life in the atmosphere, bisphenol  
6 A is unlikely to be present in the atmosphere in high concentrations (2). A study of 222 homes and 29 day  
7 care centers found bisphenol A in 31-44% of outdoor air samples with concentrations of < LOD (0.9) to  
8 51.5 ng/m<sup>3</sup> (32). Rapid biodegradation of bisphenol A in water was reported in the majority of studies  
9 reviewed by the European Union (2) and Staples et al. (3). Drinking water concentrations of bisphenol A  
10 at Louisiana and Detroit Michigan water treatment plants were below the limit of detection (<0.1 ng/L).  
11 Chlorinated congeners of bisphenol A resulting from chlorination of water may be degraded less rapidly  
12 (73). Bisphenol A is not expected to be stable, mobile, or bioavailable from soils (30). A study of 222  
13 homes and 29 day care centers found bisphenol A in 25-70% of indoor dust samples with concentrations  
14 of < LOD (20) to 707 ng/g (32). The potential for bioconcentration of bisphenol A in fish is low (2, 3).  
15 [Table 17](#) summarizes concentrations of bisphenol A detected in environmental samples and drinking  
16 water.

17

1 **Table 17. Maximum Reported Bisphenol A Concentrations in US Ambient Air and Dust Samples**

Sample	Bisphenol A concentration	Reference
Outdoor air	<52 ng/m <sup>3</sup> Monthly average 0.12-1.2 ng/m <sup>3</sup>	Wilson et al. (31); Wilson et al. (32); Matsumoto et al. (117)
Indoor air	≤ 193 ng/m <sup>3</sup>	Wilson et al. (31); Wilson et al. (32); Rudel et al. (33); Rudel et al. (34)
Indoor dust	≤17.6 µg/g	Wilson et al. (31); Wilson et al. (32); Rudel et al. (33); Rudel et al. (34)
Drinking water	< 0.1 (MDL) <0.005	Boyd et al. (25); Kuch and Ballschmiter (24)

2  
3 The highest potential for human exposure to bisphenol A is through products that directly contact food  
4 such as food and beverage containers with internal epoxy resin coatings and polycarbonate tableware and  
5 bottles, such as those used to feed infants (2). Studies examining the extraction of bisphenol A from  
6 polycarbonate bottles or tableware into food simulants are summarized in Table 4. Studies measuring  
7 bisphenol A concentrations in canned infant foods are summarized in Table 5 and studies measuring  
8 bisphenol A concentrations in canned food are summarized in Table 6. Table 18 summarizes the general  
9 findings from all the food contact-material studies. Bisphenol A concentrations were measured in canned  
10 foods produced and purchased from various countries.

11  
12 **Table 18. Maximum Reported Bisphenol A Concentrations Measured in Foods or Food Simulants**

Exposure Source	Bisphenol A concentration	Table Reference
Polycarbonate infant bottles	≤55 µg/L (<5 µg/L in US study)	Table 4
Polycarbonate tableware	≤5 µg/kg	Table 4
Canned infant formulas	≤ 113 µg/L (<6.6 µg µg/kg in U.S. study of water mixed formula; <13 µg/kg in U.S. formula concentrate)	Table 5
Canned infant foods	≤77.3 µg/kg	
Canned foods	≤ 842 µg/kg (≤ 39 µg/kg in US studies)	Table 6

13  
14 Table 19 summarizes BPA concentrations reported in human body fluids. Measurement of bisphenol A  
15 concentrations are affected by measurement technique, particularly at the very low concentrations that can  
16 now be measured. Enzyme-linked immunosorbent assay (ELISA) has poor correlation with the LC-ECD  
17 method and also the different ELISA kits correlate poorly with each other. ELISA methods may over-  
18 estimate bisphenol A in biologic samples due to lack of specificity of the antibody and effects of the  
19 biologic matrix (8, 9). In addition, contamination from labware and reagents or sample degradation during  
20 storage can impact the accuracy of measurements. [The panel therefore finds the greatest utility in  
21 studies that use sensitive and specific analytical methods for biological samples (LC-MS or GC-MS)  
22 and report quality control measures for sample handling and analysis.]  
23

24 **Table 19. Maximum Reported Biological Measures of Bisphenol A Concentrations in Humans**

Biological Medium	Population	Concentration Free BPA* (µg/L)	Total BPA* (µg/L)	Reference
Urine	Adult	≤2.36 (<0.6 in US study)	≤3950 (<19.8 US studies)	Table 8
	Children		<54 (2 US studies)	
Blood	General	< LOD (0.5)	< LOD (0.5)	Table 7
	Infertile	<0.87		Table 7
	Women			
	Women	< 1.6		Table 7
	Men	< 1		Table 7

Biological Medium	Population	Concentration Free BPA* (µg/L)	Total BPA* (µg/L)	Reference
Breast Milk	Fetal	<9.2	<7.3	Table 9
	Women	< 6.3 (U.S.)		Table 3
Amniotic fluid	Fetus	<1.96 (U.S)		Table 9
Semen	Adult	<0.5		Inoue et al.(8)
Saliva after dental sealant	Adult	<2800		Arenholt-Bindslev et al.(76)

\*Measurements by HPLC, GC/MS and LC/MS only

1  
2 Table 20 summarizes food and/or aggregate exposure estimates calculated from bisphenol A  
3 concentrations in food, environmental and toy exposures along with estimates of consumption and body  
4 weights. It was noted that dietary sources account for 99% of exposure (32). Metabolite-based estimates  
5 of bisphenol A used urinary concentrations along with estimates of urinary and/or creatinine excretion,  
6 and body weight.

7  
8 **Table 20. Summary of Reported Human Dose Estimates**

Exposure Source	Population	BPA µg/kg bw/day	Notes	Source
<b>Estimates based on Intake</b>				
Formula	Infant	1.6-8	8 assumes 700 ml formula with 50 ug/L	Table 14
Formula	Infant	1.0	Assumes 4.5 kg, 700 ml formula with 6.6 ug/L from U.S. canned formula	Expert Panel
Breast milk	Infant	1.0	Assumes 4.5 kg, 700 ml with 6.3 ug/L from breast milk	Expert Panel
Food	Infant	1.65-5	5 assumes 0.375 kg canned food at 100 ug/kg	Table 14
	Child	0.00475-1.2	1.2 assumes 1 kg canned food at 20 µg/kg	Table 14
	Adult	0.00195-1.4	1.4 assumes 1 kg canned food at 100 µg/kg	Table 14
Aggregate	Infant (formula)	0.055-0.18	assumes 0-0.17 ug/L in formula	Table 14
	Infant (breast milk)	0.028-0.16	assumes 0 exposure from breast milk	Table 14
	Child	0.042981-14.7	14.7 assumes 2 kg canned food at 100 µg/kg	Table 14
	Adult	0.36-0.43	assumes 0-602 ug/kg in canned food	Table 14
Occupational	Adult	0.043-100		EPA & Expert Panel
<b>Estimates based on Urinary Metabolites</b>				
Aggregate	Child	0.07 (2.17)	Median (max) US 6-8yr old girls	Table 15
	Adult	0.026	Median NHANES	Table 15
	Adult	0.66	Assume max 19.8 ug/L from U.S., 2 L urine/day, 60kg	Ye et al.(96)

9

## 1.0 Chemistry, Use, and Human Exposure

1 Dental sealant exposure to bisphenol A occurs primarily with use of dental sealants bisphenol A  
2 dimethylacrylate. This exposure is considered an acute and infrequent event with little relevance to  
3 estimating general population exposures.  
4

5 Very limited information is available for bisphenol A exposure in the US workplace. Data obtained from  
6 the US and Europe indicate highest potential exposures during spraying of powdered bisphenol A-  
7 containing coatings and during tank filling, plant operation activities, and maintenance work in plants  
8 where bisphenol A is manufactured. (2). One study measured total urinary bisphenol A in Japanese  
9 workers who sprayed an epoxy compound (116).

## 2.0 GENERAL TOXICOLOGY AND BIOLOGICAL EFFECTS

As discussed in **Section 1.4**, the quantified amount of free bisphenol A present in biological samples may be affected by contamination with bisphenol A in plastic laboratory ware and in reagents (6, 7). In addition, the accuracy may also be affected by measurement technique, particularly at the very low concentrations that can now be measured. Enzyme-linked immunosorbent assay (ELISA) have the potential to over-estimate bisphenol A in biologic samples due to lack of specificity of the antibody and effects of the biologic matrix (8, 9). High performance liquid chromatography (HPLC) with ultraviolet, fluorescence, or electrochemical detection is unable to make definitive identification of bisphenol A or bisphenol A glucuronides, because similar retention times may occur for the metabolites of other endogenous and exogenous compounds (7). Use of LC-tandem mass spectrometry (MS/MS) with and without hydrolysis of bisphenol A glucuronide permits determination of free and total bisphenol A with a limit of quantification of 1 µg/L (7). Gas chromatography (GC)/MS/MS has been used with solid phase extraction after treatment with glucuronidase and derivitization to measure total bisphenol A with a limit of detection of 0.1 µg/L (15). Bisphenol A glucuronide has been shown to be unstable and can be hydrolyzed to free bisphenol A at neutral pH and room temperature in diluted urine of rats and in rat placental and fetal tissue homogenates at room temperature. Bisphenol A glucuronide can also be hydrolyzed and in some cases degraded to unknown components either in acidic or basic pH solutions of diluted urine, adding another potential source of error in the measurement of sample levels of bisphenol A and its conjugates (17 2485). These considerations taken together, suggest that it is possible that free bisphenol A concentrations measured in biological samples may be overestimated.

### 2.1 Toxicokinetics and Metabolism

The studies presented in this section demonstrate that bisphenol A is absorbed in humans and experimental animals following oral exposure. In humans and experimental animals, most of the dose is present in blood as the main metabolite, bisphenol A glucuronide, and smaller percentages are present as the parent compound. Bisphenol A and its metabolites are widely distributed in humans and animals. More than 90% of unmetabolized bisphenol A is reportedly bound to plasma protein. Bisphenol A is distributed to fetal fluids in humans and experimental animals, and a limited number of studies in humans demonstrate fetal concentrations of bisphenol A within an order of magnitude of concentrations in maternal blood. None of the studies detected bisphenol A glucuronide in fetal fluids. Transfer of bisphenol A to milk was demonstrated in humans and experimental animals. One study in humans reported bisphenol A in milk at concentrations exceeding maternal blood concentrations. In humans and experimental animals, most of a bisphenol A dose is metabolized to bisphenol A glucuronide prior to absorption. Studies in humans and experimental animals demonstrated that glucuronidation of bisphenol A can occur in the liver, and one study in rats demonstrated that bisphenol A is glucuronidated upon passage through the intestine. Bisphenol A glucuronide is excreted in the bile of rats, and enterohepatic cycling is thought to occur in rats but not humans. In humans, most of a bisphenol A dose is eliminated through urine as bisphenol A glucuronide. In rats, bisphenol A is eliminated through feces as bisphenol A and in urine as bisphenol A glucuronide.

#### 2.1.1 Humans

Human toxicokinetics studies that were judged potentially important to interpret developmental and reproductive toxicity were reviewed in full. These studies include reports of potential exposure of fetuses during pregnancy or of infants through human milk and reports of toxicokinetics or metabolism following low-dose exposure of humans. Information from secondary sources was included if the information was not considered to be critical to the interpretation of developmental and reproductive toxicity data.

##### 2.1.1.1 Absorption

Two studies described here examined oral absorption of bisphenol A from dental sealants, and one study examined in vitro dermal absorption. Bisphenol A (as parent or the monoglucuronide) is absorbed in

## 2.0 General Toxicology and Biological Effects

1 humans as indicated by the detection of bisphenol A (and metabolites) in blood from the general  
2 population (Section 1) and in maternal and fetal fluids (Table 9).

3  
4 Fung et al. (77) examined the toxicokinetics of bisphenol A leaching from dental sealant. Volunteers  
5 included 18 men and 22 non-pregnant women (ages 20–55 years) who did not have dental disease,  
6 existing composite resin restorations or pit and fissure sealants, or a history of resin exposure. Volunteers  
7 were treated with a widely used commercial dental sealant (Delton Opaque Light-cure Pit and Fissure  
8 Sealant). Components of the sealant were analyzed by HPLC. The low-dose group (n = 7 men, 11  
9 women) received 8 mg dental sealant on 1 tooth, and the high-dose group (11 men, 11 women) received  
10 32 mg sealant on 4 teeth. Saliva and blood samples were collected before the procedure and at 1 and 3  
11 hours and 1, 3, and 5 days after the procedure. Blood and saliva were analyzed by HPLC. Statistical  
12 analyses of data were conducted by nonparametric test, Wilcoxon signed rank test, and chi-squared test.  
13 Analysis of the dental sealant revealed that bisphenol A concentrations were below the detection limit of  
14 5 ppb. At 1 hour following treatment, bisphenol A was detected in samples from 3 of the 18 volunteers in  
15 the low-dose group and 13 of 22 samples from volunteers in the high-dose group. At 3 hours post-  
16 treatment, bisphenol A was detected in samples from 1 of 18 volunteers in the low-dose group and 7 of 22  
17 volunteers in the high-dose group. Concentrations of bisphenol A in saliva at 1 and 3 hours following  
18 exposure were reported at 5.8–105.6 ppb [ $\mu\text{g/L}$ ]. No bisphenol A was detected in saliva samples at 24  
19 hours or in serum samples at any time point. Differences between the low-dose and high-dose groups in  
20 bisphenol A saliva concentrations and in the proportion of bisphenol A-positive saliva samples at 1 and 3  
21 hours achieved statistical significance. In the high-dose group, a significant difference in “readings” was  
22 observed between 1 and 3 hours. **[The data as presented did not illustrate possible quantitative  
23 differences in saliva bisphenol A concentrations from the 2 dose groups or at different sampling  
24 times.]**

25  
26 Joskow et al. (79) examined bisphenol A in urine and saliva of 14 adults (19–42 years old) treated with  
27 dental sealants. Excluded from the study were individuals with resin-based materials on their teeth,  
28 smokers, users of antihistamines, and patients with Gilbert syndrome. The volunteers received either  
29 Helioclear F (n = 5) or Delton LC (n = 9) sealant. Sealant was weighed before and after application to  
30 determine the amount applied, and the number of treated teeth was recorded. The mean number of teeth  
31 treated was 6/person and the mean total weight of sealant applied was 40.35 mg/person. In a comparison  
32 of the 2 sealants, no differences were reported for number of teeth treated or amount of sealant applied.  
33 Saliva samples were collected prior to treatment, immediately after, and at 1 hour following sealant  
34 application. Urine samples were collected prior to treatment and at 1 and 24 hours following sealant  
35 placement. A total of 14–15 saliva samples and 12–14 urine samples were collected at each time point.  
36 Samples were treated with  $\beta$ -glucuronidase and analyzed for bisphenol A concentrations using selective  
37 and sensitive isotope-dilution-MS-based methods. Table 21 summarizes changes in saliva and bisphenol  
38 A concentrations. Immediately and at 1 hour after sealant application, salivary concentrations of  
39 bisphenol A compared to baseline were significantly higher in the patients who received the Delton LC  
40 sealant. Bisphenol A concentrations in saliva increased more than 84-fold following application of the  
41 Delton LC sealant. Urinary concentrations of bisphenol A were increased 1 hour following application of  
42 the Delton LC sealant. Concentrations of bisphenol A in saliva and urine following application of  
43 Helioclear F were reported to be similar to baseline.

1 **Table 21. Saliva and Urinary Concentrations of Total Bisphenol A in Adults Receiving Dental**  
 2 **Sealants**

Collection time	Mean ± SD Bisphenol A concentration (ng/mL) <sup>a</sup>		
	Both sealants	Delton LC	Helioseal F
<b>Saliva</b>			
Pretreatment	0.30 ± 0.17	0.34 ± 0.19	<b>0.22 ± 0.03</b>
Immediately after treatment	26.5 ± 30.7	42.8 ± 28.9	<b>0.54 ± 0.45</b>
1 hour post-treatment	5.12 ± 10.7	7.86 ± 12.73	<b>0.21 ± 0.03</b>
<b>Urine (creatinine-adjusted)</b>			
Pretreatment	2.41 ± 1.24	2.6 ± 1.4	<b>2.12 ± 0.93</b>
1 hour post-treatment	20.1 ± 33.1	27.3 ± 39.1	<b>7.26 ± 13.5</b>
24 hours post-treatment	5.14 ± 3.96	7.34 ± 3.81	<b>2.06 ± 1.04</b>

<sup>a</sup>Samples were treated with β-glucuronidase.  
 From Joskow et al. (79).

3  
 4 The European Union (2) reviewed unpublished preliminary data from a human dermal absorption study.  
 5 Skin samples obtained from 3 human donors (6 samples/donor/dose) were exposed to 5 or 50 mg/cm<sup>2</sup>  
 6 (3.18 or 31.8 mg/mL) <sup>14</sup>C-bisphenol A in ethanol vehicle. Following evaporation of the vehicle, bisphenol  
 7 A was resuspended in artificial sweat. Radioactivity was measured in receptor fluid at various time  
 8 intervals over a 24-hour period. Radioactivity was measured in the stratum corneum and “lower” skin  
 9 layer at 24 hours. Authors of the European Union report noted that tritiated water was not used as a  
 10 marker for skin integrity. However, based on the patterns of results, they concluded that skin integrity was  
 11 likely lost after 4–8 hours. The European Union authors therefore concluded that the only reliable data  
 12 from the study were those for the cumulative percentage of the dose in receptor fluid at 8 hours, which  
 13 was reported at 0.57–1.22% at 5 mg/cm<sup>2</sup> and 0.491–0.835% at 50 mg/cm<sup>2</sup>. Because radioactivity in skin  
 14 was not measured at 8 hours, the percentage of the applied dose remaining on skin and available for  
 15 future absorption could not be determined. Based on ratios of receptor fluid concentrations and lower skin  
 16 levels (1:2 to 1:8) at 24 hours, and assuming that the higher ratio applies to skin at 8 hours, the authors of  
 17 the European Union report predicted that 10% of the dose would be present in “lower” skin layers.  
 18 Therefore, dermal absorption of bisphenol A was estimated at 10%.

#### 19 20 2.1.1.2 Distribution

21 In humans, bisphenol A was measured in cord blood and amniotic fluid, demonstrating distribution to the  
 22 embryo or fetus. Studies reporting bisphenol A concentrations in fetal and/or maternal compartments are  
 23 summarized in [Table 9](#). Detailed descriptions of those studies are also presented below.

24  
 25 Engel et al. (103) reported concentrations of bisphenol A in human amniotic fluid. Twenty-one samples  
 26 were obtained during amniocentesis conducted before 20 weeks gestation in women who were referred to  
 27 a US medical center for advanced maternal age. Bisphenol A concentrations in amniotic fluid were  
 28 measured using LC with electrochemical detection. Bisphenol A was detected in 10% of samples at  
 29 concentrations exceeding the LOD (0.5 µg/L). Bisphenol A concentration ranges of 0.5–1.96 µg/L were  
 30 reported.

31  
 32 Schönfelder et al. (104) examined bisphenol A concentrations in maternal and fetal blood and compared  
 33 bisphenol A concentrations in blood of male and female fetuses. In a study conducted at a German  
 34 medical center, blood samples were obtained from 37 Caucasian women between 32 and 41 weeks  
 35 gestation. At parturition, blood was collected from the umbilical vein after expulsion of the placenta.  
 36 Bisphenol A concentrations in plasma were measured by GC/MS. Control experiments were conducted to  
 37 verify that bisphenol A did not leach from collection, storage, or testing equipment. Bisphenol A was  
 38 detected in all samples tested, and concentrations measured in maternal and fetal blood are summarized in  
 39 [Table 9](#). Mean bisphenol A concentrations were higher in maternal (4.4 ± 3.9 [SD] µg/L) than fetal blood

## 2.0 General Toxicology and Biological Effects

1 (2.9 ± 2.5 µg/L). Study authors noted that in 14 cases fetal bisphenol A plasma concentrations exceeded  
2 those detected in maternal plasma. Among those 14 cases, 12 fetuses were male. Analysis by paired *t*-test  
3 revealed significantly higher mean bisphenol A concentrations in the blood of male than female fetuses  
4 (3.5 ± 2.7 versus 1.7 ± 1.5 ng/mL, *P* = 0.016). Bisphenol A concentrations were measured in placenta  
5 samples at 1.0–104.9 µg/kg.

6  
7 Ikezuki et al. (91) measured concentrations of bisphenol A in serum from 30 healthy premenopausal  
8 women, 37 women in early pregnancy, 37 women in late pregnancy, and 32 umbilical cord blood  
9 samples. Concentrations of bisphenol A were also measured in 32 samples of amniotic fluid obtained  
10 during weeks 15–18 of gestation, 38 samples of amniotic fluid obtained at full-term cesarean section, and  
11 36 samples of ovarian follicular fluid collected during in vitro fertilization procedures. **[It was not stated  
12 if different sample types were obtained from the same subjects.]** An ELISA method was used to  
13 measure bisphenol A concentrations and results were verified by HPLC. The mean ± SD concentration of  
14 bisphenol A in follicular fluid was reported at 2.4 ± 0.8 µg/L. As summarized in Table 7 for nonpregnant  
15 women and Table 9 for maternal and fetal samples, concentrations of bisphenol A in follicular fluid were  
16 similar to those detected in the serum of fetuses and pregnant and non-pregnant women and in amniotic  
17 fluid collected in late pregnancy (~1–2 µg/L). Bisphenol A concentrations in amniotic fluid samples  
18 collected in early pregnancy were ~5-fold higher than in other samples, and the difference achieved  
19 statistical significance (*P* < 0.0001). Study authors postulated that the higher concentrations of bisphenol  
20 A in amniotic fluid collected during gestation weeks 15–18 may have resulted from immature fetal liver  
21 function. They noted that according to unpublished data from their laboratory, the percentage of  
22 glucuronidated bisphenol A in mid-term amniotic fluid was ~34%, which is much lower than reported  
23 values for other human fluids (>90%).

24  
25 Yamada et al. (92) measured bisphenol A concentrations in maternal serum and amniotic fluid from  
26 Japanese women. Samples were collected between 1989 and 1998 in women undergoing amniocentesis  
27 around gestation week 16. One group of samples was obtained from 200 women carrying fetuses with  
28 normal karyotypes, and a second group of samples was obtained from 48 women carrying fetuses with  
29 abnormal karyotypes. An ELISA method was used to measure bisphenol A concentrations. **[As discussed  
30 in Section 1.1.5, ELISA may over-estimate bisphenol A.]** Concentrations of bisphenol A measured in  
31 maternal plasma and amniotic fluid are summarized in Table 9. Median concentrations of bisphenol A in  
32 maternal serum (~2–3 µg/L) were significantly higher [**~10-fold**] than concentrations in amniotic fluid  
33 (~0–0.26 µg/L) in the groups carrying fetuses with normal and abnormal karyotypes. However, in 8  
34 samples from women carrying fetuses with normal karyotypes, high concentrations (2.80–5.62 µg/L) of  
35 bisphenol A were measured in amniotic fluid. The study authors interpreted the data as indicating that  
36 bisphenol A does not accumulate in amniotic fluid in most cases but accumulation is possible in some  
37 individuals. Bisphenol A concentrations in maternal blood were significantly higher [**by ~33%**] in  
38 woman carrying fetuses with abnormal versus normal karyotypes. However, the study authors noted that  
39 the effect may not be related to bisphenol A exposure because there was no adjustment for maternal age,  
40 and concentrations in amniotic fluid did not differ between groups. In the group carrying fetuses with  
41 normal karyotypes, data obtained from 1989 to 1998 were summarized by year. Median bisphenol A  
42 concentrations in serum significantly decreased over that time from a concentration of 5.62 µg/L detected  
43 in 1989 to 0.99 µg/L in 1998.

44  
45 Kuroda et al. (11) used an HPLC method to measure bisphenol A concentrations in 9 sets of maternal and  
46 cord blood samples obtained from Japanese patients at the time of delivery. Bisphenol A concentrations  
47 were also measured in 21 sets of serum and ascitic fluid samples collected from sterile Japanese patients  
48 of unspecified sexes and ages. Results for pregnant women are summarized in Table 9. Mean ± SD  
49 concentrations of bisphenol A were lower in maternal (0.46 ± 0.20 ppb [**µg/L**]) than cord blood  
50 (0.62±0.13 ppb [**µg/L**]). There was a weak positive correlation (*r* = 0.626) between bisphenol A  
51 concentrations in maternal and cord blood. Concentrations of bisphenol A in the blood of sterile patients  
52 are summarized Table 7. There were no differences between pregnant and non-pregnant blood levels (11).

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1 Mean  $\pm$  SD concentrations of bisphenol A were higher in ascitic fluid ( $0.56 \pm 0.19$  ppb [ $\mu\text{g/L}$ ]) than in  
2 serum ( $0.46 \pm 0.20$  ppb [ $\mu\text{g/L}$ ]). The correlation between bisphenol A concentration in serum and ascitic  
3 fluid was relatively strong ( $r = 0.785$ ).

4  
5 Tan and Mohd (14) used a GC/MS method to measure bisphenol A concentrations in cord blood at  
6 delivery in 180 patients at a Malaysian medical center. Bisphenol A was detected in 88% of samples. As  
7 noted in Table 9 concentrations ranged from  $<0.10$  to  $4.05$   $\mu\text{g/L}$ .

8  
9 Calafat et al. (36) reported a median bisphenol A concentration of  $\sim 1.4$   $\mu\text{g/L}$  **[as estimated from a**  
10 **graph]** in milk from 32 women. Bisphenol A was measured after enzymatic hydrolysis of conjugates. Ye  
11 et al. (37) found measurable milk concentrations of bisphenol A in samples from 18 of 20 lactating  
12 women. Free bisphenol A was found in samples from 12 women. The median total bisphenol  
13 concentration in milk was  $1.1$   $\mu\text{g/L}$  (range: undetectable to  $7.3$   $\mu\text{g/L}$ ). The median free bisphenol A  
14 concentration was  $0.4$   $\mu\text{g/L}$  (range: undetectable to  $6.3$   $\mu\text{g/L}$ ).

15  
16 Sun et al. (12) used an HPLC method to measure bisphenol A concentrations in milk from 23 healthy  
17 lactating Japanese women. Bisphenol A concentrations ranged from  $0.28$  to  $0.97$   $\mu\text{g/L}$ , and the mean  $\pm$   
18 SD concentration was reported at  $0.61 \pm 0.20$   $\mu\text{g/L}$ . No correlations were observed between bisphenol A  
19 and triglyceride concentrations in milk. Values from 6 milk samples were compared to maternal and  
20 umbilical blood samples previously reported in a study by Kuroda et al. (11). Bisphenol A values were  
21 higher in milk, and the milk/serum ratio was reported at 1.3. Bisphenol A values in milk were comparable  
22 to those in umbilical cord serum. **[It was not clear whether milk and serum samples were obtained**  
23 **from the same volunteers in the two studies.]**

24  
25 Schaefer et al. (105) measured concentrations of bisphenol A and other compounds in uterine  
26 endometrium of women undergoing hysterectomy for uterine myoma at a German medical center.  
27 Endometrial and fat samples were obtained between 1995 and 1998 from 23 women (34–51 years old)  
28 with no occupational exposure. Samples were handled with plastic-free materials and stored in glass  
29 containers. Concentrations of environmental chemicals were measured in samples by GC/MS. None of 21  
30 fat samples had detectable concentrations of bisphenol A. Bisphenol A was detected in 1 of 23  
31 endometrial samples; the median concentration was reported at  $<1$   $\mu\text{g/kg}$  wet weight, and the range was  
32 reported at  $0$ – $13$   $\mu\text{g/kg}$ . **[It is not known why a median value and range were reported when**  
33 **bisphenol A was only detected in 1 sample.]**

34  
35 As part of a study to compare an ELISA and an LC/MS method for biological monitoring of bisphenol A,  
36 Inoue et al. (8) measured concentrations of bisphenol A in semen samples obtained from 41 healthy  
37 Japanese volunteers (18–38 years old). Analysis by the ELISA method indicated bisphenol A  
38 concentrations ranging from concentrations below the detection limit ( $2.0$   $\mu\text{g/L}$ ) to  $12.0$   $\mu\text{g/L}$ . The  
39 LC/MS method indicated that the bisphenol A concentration in all samples was  $<0.5$   $\mu\text{g/L}$ , the LOQ. The  
40 study authors concluded that the LC/MS method was more accurate and sensitive and that the ELISA  
41 method overestimated bisphenol A concentrations, possibly due in part to nonspecific antibody  
42 interactions.

### 43 2.1.1.3 Metabolism

44  
45 Völkel et al. (7) measured bisphenol A and metabolite concentrations in human urine following exposure  
46 to a low bisphenol A dose. The human volunteers consisted of 3 healthy females (25–32 years old) and 3  
47 healthy males (37–49 years old) who were asked to refrain from alcohol and medicine intake for 2 days  
48 prior to and during the study. Volunteers received  $25$   $\mu\text{g}$   $\text{D}_{16}$ -bisphenol A in drinking water **[0.00028–**  
49 **0.00063 mg/kg bw based on reported body weights]**, a dose reported to represent a worst-case human  
50 exposure. Urine samples were collected at 0, 1, 3, 5, and 7 hours following exposure. Analyses for  $\text{D}_{16}$ -  
51 bisphenol A and  $\text{D}_{16}$ -bisphenol A-glucuronide were conducted by LC/MS and HPLC. Recovery of  $\text{D}_{16}$ -  
52 bisphenol A-glucuronide in urine within 5 hours of dosing was 85% of dose in males and 75% of dose in

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1 females. Analysis following treatment of urine with glucuronidase resulted in recovery of 97% of the dose  
2 in males and 84% of the dose in females. The highest concentrations of bisphenol A glucuronide in urine  
3 were measured at 1 hour (221–611 pmol [**50–139 ng bisphenol A eq**]/mg creatinine) and 3 hours (117–  
4 345 pmol [**27–79 ng bisphenol A eq**]/mg creatinine) following exposure. Elimination half-life was  
5 estimated at 4 hours. Bisphenol A concentrations exceeding the detection limit were detected in only 2  
6 urine samples at concentrations of ~10 pmol [**2 ng**]/mg creatinine.

7  
8 Völkel et al. (109) examined toxicokinetics and metabolism of bisphenol A in humans administered a low  
9 dose. Volunteers in this study consisted of 3 healthy females (24–31 years of age) and 6 healthy males  
10 (28–54 years of age) who were non- or occasional smokers; volunteers were asked to refrain from alcohol  
11 and medicine intake for 2 days before and during the study. In two different studies, D<sub>16</sub>-bisphenol A was  
12 orally administered to volunteers via gelatin capsules at a dose of 5 mg (0.054–0.090 mg/kg bw). The  
13 dose was reported to be ~10-fold higher than the estimated human exposure level of 0.6 mg/day. In the  
14 first study, urine samples were collected at 6-hour intervals until 42 hours following exposure and blood  
15 samples were collected at 4-hour intervals until 32 hours following exposure in 3 males and 3 females. In  
16 a second, more detailed study conducted in 4 of the male volunteers, blood samples were collected at 30–  
17 60-minute intervals until 381 minutes following exposure. Samples were analyzed by GC/MS and  
18 LC/MS. In the first study, a terminal half-life of 5.3 hours was reported for D<sub>16</sub>-bisphenol A glucuronide  
19 clearance from blood. The half-life for urinary elimination was reported at 5.4 hours. D<sub>16</sub>-Bisphenol A  
20 glucuronide concentrations in plasma and urine fell below LOD at 24–34 hours post dosing. Complete  
21 urinary recovery (100%) was reported for the D<sub>16</sub>-bisphenol A glucuronide. In the second study,  
22 maximum plasma concentration of D<sub>16</sub>-bisphenol A glucuronide (~800 pmol [**183 ng bisphenol A**  
23 **eq**]/mL) was obtained 80 minutes after oral administration. The half-life for initial decline in plasma was  
24 reported at 89 minutes. Free D<sub>16</sub>-bisphenol A was not detected in plasma. According to study authors, the  
25 study demonstrated rapid absorption of bisphenol A from the gastrointestinal tract, conjugation with  
26 glucuronic acid in the liver, and rapid elimination of the glucuronide in urine. Study authors noted that the  
27 rapid and complete excretion of bisphenol A glucuronide in urine suggested that in contrast to rats,  
28 enterohepatic circulation did not occur in humans.

29  
30 **Table 8** in Section 1 provides information on bisphenol A and metabolites detected in human urine. A  
31 study conducted in the US used an HPLC method to examine 30 urine samples collected from a  
32 demographically diverse adult population in 2000–2004 (96). Mean urinary compound composition was  
33 9.5% bisphenol A, 69.5% bisphenol A glucuronide, and 21% bisphenol A sulfate conjugate. A study  
34 conducted in Korea used an HPLC method to examine urine collected from 15 men (mean age 42.6 years)  
35 and 15 women (mean age 43.0 years) (98). Sex-related differences were observed for urinary metabolic  
36 profiles. Mean urinary compound composition in men was reported at 29.1% bisphenol A, 66.2%  
37 bisphenol A glucuronide, and 4.78% bisphenol sulfate conjugate. The urinary metabolite profile in  
38 females was 33.4% bisphenol A, 33.1% bisphenol A glucuronide, and 33.5% bisphenol A sulfate  
39 conjugate. The study authors concluded that women had a greater ability for sulfation than men.

### 40 41 2.1.1.4 Excretion

42 As discussed in greater detail in Section 2.1.1.3, two studies in which human volunteers were  
43 administered low doses of D<sub>16</sub>-bisphenol A (~0.00028–0.090 mg/kg bw) demonstrated that most of the  
44 dose (85–100%) was eliminated through urine (7) (109). In those studies, the half-lives for urinary  
45 elimination were reported at 4–5.4 hours. As discussed in more detail in Section 2.1.1.3, examination of  
46 human urine samples revealed that bisphenol A glucuronide and sulfate conjugates are present at higher  
47 concentrations than is the parent compound (96, 98).

### 48 49 2.1.2 Experimental animal

50 Original animal studies that were potentially important for the interpretation of developmental and  
51 reproductive toxicity were reviewed thoroughly. Examples included:

## 2.0 General Toxicology and Biological Effects

- Studies examining toxicokinetics or metabolism in pregnant or lactating animals
- Studies examining toxicokinetic difference observed with different doses or exposure routes
- Studies looking at age-related differences in toxicokinetics or metabolism
- Studies in non-rodent species such as primates

Secondary sources were utilized for general information not considered critical to the interpretation of developmental and reproductive toxicity data.

### 2.1.2.1 Absorption

In rats orally exposed to bisphenol A at doses  $\leq 100$  mg/kg bw, maximum bisphenol A concentrations ( $C_{\max}$ ) were generally measured in plasma within 0.083–0.75 hours following exposure (118-122). At doses of 1 or 10 mg/kg bw, time to maximum bisphenol A concentration ( $T_{\max}$ ) in plasma was longer in postnatal day (PND) 21 rats (1.5–3 hours) than in PND 4 and 7 rats (0.25–0.75 hours) (118). In a limited number of studies in which rats were subcutaneously (sc) dosed with up to 100 mg/kg bw bisphenol A, time (0.5–4 hours) to reach  $C_{\max}$  was longer than with oral dosing, although the findings were not always consistent (119, 120). In one study,  $T_{\max}$  was comparable in oral and intraperitoneal (ip) dosing of rats (119). Another study reported that  $C_{\max}$  was attained at 0.7 hours in monkeys orally exposed to 10 or 100 mg/kg bw bisphenol A and at 0.5 hours in chimpanzees orally exposed to 10 mg/kg bw bisphenol A (120). In the same study, a longer  $T_{\max}$  (2 hours) was observed following exposure of monkeys and chimpanzees to the same doses by sc injection compared to oral intake. Additional details for these studies are presented below.

As discussed in greater detail in Section 2.1.2.3, bisphenol A is glucuronidated in the liver and intestine, and most of the dose is absorbed as bisphenol A glucuronide following oral exposure of rats (118). In ovariectomized rats gavaged with bisphenol A, bioavailability of bisphenol A was reported at 16.4% at a 10 mg/kg bw dose and 5.6% at a 100 mg/kg bw dose (123). The findings are fairly consistent with a second study in which maximum plasma values of free bisphenol A represented low percentages [ $<2$ –**8%**] of the total radioactive dose in rats orally administered bisphenol A at 10 or 100 mg/kg bw (119); maximum values of free bisphenol A represented higher percentages of the radioactive dose in rats given 10 or 100 mg/kg bw sc [**64–82% free bisphenol A**] or ip [**19–54%**] (119). Percentages of parent bisphenol A in blood were also higher in monkeys exposed intravenously (iv; 5–29%) than orally (0–1%) (124). Similarly, HPLC analysis of plasma conducted 1 hour following sc or gavage dosing of 4 female 21-day-old Sprague Dawley rats/group with bisphenol A revealed higher bisphenol A plasma concentrations with sc than with gavage dosing (Table 22) (125). One study in male and female rats gavaged with 10 mg/kg bw bisphenol A demonstrated higher plasma concentrations of bisphenol A in immature animals than in adults (10.2–48.3  $\mu\text{g/g}$  [**mg/L**] plasma at 4 days of age; 1.1–1.4  $\mu\text{g/g}$  [**mg/L**] plasma at 7 days of age; 0.2  $\mu\text{g/g}$  [**mg/L**] plasma at 21 days of age; and 0.024–0.063  $\mu\text{g/g}$  [**mg/L**] plasma in adulthood) (118).

**Table 22. Plasma Bisphenol A Concentrations in 21-day-old Rats at 1 Hour Following Oral Gavage or SC Dosing**

Dose, mg/kg bw	Plasma concentration, $\mu\text{g/L}$	
	SC injection	Oral gavage
0 (sesame oil vehicle)	Not detected	Not detected
8	94.6 $\pm$ 58.0	Not examined
40	886.3 $\pm$ 56.4	Not detected
160	2948 $\pm$ 768.8	198.8 $\pm$ 88.2
800	Not examined	2879.0 $\pm$ 2328.3

Values presented as mean  $\pm$  SD.  
From Yamasaki et al. (125).

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1 A review by the European Union (2) noted that in the study by Pottenger et al. (119), fecal excretion  
 2 represented the highest proportion of the eliminated dose (74–83% in males and 52–72% in females)  
 3 following oral or parenteral exposure of rats to 10 or 100 mg/kg bw bisphenol A. The authors of the  
 4 European Union report therefore concluded that absorption [assumed to be of the radioactive dose] is  
 5 likely extensive following oral intake. Adding to the proof of extensive oral absorption is the observation  
 6 that more than 50% of fecal elimination occurred at 24 hours post dosing, a time period beyond the  
 7 average gastrointestinal transit time of 12–18 hours for rats. Possible explanations provided for the  
 8 detection of parent compound in feces were cleavage of conjugates within intestines and enterohepatic  
 9 circulation.

### 11 2.1.2.2 Distribution

#### 13 2.1.2.2.1 Pregnant or lactating animals

14 Information on distribution in pregnant or lactating rats is presented first followed by other species.  
 15 Studies including oral exposures are summarized before those with parenteral exposures.

17 Takahashi and Oishi (121) examined disposition and placental transfer of bisphenol A in F344 rats. Rats  
 18 were orally administered 1000 mg/kg bw bisphenol A (>95% purity) in propylene glycol on gestation day  
 19 (GD) 18 (GD 0 = day of vaginal plug). Rats were killed at various time points between 10 minutes and 48  
 20 hours after bisphenol A dosing. At each time point, 2–6 dams and 8–12 fetuses obtained from 2–3 dams  
 21 were analyzed. Blood was collected from dams and kidneys, livers, and fetuses were removed for  
 22 measurement of bisphenol A concentrations by HPLC. Results are summarized in Table 23

24 . Study authors noted the rapid appearance of bisphenol A in maternal blood and organs and in fetuses.  
 25 Concentrations of bisphenol A at 6 hours following dosing were 2% of peak concentrations in maternal  
 26 blood and 5% of peak concentrations in fetuses. It was noted that in fetuses, area under the time-  
 27 concentration curve (AUC) was higher and mean retention time, variance of retention time, and terminal  
 28 half-life were longer than in maternal blood.

30 **Table 23. Toxicokinetic Endpoints for Bisphenol A in Rats Dosed with 1000 mg/kg bw Bisphenol A**  
 31 **on GD 18**

Endpoint	Maternal tissue			Fetus
	Blood	Liver	Kidney	
C <sub>max</sub> , mg/L	14.7	171	36.2	9.22
T <sub>max</sub> , minutes	20	20	20	20
AUC, mg·hour/L	13.1	700	84.0	22.6
Mean retention time, hours	10.6	29.3	12.0	20.0
Variance in retention time, hours squared	203	657	227	419
Half-life, hours				
From 20 to 40 minutes	0.0952	0.178	0.245	0.55
From 40 minutes to 6 hours	2.58	1.75	2.98	1.60
From 6 to 48 hours	4.65	No data	No data	173

32 From Takahashi and Oishi (121)

33 Dormoradzki et al. (126) examined metabolism, toxicokinetics, and embryo-fetal distribution of bisphenol  
 34 A in rats during 3 different gestation stages. Sprague Dawley rats were gavaged with bisphenol A (99.7%  
 35 purity)/radiolabeled <sup>14</sup>C-bisphenol A (98.8% radiochemical purity) at 10 mg/kg bw. Bisphenol A was  
 36 administered to 1 group of non-pregnant rats and 3 different groups of pregnant rats on GD 6 (early  
 37 gestation), 14 (mid gestation), or 17 (late gestation). GD 0 was defined as the day that sperm or a vaginal  
 38 plug were detected. Blood, urine, and feces were collected at multiple time points between 0.25 and 96  
 39 hours post dosing. It appears that most and possibly all samples were pooled. Four rats in each group

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1 were killed at 96 hours post dosing. Maternal organs, 6 embryos or fetuses/dam (when possible), and  
2 placentas were collected. Samples were analyzed for radioactivity and bisphenol A and/or bisphenol A  
3 glucuronide by HPLC/liquid scintillation spectrometry.

4  
5 In all groups, 90–94% of radioactivity was recovered. Elimination of bisphenol A and its metabolites is  
6 discussed in Section 2.1.2.4. At 96 hours following dosing, low percentages of the dose were present in  
7 carcass (~1–6%) and tissues such as brain, fat, liver, kidney, ovary, uterus, and skin. The only  
8 quantifiable data in placentas and fetuses at 96 hours were obtained in the GD 17 group, and those  
9 samples contained 0.01–0.07% of the bisphenol A dose. Standard deviations for maternal and fetal tissues  
10 generally exceeded 50% of the mean. Study authors concluded that disposition of radioactivity was  
11 similar in pregnant and non-pregnant rats.

12  
13 Toxicokinetic data obtained from plasma profiles are summarized in [Table 24](#). The authors stated that  
14 there was high inter-animal variability. The presence of 2  $C_{max}$  values was noted by the authors, and they  
15 stated that it was the result of enterohepatic circulation of radioactivity. Bisphenol A was not quantifiable  
16 in most plasma samples. Because bisphenol A glucuronide represented most (~95–99%) of the  
17 radioactivity, plasma profiles for that metabolite were nearly identical to profiles for radioactivity.

18  
19 **Table 24. Toxicokinetic Data for Radioactivity in Pregnant and Non-pregnant Rats Gavaged with 10**  
20 **mg/kg bw  $^{14}\text{C}$ -bisphenol A**

Endpoint	Non-pregnant	GD 6–10	GD 14–18	GD 17–21
$C_{max1}$ , mg eq/L	0.716	0.370	0.482	1.006
$T_{max1}$ , hours	0.25	0.25	0.25	0.25
$C_{max2}$ , mg eq/L	0.171	0.336	0.211	0.278
$T_{max2}$ , hours	18	12	24	12
Time to non-quantifiable level, hours	72	Not determined	72	96
AUC				
$^{14}\text{C}$ , mg-eq·hour/L	6.1	12.4	7.1	10.2
Bisphenol A glucuronide, mg-eq·hour/L	5.8	12.3	6.8	9.7
Percent as bisphenol A glucuronide	95.1	99.2	95.8	95.1

From Dormoradzki et al. (126)

21  
22 A second study was conducted by Dormoradzki et al. (126) to measure bisphenol A and bisphenol A  
23 glucuronide concentrations in maternal and fetal tissues. Rats were gavaged with radiolabeled bisphenol  
24 A at 10 mg/kg bw on GD 11, 13, or 16. Blood was collected over a 24-hour period. Five rats/group/time  
25 period were killed at 0.25, 12, and 96 hours post dosing. Maternal blood and organs, yolk sacs/placentas,  
26 and embryos/fetuses were removed for measurement of bisphenol A and bisphenol A glucuronide. Yolk  
27 sacs/placentas and fetuses were pooled at most time periods. Results are summarized in [Table 25](#).

28  
29 At 0.25 hours following dosing, bisphenol A glucuronide concentrations in maternal plasma were similar  
30 in groups dosed on GD 11 and 13 but concentrations were 1.7–2 times higher in the group dosed on GD  
31 16. At 12 hours post dosing in all exposure groups, bisphenol A glucuronide concentrations in maternal  
32 plasma were reduced 7- to 11-fold from values observed at 0.25 hours. Levels of radioactivity in plasma  
33 were not sufficient for analysis at 96 hours post dosing. Bisphenol A was detected in maternal plasma at  
34 0.25 hours post dosing in rats that were exposed to a higher radioactive concentration (0.5 mCi compared  
35 to 0.2 mCi) on GD 16; bisphenol A concentrations were 26.5-fold lower than bisphenol A glucuronide  
36 concentrations.

37  
38 In animals dosed on GD 11, bisphenol A glucuronide was only detected in yolk sac/placenta at 0.25 hours  
39 post dosing and the concentration was ~17 times lower than the concentration detected in maternal blood  
40 for the same time period. With dosing on GD 11, bisphenol A glucuronide was not detected in embryos

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1 and bisphenol A was not detected in yolk sac/placenta or embryos. In animals dosed on GD 13, bisphenol  
 2 A glucuronide was detected in yolk sac/placenta at 0.25 and 12 hours post dosing and concentrations were  
 3 9–24-fold lower than those detected in maternal plasma for the same time period. Bisphenol A was also  
 4 detected in yolk sac/placenta at 0.25 and 12 hours after dosing on GD 13 and concentrations were similar  
 5 to those detected in the blood of 2 dams. A lower concentration of bisphenol A was detected in embryos  
 6 of dams at 0.25 hours following dosing on GD 13, and bisphenol A was the only moiety detected in  
 7 embryos. Following dosing on GD 16, bisphenol A glucuronide and bisphenol A were detected in yolk  
 8 sac/placenta at 0.25 and 12 hours post dosing. Concentrations of bisphenol A glucuronide in yolk  
 9 sac/placenta were 7- to 8-fold lower than concentrations detected in maternal plasma. From 0.25 to 12  
 10 hours, concentrations of bisphenol A decreased 4.9–fold and concentrations of bisphenol A glucuronide  
 11 decreased 9-fold. Mean concentrations of bisphenol A in yolk/sac placenta following exposure on GD 16  
 12 were similar to the blood concentration detected in 1 of 2 dams.

13  
 14 In yolk sac/placenta and fetuses of dams dosed with a higher level of radioactivity (0.5 mCi) on GD 16,  
 15 bisphenol A glucuronide and bisphenol A were detected at 0.25 hours following dosing. Compared to  
 16 concentrations detected in placenta, fetal concentrations of bisphenol A glucuronide were ~26-fold lower  
 17 and bisphenol A concentrations were 5-fold lower. Bisphenol A concentrations were lower than bisphenol  
 18 A glucuronide concentrations by 3.6-fold in yolk sac/placenta and by 0.7-fold in fetuses. Study authors  
 19 concluded that there is no selective affinity for bisphenol A or bisphenol A glucuronide by the yolk  
 20 sac/placenta or embryo/fetus.

21  
 22 **Table 25. Maternal and Fetal Concentrations of Bisphenol A Following Gavage Dosing of Dams with**  
 23 **10 mg/kg bw Bisphenol A**

Exposure	Bisphenol A concentration, mg/L or mg/kg					
	Maternal plasma		Yolk sac/placenta		Embryo/fetus	
	Glucuronide	Parent	Glucuronide	Parent	Glucuronide	Parent
GD 11, 0.2 mCi						
0.25 hours	1.060 ± 0.258	0.041	0.062	<LOD <sup>a</sup>	<LOD	<LOD
12 hours	0.099 ± 0.036	<LOD	<LOD	<LOD	<LOD	<LOD
96 hours	NA	NA	<LOD	<LOD	<LOD	<LOD
GD 13, 0.2 mCi						
0.25 hours	0.868 ± 0.189	0.078	0.036	0.019	<LOD	0.013
12 hours	0.117 ± 0.033	0.008	0.013	0.009	<LOD	<LOD
96	Not analyzed due to insufficient radioactivity					
GD 16, 0.2 mCi						
0.25 hours	1.768 ± 0.783	0.485, 0.129 <sup>b</sup>	0.223 ± 0.104	0.166 ± 0.069	0.031, 0.009 <sup>b</sup>	0.122, 0.020 <sup>b</sup>
12 hours	0.174 ± 0.045	<LOD	0.025 ± 0.005	0.034 ± 0.002	NA	NA
96 hours	Not analyzed due to insufficient radioactivity				0.016	0.008
GD 16, 0.5 mCi						
0.25 hours	1.699 ± 0.501	0.064 ± 0.025	0.342 ± 0.104	0.095 ± 0.031	0.013 ± 0.008	0.018 ± 0.011

Data expressed as mean ± SD or single values for individual or pooled data.

<sup>a</sup>Limit of detection (LOD) for bisphenol A reported at 0.005–0.029.

<sup>b</sup>Detected only in 2 animals at the concentrations listed.

From Dormoradzki et al. (126).

24  
 25 Kurebayashi et al. (127) examined distribution of radioactivity in pregnant and lactating rats dosed with  
 26 <sup>14</sup>C-bisphenol A. Pregnant rats were orally dosed with 0.5 mg/kg bw <sup>14</sup>C-bisphenol A on GD 12, 15, or  
 27 18. The rats were killed at 30 minutes or 24 hours following dosing (n = 1/time period) and examined by  
 28 whole-body radioluminography. Study authors noted that the distribution of label was nearly identical in  
 29 dams at each gestation time point. At 30 minutes following dosing, the concentration of radioactivity in  
 30 dam blood was ~31–43 µg bisphenol A eq/L. The highest concentration of radioactivity was detected in

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maternal liver (~219–317 µg bisphenol A eq/kg) and kidney (~138–270 µg bisphenol A eq/kg); concentrations in other tissues (lung, ovary, placenta, skin, and uterus) were ~10-fold lower. Fetuses, fetal membranes, and yolk sacs did not contain quantifiable levels of radioactivity at 30 minutes following maternal exposure at any gestation time point. At 24 hours following exposure of dams, radioactivity concentrations in blood (~4–11 µg bisphenol A eq/L) were nearly 3–10-fold lower than values obtained at 30 minutes following exposure. Levels of radioactivity remained highest in liver. At 24 hours following exposure, radioactivity was only detected in fetuses and fetal tissues from dams dosed on GD 18. Radioactivity levels in fetuses or fetal tissues compared to maternal blood were ~30% in fetuses, nearly equal in fetal membranes, and ~5-fold higher in yolk sacs. Study authors concluded that there was limited distribution of radiolabel to fetuses.

In another study by Kurebayashi et al. (127), a lactating rat was orally dosed with 0.5 mg/kg bw <sup>14</sup>C-bisphenol A on PND 11 and caged with 5 neonatal rats for 24 hours. One male and one female neonatal rat were killed at the end of the 24-hour period and examined by whole-body radioluminography. The 3 remaining neonates were caged for 24 hours with a dam that was not exposed to bisphenol A. One male and one female neonate were then killed and examined by whole-body radioluminography. In pups killed immediately after being nursed by the lactating dam exposed to <sup>14</sup>C-bisphenol A, most of the radioactivity was detected in intestinal contents (~30–46 µg bisphenol A eq/kg) and lower levels were found in gastric contents and urinary bladder (< 10 µg bisphenol A eq/kg). After being nursed for 24 hours by a dam that was not exposed to bisphenol A, radioactivity was only detected in intestinal contents and the level was ~20–40% of that measured in pups examined immediately after being nursed by dams receiving <sup>14</sup>C-bisphenol A.

An additional 3 lactating dams were dosed with 0.5 mg/kg bw <sup>14</sup>C-bisphenol A on PND 11 for examination of radioactivity in plasma and milk over a 48-hour period. Table 26 summarizes toxicokinetic endpoints for radioactivity in milk and plasma. Study authors concluded that there was significant secretion of <sup>14</sup>C-associated radioactivity into milk.

**Table 26. Toxicokinetic Endpoints for Radioactivity in Lactating Rats Orally Administered 0.5 mg/kg bw <sup>14</sup>C-bisphenol A on PND 11**

Endpoint	Milk	Maternal plasma
C <sub>max</sub> , µg-eq/L	4.46	27.2
T <sub>max</sub> , hours	8	4
Elimination half-life, hours	26	31
AUC (0–48 hours), µg-eq·hour/L)	156	689

From Kurebayashi et al. (127)

Miyakoda et al. (128) examined placental transfer of bisphenol A in rats. Wistar rats were administered an oral dose of bisphenol A (99% purity) at 10 mg/kg bw on GD 19. Blood was collected and fetuses were removed at 1, 3, and 24 hours following dosing. Bisphenol A concentrations were measured in plasma and fetuses by GC/MS. **[A statement in Figure 3 of the study indicated that values were the means of 5 or 7 experiments; it is possible the authors meant that 5 or 7 dams were dosed.]** Concentrations of bisphenol A peaked in maternal plasma and fetuses within 1 hour of dosing, with bisphenol A concentrations measured at ~34 ppb [µg/L] in maternal plasma and 11 ppb [µg/kg] in fetuses. At 3 hours after dosing, bisphenol A concentrations were ~10% of peak concentrations in maternal plasma and 40% of peak concentrations in fetuses. At 24 hours post dosing, bisphenol A concentrations in fetuses were detected at 70% of peak value and concentrations in fetuses were more than twice the concentrations in maternal plasma. Study authors concluded that bisphenol A is rapidly transferred to the fetus and tends to remain longer in fetuses than in maternal blood.

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1 Snyder et al. (129) examined the toxicokinetics of bisphenol A in lactating rats. On PND 14, lactating CD  
2 rats were gavaged with 100 mg/kg bw <sup>14</sup>C-bisphenol A. Milk, blood, and organs were collected from 2–4  
3 dams/group at 1, 8, 24, or 26 hours after dosing. **[While the text indicates collection of samples at 26**  
4 **hours, Table 3 of the study indicates collection at 24 hours. The collection time reported in the**  
5 **study table was used when there were discrepancies between text and table.]** Animals were injected  
6 with oxytocin prior to milk collection. Radioactivity in pup carcasses was measured at 2, 4, 6, and 24  
7 hours following exposure of dams; 8–16 pups/time period were examined **[pup data does not appear to**  
8 **analyzed by litter]**. Samples were analyzed by scintillation counting, HPLC, and/or nuclear magnetic  
9 resonance. At 1 and 8 hours following exposure, the highest percentage of the radioactive dose was  
10 detected in intestine with contents (75–83%). Among the other organs examined, the highest percentage  
11 of the radioactive dose was detected in liver (0.38–0.74%) and much lower percentages were detected in  
12 kidney and lung (≤0.02%). Low percentages of the radioactive dose were also detected in milk  
13 (≤0.0020%), blood (~0.006%), plasma (~0.01%), and fat (≤0.004%). Compared to earlier time periods,  
14 radioactivity levels were lower at 24 hours post dosing (26% of the dose detected in intestine and  
15 contents), but distribution was similar. At all 3 sampling time points, radioactivity levels were highest in  
16 plasma > blood > milk. The major radioactivity peak in plasma was represented by bisphenol A  
17 glucuronide at 1, 8, and 26 hours following exposure. Bisphenol A glucuronide also represented the major  
18 radioactive peak detected in milk. Radioactivity levels in pups amounted to <0.01% of the maternal dose.  
19 Radioactivity levels in pups tended to increase over time. From 2 to 24 hours following exposure, mean ±  
20 SD radioactivity levels rose from 44 ± 24 to 78 ± 11 µg bisphenol A eq/pup.

21  
22 Yoshida et al. (130) compared bisphenol A concentrations in rats and their offspring during the lactation  
23 period. The main focus of the study was developmental toxicity, which is discussed in Section 3.2.3.2. In  
24 the distribution study, Donryu rats (12–19/group) were gavaged with bisphenol A at 0  
25 (carboxymethylcellulose solution), 0.006, or 6 mg/kg bw/day from GD 2 to the day before weaning (21  
26 days post-delivery). Bisphenol A concentrations were measured in maternal and pup serum, milk, and pup  
27 liver by GC/MS on PND 10, 14, and/or 21. Milk samples were obtained from pup stomachs. Pup serum  
28 and liver samples were pooled. Two to six dams/litter were examined in each dose group and time period.  
29 Samples of tap water, drinking water from plastic containers, and feed were measured for bisphenol A  
30 content by HPLC. Bisphenol A was not detected in fresh tap water but was detected at ~3 µg/L following  
31 storage of that water in plastic containers. Bisphenol A concentration in feed was ~40 µg/kg. Results for  
32 maternal and fetal tissues are summarized in Table 27. Bisphenol A concentrations in the serum of high  
33 dose-dams were significantly elevated compared to the control group on PND 21. No other significant  
34 differences were observed in bisphenol A concentrations in samples between treated and control groups.

35  
36 Kim et al. (131) used an HPLC method to measure bisphenol A concentrations in rat dams and their  
37 offspring. Dams were gavaged with bisphenol A (>99.7% purity) at doses of 0 (corn oil vehicle), 0.002,  
38 0.020, 0.200, 2, or 20 mg/kg bw/day on GD 7–17. Dams and offspring were killed at 21 days following  
39 parturition, and serum was collected for measurement of bisphenol A. Development effects observed in  
40 this study are summarized in Section 3.2.1.1. Bisphenol A was not detected in the serum of dams at the  
41 two lowest doses. Respective concentrations of bisphenol A in the serum of dams at the 3 highest doses  
42 were 0.900, 0.987, and 1.00 mg/L. In offspring, bisphenol A was not detected in serum at the 3 lowest  
43 doses. At the 2 highest doses, the respective concentrations of bisphenol A in offspring were 0.69 and  
44 0.74 mg/L in males and 0.71 and 0.82 mg/L in females.

45

1 **Table 27. Bisphenol A Concentrations in Maternal and Pup Samples During Lactation in Rats**  
 2 **Gavaged with Bisphenol A**

Sample	Time of analysis	Sex	Dose group, mg/kg bw/day		
			0	0.006	6
Bisphenol A concentration, ppb [ $\mu\text{g/L}$ or $\mu\text{g/kg}$ ]					
<b>Dam<sup>a</sup></b>					
Serum	PND 21		3 $\pm$ 0	4 $\pm$ 0	11 $\pm$ 4
Milk	PND 10		28 $\pm$ 9	8 $\pm$ 21	8 $\pm$ 3
	PND 14		255 $\pm$ 78	205 $\pm$ 7	185 $\pm$ 50
<b>Pup<sup>b</sup></b>					
Serum	PND 10	Female	4	10	23
		Male	15	5	7
	PND 14	Female	5	4	3
		Male	4	5	4
	PND 21	Female	9	3	9
		Male	14	9	20
Liver	PND 10	Female	13	12	17
		Male	9	9	14
<b>Pup<sup>b</sup></b>					
Liver	PND 14	Female	22	100	18
		Male	45	14	16
	PND 21	Female	60	70	37
		Male	69	9	60

<sup>a</sup>Values are presented as mean  $\pm$  SD.

<sup>b</sup>Pup samples were pooled.

From Yoshida et al. (130).

3  
 4 Shin et al. (132) examined elimination of bisphenol A from maternal-fetal compartments of rats. On 1 day  
 5 between GD 17 and 19, four Sprague Dawley rats were iv injected with 2 mg/kg bw bisphenol A.  
 6 Amniotic fluid, placenta, and fetuses were collected at multiple intervals between 5 minutes and 8 hours  
 7 following injection. Bisphenol A concentrations in samples were measured by HPLC. Transfer rate  
 8 constants and clearance rates were determined using a 5-compartment model consisting of maternal  
 9 central, maternal tissue, placental, fetal, and amniotic fluid compartments. Toxicokinetic findings are  
 10 summarized in Moors et al. (133) evaluated the kinetics of bisphenol A in pregnant rats on GD 18 after a  
 11 single intravenous dose of 10 mg/kg bw. Unconjugated bisphenol A represented almost 80% of total  
 12 bisphenol A 5 minutes after injection, 50% of total bisphenol A 20 minutes after injection, and ~10% of  
 13 total bisphenol A 6 hours after the injection. The half life of free bisphenol A in the dam's blood was 0.34  
 14 hours, and the half-life of total bisphenol A was 0.58 hours. Bisphenol A in fetal tissues peaked 20–30  
 15 minutes after maternal injection at 4.0 mg/kg in placenta, 3.4 mg/kg in fetal liver, and 2.4 mg/kg in  
 16 remaining fetal tissues. Peak maternal blood bisphenol A had been 3.8 mg/L shortly after injection.  
 17 Rapid distribution of bisphenol A was observed in placenta, fetus, and amniotic fluid. Bisphenol A  
 18 concentrations in placenta and fetus remained higher than those in maternal serum over most of the  
 19 sampling period. Amniotic fluid contained the lowest concentration of bisphenol A. Decay curves in  
 20 amniotic fluid, fetus, and placenta paralleled decay curves in maternal serum. Transfer rate constants and  
 21 clearance rates are summarized in Table 29. Transfer rate constants were greater in the direction of  
 22 amniotic fluid to fetus or placenta than in the opposite direction. The elimination rate constant and  
 23 clearance rate from the fetal compartment were much lower than for the maternal central compartment.  
 24 The clearance rate from placenta to fetus was higher than clearance rate from fetus to placenta. The  
 25 authors calculated that 65.4% of the bisphenol A dose was delivered to the fetus, 33.2% to the maternal  
 26 central compartment, and 1.4% to amniotic fluid. According to the study authors, the low transfer rate  
 27 from the fetal to amniotic compartment suggested minimal fetal excretion of unchanged bisphenol A

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1 through urine and feces into the amniotic fluid. They also noted that the small fetal compartment transfer  
 2 constant compared to the relative fetal-placental transfer constant indicated minimal metabolism by the  
 3 fetus. Authors estimated that 100% of bisphenol A was eliminated from the fetus via the placental route  
 4 and concluded that fetal elimination represents 0.05% of total elimination from the maternal-fetal unit.  
 5

6 Moors et al. (133) evaluated the kinetics of bisphenol A in pregnant rats on GD 18 after a single  
 7 intravenous dose of 10 mg/kg bw. Unconjugated bisphenol A represented almost 80% of total bisphenol  
 8 A 5 minutes after injection, 50% of total bisphenol A 20 minutes after injection, and ~10% of total  
 9 bisphenol A 6 hours after the injection. The half life of free bisphenol A in the dam's blood was 0.34  
 10 hours, and the half-life of total bisphenol A was 0.58 hours. Bisphenol A in fetal tissues peaked 20–30  
 11 minutes after maternal injection at 4.0 mg/kg in placenta, 3.4 mg/kg in fetal liver, and 2.4 mg/kg in  
 12 remaining fetal tissues. Peak maternal blood bisphenol A had been 3.8 mg/L shortly after injection.

13 **Table 28. Toxicokinetic Endpoints for Bisphenol A in Pregnant Rats iv Dosed with 2 mg/kg bw**  
 14 **Bisphenol A**

Endpoint	Compartment			
	Maternal serum	Placenta	Fetus	Amniotic fluid
AUC, $\mu\text{g}\cdot\text{hour/L}$	905.5 $\pm$ 275.8	4009 $\pm$ 962.7	1964.7 $\pm$ 678.5	180.4 $\pm$ 102.0
Elimination half-life, hours	2.5 $\pm$ 0.9	2.2 $\pm$ 0.8	2.2 $\pm$ 0.8	3.9 $\pm$ 3.1
Mean residence time, hours	3.0 $\pm$ 1.1	2.0 $\pm$ 0.5	3.0 $\pm$ 0.9	5.6 $\pm$ 4.7
$C_{\text{max}}$ , $\mu\text{g/L}$	927.3 $\pm$ 194.3	1399.2 $\pm$ 323.7	794 $\pm$ 360.6	75.1 $\pm$ 59.7
$T_{\text{max}}$ , hours	No data	0.1 $\pm$ 0.1	0.6 $\pm$ 0.3	0.3 $\pm$ 0.2

Values presented as mean  $\pm$  SD. From Shin et al. (132)

15  
 16 **Table 29. Intercompartmental Transfer and Clearances in Pregnant Rats After iv Bisphenol A**

Compartment	Transfer rate constant, $\text{hour}^{-1}$	Clearance rate, mL/minute
Maternal central to maternal tissue	3.4 $\pm$ 2.6	38.2 $\pm$ 26.5
Maternal tissue to maternal central	1.7 $\pm$ 1.3	50.2 $\pm$ 36.7
Maternal central to placental	0.7 $\pm$ 0.5	8.3 $\pm$ 5.4
Placental to maternal central	23.6 $\pm$ 14.7	2.2 $\pm$ 1.3
Placental to fetal	46.4 $\pm$ 29.2	4.1 $\pm$ 2.1
Fetal to placental	22.8 $\pm$ 28.0	7.6 $\pm$ 6.0
Fetal to amniotic fluid	0.00001 $\pm$ 0.00002	0.00001 $\pm$ 0.00001
Fetal	0.0062 $\pm$ 0.0044	0.0024 $\pm$ 0.0015
Amniotic fluid to fetal	14.0 $\pm$ 21.0	0.8 $\pm$ 1.1
Amniotic fluid to placental	7.9 $\pm$ 6.7	0.7 $\pm$ 0.7
Placental to amniotic fluid	1.0 $\pm$ 1.3	0.1 $\pm$ 0.1
Maternal central	0.9 $\pm$ 0.6	9.7 $\pm$ 5.3

Values presented as mean  $\pm$  SD. From Shin et al. (132)

17  
 18 Yoo et al. (122) examined mammary excretion of bisphenol A in rats. At 4–6 days postpartum, 4–6  
 19 lactating female Sprague Dawley rats/group were iv injected with bisphenol A at 0.47, 0.94, or 1.88  
 20 mg/kg bw and then infused with bisphenol A over a 4-hour period at rates of 0.13, 0.27, or 0.54 mg/hour.  
 21 Blood samples were collected at 2, 3, and 4 hours, and milk was collected at 4 hours following initiation  
 22 of infusion. Prior to collection of milk, rats were injected with oxytocin to increase milk production.  
 23 HPLC was used to measure bisphenol A concentrations in serum. Differences in data for mean systemic  
 24 clearance were analyzed by analysis of variance (ANOVA). Results are summarized in  
 25 [Table 30](#). The study authors noted extensive excretion of bisphenol A into milk, with milk concentrations  
 26 exceeding serum concentrations. No significant differences were reported for systemic clearance rates

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1 between the 3 doses. Steady state concentrations of bisphenol A in maternal serum and milk increased  
2 linearly according to dose.

3

4 **Table 30. Toxicokinetic Endpoints in Lactating Rats Infused with Bisphenol A**

Endpoint	Bisphenol A infusion rate, mg/hour		
	0.13	0.27	0.54
Systemic clearance, mL/minute/kg	119.2 ± 23.8	142.4 ± 45.3	154.1 ± 44.6
Steady state serum bisphenol A concentration, ng/mL	66.1 ± 15.5	120.0 ± 34.7	217.1 ± 65.0
Steady state milk bisphenol A concentration, ng/mL	173.1 ± 43.3	317.4 ± 154.4	493.9 ± 142.2
Milk/serum ratio	2.7 ± 0.9	2.6 ± 1.2	2.4 ± 0.6

Data presented as mean ± SD.

From Yoo et al. (122).

5

6 Kabuto et al. (134) reported bisphenol A concentrations in mice indirectly exposed to bisphenol A during  
7 gestation and lactation. The focus of the study was oxidative stress; more details are presented in Section  
8 3.2.7. Six ICR mouse dams were given drinking water containing 1% ethanol vehicle or bisphenol A at 5  
9 or 10 µg/L. **[Based on the reported water intake of 5 mL/day and an assumed body weight of 0.02 kg**  
10 **(115), it is estimated that bisphenol A intakes in mice at the start of pregnancy were 0.0013 and**  
11 **0.0025 mg/kg bw/day.]** Mice gave birth about 3 weeks following mating and pups were housed with  
12 dams for 4 weeks. **[Based on an assumed body weight of 0.0085 kg and assumed water intake rate of**  
13 **0.003 L/day (115), it is estimated that intake of bisphenol A in weanling males was 0.0018 and**  
14 **0.0035 mg/kg bw/day.]** At 4 weeks of age, male pups were killed and a GC/MS technique was used to  
15 measure bisphenol A concentrations in brain, kidney, liver, and testis in an unspecified number of control  
16 pups and in 4 pups from the 10 µg/L group. Study authors reported that they could not detect bisphenol A  
17 in control pups. In pups from the 10 µg/L group, the highest concentration of bisphenol A was detected in  
18 kidney (~24 µg/kg wet weight), followed by testis (~20 µg/kg wet weight), brain (~18 µg/kg wet weight),  
19 and liver (~11 µg/kg wet weight).

20

21 Zalko et al. (135) examined metabolism and distribution of bisphenol A in pregnant CD-1 mice. A series  
22 of studies was conducted in which mice were treated with <sup>3</sup>H-bisphenol A (>99.9% purity)/unlabeled  
23 bisphenol A (>99% purity). Mice were exposed to different regimens; biological samples examined  
24 included blood, liver, fat, gall bladder, uterus, ovaries, digestive tract and contents, urine, and feces. In the  
25 first exposure scenario, mice were sc injected with 0.025 mg/kg bw labeled/unlabeled bisphenol A on GD  
26 17; three animals/time period were examined at 0.5, 2, and 24 hours following dosing. In the second  
27 exposure scenario, 2 mice/group were sc injected with 50 mg/kg bw bisphenol A on GD 17 and killed 24  
28 hours following dosing. In the third scenario, 3 non-pregnant female mice/group were “force-fed” a single  
29 oral dose of 0.025 mg/kg bw bisphenol A; urine and feces were collected over 24 hours, and animals were  
30 killed at 24 hours. Biological samples were analyzed by scintillation analysis, HPLC, MS, and/or nuclear  
31 magnetic resonance.

32

33 In pregnant mice injected with 0.025 mg/kg bw/day bisphenol A and examined 24 hours later, 85.68% of  
34 the radioactivity was recovered. The highest percentages of radioactivity were detected in the digestive  
35 tract and contents (~45%) and feces (~21%). Less radioactivity was detected in the litter (~4%), liver  
36 (~2%), bile (~2%), urine (~6%), and carcass (~3%). Blood, ovaries, uterus, placenta, amniotic fluid, fat,  
37 and cage washes each contained <1% of the radioactive dose. At 0.5 hours following dosing, levels of  
38 radioactivity were highest in uterus > liver > placenta > fetus > amniotic fluid > ovaries > carcass >  
39 blood. Radioactivity levels in tissues were lower by 24 hours following exposure. **[Compared to**  
40 **radioactive levels detected in tissues at 24 hours, levels detected at 0.5 hours were ~12-fold higher in**  
41 **uterus, 3-fold higher in liver, 8-fold higher in placenta, 3.5-fold higher in fetuses, 2-fold higher in**  
42 **amniotic fluid, and 3.5-fold higher in ovaries.]** The only information provided for mice sc dosed with

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1 50 mg/kg bw bisphenol A and examined 24 hours later was for radioactivity levels in organs; the highest  
2 levels (pg/g) were detected in uterus >blood> ovary >carcass> liver. Study authors stated that distribution  
3 of radioactivity was comparable in mice treated with 50 and 0.025 mg/kg bw bisphenol A. In the mice  
4 orally dosed with 0.025 mg/kg bw bisphenol A and examined 24 hours later, levels of radioactivity in  
5 blood, ovaries, and uterus were reported to be significantly lower [**by ~1–2 orders of magnitude**] than  
6 levels in animals exposed by sc injection, but the level in the liver was not significantly different. There  
7 was significantly more residue in mouse carcass after oral than sc dosing (~2.5 fold, A. Soto, personal  
8 communication, March 2, 2007). No qualitative differences in metabolites were observed following oral  
9 or sc exposure. [**Data were not shown by study authors.**] Distribution of parent compound and  
10 metabolites detected in maternal and fetal tissues is summarized in [Table 31](#). Further discussion on  
11 metabolites is included in Section 2.1.2.3.

12  
13 Uchida et al. (136) examined distribution of bisphenol A in pregnant mice and monkeys. On GD 17 (GD  
14 0 = day of vaginal plug), ICR mice were sc injected with bisphenol A 100 mg/kg bw in sesame oil  
15 vehicle. More than 3 mice/time point were killed at various points between 0.5–24 hours following  
16 injection. An untreated control group consisted of 6 mice. [**Data were not presented for controls.**]  
17 Maternal and fetal serum and organs were collected. Among organs collected were fetal uteri and testes,  
18 which were pooled. On GD 150, 2 Japanese monkeys (*Macaca fuscata*) were sc injected with 50 mg  
19 bisphenol A/kg bw and at 1 hour following injection, fetuses were removed by cesarean section. Two  
20 untreated fetuses were used as controls. Maternal and fetal serum and organs, not including reproductive  
21 organs, were collected from monkeys. Bisphenol A concentrations were measured by GC/MS in mouse  
22 and monkey samples.

23  
24 In mice, bisphenol A was detected within 0.5 hours of exposure in all tissues examined, including  
25 placenta; maternal and fetal serum, liver, and brain; and fetal uterus and testis. Bisphenol A  
26 concentrations were higher in fetal than maternal serum and liver. [**Peak concentrations were observed**  
27 **within 0.5–1 hour in most tissues, with the exception of fetal brain (2 hours), and concentrations**  
28 **remained elevated for 1–6 hours, depending on tissue. More than 1 peak was observed in fetal**  
29 **serum, uterus, and testis.**] In exposed monkeys, bisphenol A was found at the highest concentrations  
30 (15.6–72.50 mg/kg) in fetal heart, intestine, liver, spleen, kidney, thymus, muscle, cerebrum, pons, and  
31 cerebellum; bisphenol A concentrations in the same organs from control monkeys were measured at 3.70–  
32 22.80 mg/kg. Lower concentrations of bisphenol A were detected in umbilical cord and maternal and fetal  
33 serum of the exposed group (1.70–6.10 mg/kg) and control group (0.02–0.25 mg/kg). The study authors  
34 stated that the most likely source of bisphenol A in control monkeys was the feed, which was found to  
35 contain bisphenol A. The study authors concluded that the placental barrier does not protect the fetus  
36 from bisphenol A exposure.

37  
38 Halldin et al. (137) examined distribution of bisphenol A in quail eggs or hens. After injection of  
39 fertilized quail egg yolk sacs with 67 µg/g <sup>14</sup>C-bisphenol A egg on incubation day 3, <1% of radioactivity  
40 was detected in embryos at incubation day 6 or 9. A similar finding was reported for diethylstilbestrol. At  
41 incubation day 6, no specific localization was observed in the embryo but in 10 and 15 day-old embryos a  
42 high amount of radioactivity was observed in liver and bile. [**Low transfer of labeled bisphenol A to the**  
43 **egg was reported after oral or iv dosing of quail hens (with apparently 105 µg bisphenol A), but**  
44 **concentrations in eggs were not quantified by study authors.**]

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1 **Table 31. Qualitative Analysis of Maternal and Fetal Tissues Following Injection of Mice with 0.025 mg/kg bw Radiolabeled Bisphenol A on**  
 2 **GD 17**

Hours after dose	Bisphenol A-associated compound detected										
	Hydroxylated glucuronide		Double glucuronide		Metabolite F <sup>a</sup>		Glucuronide		Parent		Others
	11.5 ng/g	%	17.5 ng/g	%	24.0 ng/g	%	25.0 ng/g	%	33.5 ng/g	%	%
Maternal plasma											
0.5	0.07 ±0.01	3	0.11 ±0.02	4	0.11 ±0.02	4	1.01 ±0.19	39	1.06 ±0.19	41	9
2	0.02 ±0.01	2	0.03 ±0.01	4	0.03 ±0.01	4	0.55 ±0.14	63	0.15 ±0.04	17	10
24	0.04 ±0.04	20		0		0	0.13 ±0.05	65		0	15
Placenta											
0.5		0		0	0.46 ±0.48	2	5.50 ±4.24	25	15.98 ±12.02	72	1
2	0.03 ±0.02	1	0.04 ±0.03	1	0.37 ±0.07	7	3.13 ±2.34	62	1.32 ±0.95	26	3
24	0.05 ±0.04	5	0.04 ±0.02	4	0.64 ±0.19	59	0.21 ±0.22	19	0.06 ±0.04	6	6
Fetus											
0.5	0.05 ±0.03	1	0.04 ±0.04	0	0.46 ±0.27	5	3.83 ±2.65	44	4.20 ±2.16	49	1
2	0.02 ±0.02	1	0.01 ±0.02	0	0.37 ±0.22	13	1.93 ±0.45	66	0.48 ±0.55	16	3
24	0.01 ±0.01	1		0	0.11 ±0.07	13	0.51 ±0.12	60	0.13 ±0.16	15	2
Amniotic fluid											
0.5	0.10 ±0.14	1	0.19 ±0.14	2	0.09 ±0.13	1	8.17 ±6.55	83	0.90 ±0.89	9	4
2	0.06 ±0.03	1	0.07 ±0.03	1	0.26 ±0.15	5	4.82 ±4.81	88	0.10 ±0.07	2	2
24	0.13 ±0.05	8	0.01 ±0.02	1	0.37 ±0.09	24	0.70 ±0.13	44	0.03 ±0.03	2	20
Maternal liver											
0.5	0.12 ±0.12	0	0.18 ±0.24	0	6.22 ±1.75	18	12.90 ±2.81	37	10.85 ±2.77	31	12
2	0.08 ±0.08	1	0.77 ±0.25	8	2.16 ±0.91	20	4.95 ±1.82	45	1.51 ±0.97	13	13
24	0.16 ±0.14	2	0.35 ±0.13	7	0.99 ±0.42	16	2.56 ±1.62	36	1.72 ±1.18	23	17

<sup>a</sup>Most likely bisphenol A glucuronide conjugated to acetylated galactosamine or glucosamine.

Data presented as mean ± SD

From Zalko et al. (135).

## 2.0 General Toxicology and Biological Effects

### 2.1.2.2.2 Non-pregnant and non-lactating animals

Domoradzki et al. (118), examined the effects of dose and age on toxicokinetics and metabolism of bisphenol A in rats. Neonatal and adult male and female Sprague Dawley rats were gavaged with  $^{14}\text{C}$ -bisphenol A (~99% radiochemical purity)/non-radiolabeled bisphenol A (99.7% purity). Three neonatal rats/age/sex/time period were dosed on PND 4, 7, and 21 with 1 or 10 mg/kg bw bisphenol A. Adult rats (11 weeks old) **[number treated not specified]** were dosed with 10 mg/kg bw bisphenol A. Blood samples were collected at various time points from 0.25 to 24 hours post dosing in neonatal rats and from 0.25 to 96 hours in adult rats. Plasma samples were pooled on PND 4. Immature rats were killed at 24 hours post-dosing, and adult rats were killed at 96 hours post dosing. Brain, liver, kidneys, skin, and reproductive organs were collected from neonatal rats. Levels of radioactivity, bisphenol A, and/or metabolites were analyzed in blood and tissue samples using HPLC and liquid scintillation spectrometry.

In neonatal and adult rats, radioactivity levels in plasma generally peaked within 0.25–0.75 hours. With the exception of 0.25 hours post dosing on PND 4, when plasma radioactivity levels were ~4-fold higher in males than females, plasma radioactivity levels were generally similar in male and female rats. At 24 hours post dosing, plasma radioactivity levels were 4–100 times lower in all groups of neonatal rats. Trends were noted for decreasing radioactivity levels with increasing age. Information related to dose- and age-related effects on metabolism is presented in Section 2.1.2.3.

Toxicokinetic values for bisphenol A are listed in Table 32.  $C_{\text{max}}$  and AUC values for bisphenol A decreased with increasing age, especially following dosing with 10 mg/kg bw. Bisphenol A concentrations were lower in adults than neonates. No patterns were observed for half-lives, and the authors stated that values in neonates may not have been reliable because bisphenol A concentrations were near the LOD at the end of the 24-hour observation period. Ratios of  $C_{\text{max}}$  and AUC values for the 10 and 1 mg/kg bw doses were different at each age and generally decreased with age. Plasma bisphenol A concentrations were very low in adults dosed with 10 mg/kg bw; therefore, few data were available.

Toxicokinetic values for bisphenol A glucuronide are listed in Table 33. Peak plasma concentrations of bisphenol A glucuronide were 9–22 times higher in neonates than adult rats dosed with 10 mg/kg bw bisphenol A. AUC values for bisphenol A glucuronide were also higher in neonates than adults [**~2–6 times higher**]. In neonates dosed with 1 mg/kg bw, AUC values and elimination half-lives for bisphenol A glucuronide decreased with age. Ratios of  $C_{\text{max}}$  and AUC values for the 10 and 1 mg/kg bw doses were nearly proportional. In adults dosed with 10 mg/kg bw, bisphenol A glucuronide concentrations peaked at 0.25 hours and secondary peaks were observed at 18 and 24 hours. In neonates dosed with 10 mg/kg bw, concentrations of bisphenol A glucuronide peaked at 0.75–1.5 hours and then bisphenol A glucuronide was eliminated in an apparently monophasic manner. Half-lives of elimination were shorter in neonates compared to adults. In neonatal rats, the bisphenol A glucuronide represented 94–100% of the 1 mg/kg bw dose and 71–97% of the 10 mg/kg bw/day dose. In adult rats, ~100% of the dose was represented by bisphenol A glucuronide.

Half-life and AUC data for bisphenol A-derived radioactivity in organs of neonatal rats are summarized in Table 34. Radioactivity was distributed to all organs and dose-related increases were observed. The study authors noted lower concentrations in brain than in other tissues. **[Levels of radioactivity in reproductive organs compared to those in plasma varied at each evaluation period but were usually within the same or 1 order of magnitude lower.]** With the exception of males dosed with 10 mg/kg bw bisphenol A, half-lives decreased with age. There were some disproportionate increases in ratios of AUC at 10 and 1 mg/kg bw.

The study authors concluded:

- Metabolism of bisphenol A to its glucuronide conjugate occurred as early as PND 4 in rats,

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- Dose-dependent differences occurred in neonatal rats, as noted by a larger fraction of the lower dose being metabolized to the glucuronide, and
- There were no major sex differences in metabolism or toxicokinetics of bisphenol A.

**Table 32. Toxicokinetic Values for Bisphenol A in Rats Following Gavage Dosing with 1 or 10 mg/kg bw**

Endpoint	Age at exposure and sex							
	PND 4		PND 7		PND 21		Adult	
	Male	Female	Male	Female	Male	Female	Male	Female
Bisphenol A dose: 1 mg/kg bw								
T <sub>max</sub> , hours	0.25	0.25	0.25	0.25	3	3		
C <sub>max</sub> , mg/L	0.03	0.06	0.04	0.08	0.005	0.006		
Half-life, hours	7.2	7.3	21.8	8.8				
AUC, mg·hour/L	0.2	0.1	0.1	0.1				
Bisphenol A dose: 10 mg/kg bw								
T <sub>max</sub> , hours	0.25	0.25	0.25	0.25	1.5	1.5	0.25	0.75
C <sub>max</sub> , mg/L	48.3	10.2	1.1	1.4	0.2	0.2	0.024	0.063
Half-life, hours	17	6.7	11.4	8.5	4.3	6.6	“0”	“0”
AUC, mg·hour/L	23.1	7.2	1.9	1.7	1.1	1	“0”	“0”
Ratio of value at 10 to 1 mg/kg bw/day								
C <sub>max</sub>	1610	170	27.5	17.5				
AUC	115.2	72	19	17				

Data missing from table cells were not determined.  
From Domoradzki et al. (118)

**Table 33. Toxicokinetic Values for Bisphenol A Glucuronide in Rats following Gavage Dosing with 1 or 10 mg/kg bw Bisphenol A**

Endpoint	Age at exposure and sex							
	PND 4		PND 7		PND 21		Adult	
	Male	Female	Male	Female	Male	Female	Male	Female
Bisphenol A dose: 1 mg/kg bw								
T <sub>max</sub> , hours	0.75	0.75	0.75	0.25	0.25	0.25		
C <sub>max</sub> , mg/L	1.3	1.5	2	1.1	0.8	0.8		
Half-life, hours	26.1	24.2	6.6	6.4	4.2	4.1		
AUC, mg·hour/L	9	9.6	7.7	7.7	4.1	3.3		
AUC <sub>BPA-glucuronide</sub> /AUC <sub>BPA</sub>	45	96	77	77				
Bisphenol A dose: 10 mg/kg bw								
T <sub>max</sub> , hours	1.5	1.5	1.5	0.75	0.75	0.75	0.25	0.25
C <sub>max</sub> , mg/L	13.1	6.3	6.6	10.3	10.4	7.8	0.6	0.7
Half-life, hours	7.3	9.8	9.1	8.4	4.4	4.4	22.5	10.8
AUC, mg·hour/L	80	50.3	58.9	60.9	60.3	56.1	31.5	9.8
AUC <sub>BPA-glucuronide</sub> /AUC <sub>BPA</sub>	3.5	7	31	36	55	56		
Ratio of value at 10 to 1 mg/kg bw/day								
C <sub>max</sub>	10.1	4.2	3.3	9.4	13	9.8		
AUC	8.9	5.2	7.6	7.9	14.7	17		

Data missing from table cells were not determined.  
From Domoradzki et al. (118).

10  
11

1 **Table 34. Distribution of Radioactivity to Tissues at 24 Hours Following Dosing with Radiolabeled**  
 2 **Bisphenol A**

Tissue	PND 4			PND 7			PND 21		
	Half-life, hours	AUC, mg·h/kg	AUC ratio of doses	Half-life, hours	AUC, mg·h/kg	AUC ratio of doses	Half-life, hours	AUC, mg·h/kg	AUC ratio of doses
Females, 1 mg/kg bw									
Brain	11.7	0.4		6.7	0.2		3.6	0.1	
Liver	18	7.5		7.9	7.1		3.6	2.9	
Kidney	18.1	9.4		7.3	9.5		5.0	3.0	
Ovary	11.7	7.3		6.0	3.5		3.7	0.9	
Uterus	7.4	8.3		6.2	3.0		3.4	1.0	
Carcass	11.2	22.2		10.0	16.6		4.0	8.3	
Plasma	19.5	9.4		6.4	7.8		3.6	3.5	
Females, 10 mg/kg bw									
Brain	7.2	3.3	8.3	8.0	2.5	12.5	4.9	1.7	17.0
Liver	11.1	44.8	6.0	10.0	59.6	8.4	4.5	39.1	13.5
Kidney	15.2	43.9	4.7	8.6	66.6	7.0	5.3	36.5	12.2
Ovary	6.5	136.2	18.7	5.0	69.7	19.9	3.6	21.1	23.4
Uterus	15.2	127.0	15.3	4.8	108.5	36.2	3.4	30.6	30.6
Carcass	6.6	112.8	5.1	7.0	130.7	7.9	4.8	100.9	12.2
Plasma	9.2	61.0	6.5	8.1	67.0	8.6	3.7	59.0	16.9
Males, 1 mg/kg bw									
Brain	14.1	0.3		6.0	0.3		3.4	0.1	
Liver	19.7	6.1		6.6	7.3		3.7	3.2	
Kidney	19.3	8.5		7.0	8.6		4.6	3.4	
Testis	10.3	3.4		5.7	2.0		3.4	0.8	
Carcass	11.1	22.2		9.0	17.3		4.1	9.0	
Plasma	24.0	9.2		6.6	7.7		3.4	4.2	
Males, 10 mg/kg bw									
Brain	3.1	4.7	15.7	8.0	2.9	9.7	4.7	1.7	17.0
Liver	11.6	48.4	7.9	11.8	62.0	8.5	5.1	40.9	12.8
Kidney	5.4	68.9	8.1	9.8	59.6	6.9	6.9	30.4	8.9
Testes	5.8	36.8	10.8	7.6	22.1	11.1	5.2	8.1	10.1
Carcass	8.3	111.7	5.0	8.6	135.5	7.8	4.8	95.2	10.6
Plasma	6.9	113.0	12.3	9.9	69.0	9.0	4.0	62.0	14.8

From Domoradzki et al. (118).

3  
 4 Pottenger et al. (119) examined the effects of dose and route on metabolism and toxicokinetics of  
 5 bisphenol A in rats. Information focusing on toxicokinetics is primarily summarized in this section, while  
 6 metabolic data are primarily summarized in Section 2.1.2.3. Adult male and female F344 rats were dosed  
 7 with <sup>14</sup>C-bisphenol A (99.3% radiochemical purity)/non-radiolabeled bisphenol A (99.7% purity) at doses  
 8 of 10 or 100 mg/kg bw by oral gavage or ip or sc injection. Blood was collected at multiple time points  
 9 between 0.083 and 168 hours post dosing, and excreta were collected for 7 days. Animals were killed 7  
 10 days post dosing. Blood, brain, gonads, kidneys, liver, fat, skin, uterus, and carcass were analyzed by  
 11 liquid scintillation counting and HPLC. Some samples were analyzed by HPLC/electrospray  
 12 ionization/MS.  
 13  
 14 Toxicokinetic endpoints for bisphenol A in blood are summarized in Table 35. Study authors noted that  
 15 concentration-time profiles of bisphenol were dependent on dose, exposure route, and sex. The longest  
 16 T<sub>max</sub> was observed with sc dosing. C<sub>max</sub> and AUC values were lowest following oral administration. Time  
 17 to non-quantifiable concentrations of bisphenol A was longest following sc exposure. The only sex-

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1 related difference was a higher  $C_{\max}$  value in females than males following oral dosing. In most cases,  
 2 bisphenol A toxicokinetics were linear across doses within the same administration route, as noted by  
 3 approximate proportionate increases in  $C_{\max}$  and AUC values from the low to the high dose.  
 4 Toxicokinetics data for radioactivity in plasma are summarized in Table 36. Concentrations of  
 5 radioactivity were dependent on exposure route and to a lesser extent, dose and sex. AUC values for  
 6 radioactivity were lowest following oral exposure. Time to non-quantifiable concentration was longest  
 7 following sc dosing. For most groups,  $C_{\max}$  and AUC values were proportionate across doses within the  
 8 same exposure route. A second part of the study examined metabolites and is summarized in Section  
 9 2.1.2.3

10 .  
 11 **Table 35. Toxicokinetic Endpoints for Bisphenol A in Blood Following Dosing of Rats by Gavage or**  
 12 **Injection**

Endpoint	Exposure route and doses (mg/kg bw)					
	10 oral	100 oral	10 ip	100 ip	10 sc	100 sc
<b>Males</b>						
$T_{\max}$ , hours	N/A	0.083	0.5	0.25	0.75	0.5
$C_{\max}$ , mg/L, hours <sup>a</sup>	<sup>b</sup>	0.22 ± 0.09	0.69 ± 0.08	9.7 ± 1.27	0.39 ± 0.16	5.19 ± 0.98
Time to non-quantifiable concentration, hours	0.083	0.75	8	12	18	24
AUC, mg-hour/L		0.1	1.1	16.4	2.6	24.5
<b>Females</b>						
$T_{\max}$ , hours	0.25	0.25	0.25	0.25	4	0.75
$C_{\max}$ , mg/L, hours <sup>a</sup>	0.04 ± 0.03	2.29 ± 1.82	0.87 ± 0.15	13.13 ± 4.13	0.34 ± 0.06	3.97 ± 0.6
Time to non-quantifiable concentration, hours	1		24	72	48	72
AUC, mg-hour/L	0.42	4.4	1.4	26.2	3.1	31.5

<sup>a</sup>Mean ± SD.

<sup>b</sup>Non-quantifiable (0.01 µg/g at 10 mg/kg bw and 0.1 µg/g at 100 mg/kg bw).

Missing values were not determined.

From Pottenger et al. (119)

13  
 14 **Table 36. Toxicokinetics for Radioactivity Following Dosing of Rats with Bisphenol A through**  
 15 **Different Exposure Routes**

Endpoint	Exposure route and doses (mg/kg bw)					
	10 oral	100 oral	10 ip	100 ip	10 sc	100 sc
<b>Males</b>						
$T_{\max}$ , hours	0.25	0.25	0.5	0.25	1	0.75
$C_{\max}$ , mg eq/L, hours	0.73 ± 0.22	3.92 ± 1.93	1.26 ± 0.09	29.3 ± 11.7	0.61 ± 0.24	6.33 ± 0.43
Time to non-quantifiable concentration, hours	72	72	96	96	96	144
AUC, mg-eq-hour/L	8.1	66.5	16.9	170	15.5	218
<b>Females</b>						
$T_{\max}$ , hours	0.083	0.25	0.25	0.5	0.75	0.75
$C_{\max}$ , mg eq/L, hours	1.82 ± 0.66	28.33 ± 8.64	2.27 ± 0.19	67.81 ± 7.33	0.52 ± 0.06	5.66 ± 0.95
Time to non-quantifiable concentration, hours	72	72	72	120	120	168
AUC, mg-eq-hour/L	9.54	94.9	15.3	247	21.6	297

From Pottenger et al. (119).

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1 Upmeier et al. (123) examined toxicokinetics in rats exposed to bisphenol A through the oral or iv route.  
 2 Ovariectomized DA/Han rats (130–150 g bw) were exposed to bisphenol A by iv injection with 10 mg/kg  
 3 bw or oral gavage with 10 or 100 mg/kg bw. Blood was collected from treated rats at multiple time points  
 4 until 2 hours following iv dosing and 3 hours following oral dosing. The number of rats sampled during  
 5 each time period was 3–5. To reduce stress, only some of the rats were sampled at each time point. In  
 6 control animals, blood was collected 2 hours following dosing with vehicle. Bisphenol A concentrations  
 7 in plasma were measured by GC/MS. Dosing with 10 mg/kg bw iv resulted in a maximum plasma  
 8 concentration of 15,000 µg/L bisphenol A. Concentrations rapidly decreased to 700 µg/L within 1 hour,  
 9 100 µg/L within 2 hours, and non-detectable concentrations by 24 hours following exposure. The  
 10 apparent final elimination half-life was estimated at 38.5 hours. In rats gavaged with 10 mg/kg bw, an  
 11 initial maximum blood concentration of 30 µg/L was obtained at 1.5 hours. A decrease in bisphenol A  
 12 blood concentration at 2.5 hours was followed by a second peak of 40 µg/L at 6 hours, leading study  
 13 authors to conclude that enterohepatic cycling was occurring. The same patterns of bisphenol A  
 14 concentrations in blood were observed following gavage dosing with 100 mg/kg bw. Peak concentrations  
 15 were observed at 30 minutes (150 µg/L) and 3 hours (134 µg/L) following exposure. According to the  
 16 study authors, the differences in peak concentrations observed between the 2 doses suggested lower  
 17 bioavailability at the high dose than at the low dose. Oral bioavailability of bisphenol A was estimated at  
 18 16.4% at the low dose and 5.6% at the high dose.

19  
 20 Yoo et al. (122) examined toxicokinetics of a low iv dose and a higher gavage dose of bisphenol A in  
 21 male rats. Five adult male Sprague Dawley rats/group were administered bisphenol A by iv injection at a  
 22 dose of 0.1 mg/kg bw or by gavage at a dose of 10 mg/kg bw. Multiple blood samples were collected  
 23 until 3 hours following iv dosing and 24 hours following gavage dosing. HPLC was used to measure  
 24 bisphenol A concentrations in serum. Route-specific differences in mean systemic clearance were  
 25 analyzed by Student *t*-test. Results are summarized in Table 37. The study authors noted bi-exponential  
 26 decay of serum bisphenol A concentrations following iv dosing, significantly longer elimination half-life  
 27 with oral than iv exposure, and low oral bioavailability of bisphenol A.

28  
 29 **Table 37. Toxicokinetic Values for Bisphenol A in Adult Rats Exposed to Bisphenol A through the**  
 30 **IV or Oral Route**

Endpoint	Bisphenol A dosing	
	0.1 mg/kg bw, iv	10 mg/kg bw, gavage
Distribution half-life, minutes	6.1 ± 1.3	
Terminal elimination half-life, hours	0.9 ± 0.3	21.3 ± 7.4
AUC, µg-hour/L	16.1 ± 3.2	85.6 ± 33.7
Systemic clearance, mL/minute/kg	107.9 ± 28.7	
Steady-state volume of distribution, L/kg	5.6 ± 2.4	
C <sub>max</sub> , µg/L		14.7 ± 10.9
T <sub>max</sub> , hours		0.2 ± 0.2
Apparent volume of distribution, L/kg		4273 ± 2007.3
Oral clearance, mL/minute/kg		2352.1 ± 944.7
Absolute oral bioavailability, %		5.3 ± 2.1

Data presented as mean ± SD.

From Yoo et al. (122).

31  
 32 Kurebayashi et al. (138) conducted a series of studies to examine toxicokinetics and metabolism of  
 33 bisphenol A in adult F344N rats exposed through the oral or iv route. In these studies, radioactivity levels  
 34 were measured by scintillation counting. Bisphenol A or its metabolites were quantified by HPLC,  
 35 electrospray ionization/ MS, or nuclear magnetic resonance. As discussed in greater detail in Section  
 36 2.1.2.4, fecal excretion was the main route of elimination for radioactivity following oral or iv dosing of  
 37 rats with 0.1 mg/kg bw <sup>14</sup>C-bisphenol A. A study describing biliary excretion and metabolites in bile is  
 38 summarized in Section 2.1.2.3. Toxicokinetic endpoints were determined in a study in which blood was

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1 drawn from 3 male rats/group at various time points between 0.25 and 48 hours following oral gavage or  
 2 iv dosing with 0.1 mg/kg bw bisphenol A. Results of the study are summarized in [Table 38](#). Rapid  
 3 absorption of radioactivity was observed following oral dosing. AUC values were significantly lower for  
 4 oral than iv dosing. In a another study, rats were administered <sup>14</sup>C-bisphenol A by iv injection and blood  
 5 was collected 30 minutes later for determination of blood/plasma distribution and protein binding. At a  
 6 blood radioactivity level of 80 nM [**18 µg bisphenol A eq/L**], preferential distribution to plasma was  
 7 observed, with the blood/plasma ratio reported at 0.67. At radioactivity levels of 6–31 µg-eq/L (27–135  
 8 nM), plasma protein binding was reported at 95.4%. Additional studies reviewed by Teeguarden et al.  
 9 (*139*) reported plasma protein binding of bisphenol A at ~90–95%. An additional study by Kurebayashi et  
 10 al. (*138*) compared metabolic patterns and excretion following exposure to a higher bisphenol A dose;  
 11 that study is discussed in Section 2.1.2.3.

12  
 13 **Table 38. Toxicokinetic Endpoints for <sup>14</sup>C-Bisphenol A-Derived Radioactivity in Rats Exposed to**  
 14 **0.1 mg/kg bw <sup>14</sup>C-Bisphenol A Through the Oral or IV Route**

Endpoint	IV exposure	Oral exposure
T <sub>max</sub> , hour		0.38 ± 0.10
C <sub>max</sub> , µg-eq/L		5.5 ± 0.3
Half-life-α, hours	0.59 ± 0.09	No data
Half-life-β, hours	39.5 ± 2.1	44.5 ± 4.1
Absorbance rate, hour <sup>-1</sup>		3.6 ± 1.0
Volume of distribution, L/kg	27.0 ± 0.7	No data
Total body clearance, L/hour/kg	0.522 ± 0.011	0.544 ± 0.049
Mean residence time, hour	51.7 ± 2.4	No data
AUC, µg-eq·hour/L		
0–6 hours	33.9 ± 1.6	18.4 ± 0.7 <sup>a</sup>
0–24 hours	79.3 ± 3.3	60.0 ± 7.1 <sup>a</sup>
0–48 hours	118 ± 4	102 ± 13 <sup>a</sup>
0–∞	192 ± 4	185 ± 16
Oral bioavailability <sup>b</sup>		
0–6 hours		0.54
0–24 hours		0.76
0–48 hours		0.86
0–∞		0.97

Data presented as mean ± SD.

Missing values are not applicable or were not reported.

<sup>a</sup>P < 0.05 compared to iv exposure.

<sup>b</sup>Variances not reported.

From Kurebayashi et al. (*138*).

15  
 16 Kurebayashi et al. (*127*) administered <sup>14</sup>C-bisphenol A to adult male and female F344 rats (3/dose/sex) at  
 17 doses of 0.020, 0.1, or 0.5 mg/kg bw orally or 0.1 or 0.5 mg/kg bw by iv injection. Plasma samples were  
 18 analyzed for radioactivity over a 72-hour period to determine toxicokinetic endpoints. Results are  
 19 summarized in [Table 39](#). Study authors noted that the AUC was almost linearly correlated with dose.  
 20 Several peaks were observed with oral or iv exposure, indicating enterohepatic cycling, according to the  
 21 study authors. Study authors noted that substantially lower AUC values in females than in males  
 22 following oral exposure could have resulted from lower absorption and/or a higher elimination rate.  
 23 Distribution of radioactivity was evaluated 0.5, 24, and 72 hours following oral administration of 0.1  
 24 mg/kg bw bisphenol A to adult male and female Wistar rats (3/sex/time point). At 0.5 hours following  
 25 exposure, most of the radioactivity (~12–51 µg bisphenol A eq/kg) was found in kidney and liver. **[A**  
 26 **large amount of radioactivity was also reported for intestinal contents, but those data were not**  
 27 **shown by the study authors.]** Lower amounts of radioactivity (~2–7 µg bisphenol A eq/kg or L) were  
 28 detected in adrenal gland, blood, lung, pituitary gland, skin, and thyroid gland of both sexes; uterus; and

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bone marrow, brown fat, and mandibular gland of males. In males, <1 µg bisphenol A eq/kg was detected in skeletal muscle and testis. Radioactivity was non-quantifiable in brain and eye of both sexes; epididymis, prostate gland, and heart of males; and bone marrow, brown fat, skeletal muscle, and mandibular gland of females. At ≥24 hours following exposure, radioactivity was primarily detected only in kidney, liver, and intestinal contents, with the exception of ~3 µg bisphenol A eq/L detected in blood of males at 24 hours following dosing. Study authors noted that elimination of radioactivity from some tissues appeared to occur more rapidly in females than in males. Distribution in pregnant animals was also examined and is described in Section 2.1.2.2.1.

**Table 39. Toxicokinetic Endpoints for Plasma Radioactivity in Rats Dosed with <sup>14</sup>C Bisphenol A**

Endpoints	Route and dose (mg/kg bw)				
	Oral			IV	
	20	100	500	100	500
<b>Males</b>					
Elimination half-life, hours	78 ± 52	18 ± 3	21 ± 3	19 ± 2	21 ± 3
AUC, µg·eq·h/L	36 ± 6	178 ± 44	663 ± 164	266 ± 46	865 ± 97
Apparent absorption, %	82	81	60		
<b>Females</b>					
Elimination half-life, hours	20 ± 7	22 ± 13	18 ± 8	13 ± 3	16 ± 2
AUC, µg·eq·h/L	14 ± 5	99 ± 19	500 ± 43	190 ± 45	1029 ± 81
Apparent absorption, %	35	50	50		

Data presented as mean ± SD.

From: Kurebayashi et al. (127).

Kabuto et al. (140) reported distribution of bisphenol A in mice. Male ICR mice were ip dosed with bisphenol A at 0, 25, or 50 mg/kg bw/day for 5 days and killed 6 hours following the last dose. Bisphenol A concentrations in tissues of animals from the high-dose group were determined by GC/MS. In mice of the high-dose group, the highest concentrations of bisphenol A were detected in kidney (~2.02 mg/kg wet weight) and body fat (~1.25 mg/kg wet weight). Lower concentrations of bisphenol A (≤0.42 mg/kg wet weight or mg/L) were detected in brain, lung, liver, testis, and plasma.

Kurebayashi et al. (124) examined the toxicokinetics of a low bisphenol A dose in Cynomolgus monkeys following gavage or iv dosing. Three adult male and female monkeys were dosed with 0.1 mg/kg bw <sup>14</sup>C-bisphenol A (99% radiochemical purity)/non-radiolabeled bisphenol A [**purity not reported**]. Monkeys were dosed by iv injection on day 1 of the study and by gavage on day 15 of the study. Urine and feces were collected for 7 days post dosing. Blood samples were collected at various time points from 0.083 to 72 hours following iv dosing and for 0.25 to 71 hours after oral dosing. Binding to plasma protein was determined at some time points over 0.25–4 hours. Samples were analyzed by liquid scintillation counting and HPLC. Following oral or iv exposure, the percentage of radioactivity recovered in excreta and cage washes was 81–88% over a 1-week period. As discussed in greater detail in Section 2.1.2.4, most of the radioactivity was excreted in urine and very little was excreted in feces. Toxicokinetic endpoints are summarized in Table 40. Based on the toxicokinetic values, study authors concluded that absorption of bisphenol A following oral exposure was rapid and high, and terminal elimination half-lives of bisphenol A/metabolites were longer following iv than oral exposure. As discussed in more detail in Section 2.1.2.3, glucuronide compounds were the major metabolites detected in urine, and higher percentages of the radioactive dose in plasma were represented by bisphenol A following iv than oral dosing.

1 **Table 40. Toxicokinetic Endpoints for Radioactivity in Male and Female Cynomolgus Monkeys**  
 2 **Exposed to <sup>14</sup>C-Bisphenol A Through iv Injection or by Gavage**

Endpoint	Male	Female
<b>Intravenous exposure</b>		
AUC, µg·eq·hour/L	377 ± 85	382 ± 96
Volume of distribution, L/kg	1.58 ± 0.11	1.82 ± 0.41
Half-life, hours	13.5 ± 2.6	14.7 ± 2.1
Total body clearance, L/hours/kg	0.27 ± 0.05	0.28 ± 0.08
Mean residence time, hours	5.93 ± 0.91	6.68 ± 0.72
<b>Oral exposure</b>		
AUC, µg·eq·hour/L	265 ± 74	244 ± 21
T <sub>max</sub> , hours	1.00 ± 0.87	0.33 ± 0.14
C <sub>max</sub> , µg·eq/L	104 ± 85	107 ± 37
Half-life, hours	9.63 ± 2.74	9.80 ± 2.15
Bioavailability	0.70 ± 0.16	0.66 ± 0.13

[Mean ± SD assumed based on data presentations elsewhere in this paper.]

From Kurebayashi et al. (124).

3  
 4 Negishi et al. (120) compared toxicokinetics of bisphenol A in female F344/N rats, Cynomolgus  
 5 monkeys, and Western chimpanzees. Bisphenol A was administered by oral gavage and sc injection at  
 6 doses of 10 or 100 mg/kg bw/day to rats and monkeys and 10 mg/kg bw to chimpanzees. Three  
 7 rats/dose/time point were killed before and at various times between 0.5 and 24 hours following bisphenol  
 8 A administration. Three monkeys/group and 2 chimpanzees were first exposed orally and 1 week later by  
 9 sc injection. In monkeys, blood samples were drawn before and at various times from 0.5 to 24 hours  
 10 after dosing. In chimpanzees, blood was drawn before and at multiple time points between 0.25 and 24  
 11 hours following dosing. Bisphenol A was measured in serum by ELISA, and toxicokinetics endpoints  
 12 were determined. Results are summarized in Table 41. The study authors noted that the bioavailability of  
 13 bisphenol was lowest in rats < chimpanzees < monkeys following exposure through either route. In most  
 14 cases, bisphenol A was not detected in rat serum following oral administration of the 10 mg/kg bw dose.  
 15 In all species, higher bioavailability was observed with sc than oral dosing.

16  
 17 **Table 41. Toxicokinetic Endpoints for Bisphenol A by ELISA in Rats, Monkeys, and Chimpanzees**

Endpoints	10 mg/kg bw		100 mg/kg bw	
	Oral	SC	Oral	SC
<b>Rat (data presented as mean ± SD)</b>				
C <sub>max</sub> , µg/L		872 ± 164	580 ± 398	3439 ± 679
T <sub>max</sub> , hours		1.0	0.5	1.0
AUC <sub>0-4h</sub> , µg·hour/L		1912 ± 262	506 ± 313	9314 ± 2634
AUC <sub>0-24h</sub> , µg·hour/L		3377 ± 334	1353 ± 462	23,001 ± 6387
<b>Monkey (data presented as mean ± SD)</b>				
C <sub>max</sub> , µg/L	2793 ± 920	57,934 ± 1902	5732 ± 525	10,851 ± 3915
T <sub>max</sub> , hours	0.7 ± 0.2	2.0 ± 0.0	0.7 ± 0.2	2.0 ± 0.0
AUC <sub>0-4h</sub> , µg·hour/L	3209 ± 536	15,316 ± 5856	14,747 ± 2495	48,010 ± 11,641
AUC <sub>0-24h</sub> , µg·hour/L	3247 ± 587	39,040 ± 10,738	52,595 ± 8951	189,627 ± 21,790
<b>Chimpanzee (data presented for 2 animals)</b>				
C <sub>max</sub> , µg/L	325; 96	2058; 1026	Dose not administered	
T <sub>max</sub> , hours	0.5; 0.5	2.0; 2.0		
AUC <sub>0-4h</sub> , µg·hour/L	491; 235	5658; 3109		
AUC <sub>0-24h</sub> , µg·hour/L	1167; 813	21,141; 12,492		

Data were not reported in cases where table cells are empty.

From Negishi et al. (120).

## 2.0 General Toxicology and Biological Effects

In a subsequent report (141), these authors noted that ELISA may over-estimate bisphenol A concentrations due to non-specific binding. They reported measurements by LC-MS/MS in animals evaluated using the same study design [possibly the same specimens reported previously]. These results are summarized in Table 42. The authors proposed that primates, including humans, may completely glucuronidate orally-administered bisphenol A on its first pass through the liver and excrete it in the urine whereas bisphenol A remains in the rat for a more extended period due to enterohepatic recirculation. They suggested that the rat may not be a good model for human bisphenol A kinetics.

**Table 42. Toxicokinetic Endpoints for Bisphenol A by LC-MS/MS in Rats, Monkeys, and Chimpanzees**

Endpoints	10 mg/kg bw		100 mg/kg bw	
	Oral	SC	Oral	SC
Rat (data presented as mean ± SD)				
C <sub>max</sub> , µg/L	2.1 ± 1.6	746 ± 80	47.5 ± 10.6	2631 ± 439
T <sub>max</sub> , hours	0.7 ± 0.3	0.8 ± 0.3	0.5 ± 0.0	1.2 ± 0.8
t <sub>1/2</sub> , hours	not calculated	3.2 ± 0.7	not calculated	4.5 ± 0.7
AUC <sub>0-4h</sub> , µg·hour/L	4.2 <sup>a</sup>	1542 ± 200	43.2 ± 9.7	6926 ± 1071
AUC <sub>0-24h</sub> , µg·hour/L	7.2 <sup>a</sup>	1977 ± 182	350 ± 294	15576 ± 2263
Monkey (data presented as mean ± SD)				
C <sub>max</sub> , µg/L	11.5 ± 2.2	4213 ± 3319	28.6 ± 3.9	7010 ± 3045
T <sub>max</sub> , hours	1.0 ± 0.9	1.7 ± 0.6	3.3 ± 1.2	2.7 ± 1.2
t <sub>1/2</sub> , hours	8.9 ± 3.0	3.8 ± 0.8	4.5 ± 0.7	12.9 ± 3.6
AUC <sub>0-4h</sub> , µg·hour/L	21.4 ± 6.1	8828 ± 4309	85.3 ± 18.6	19981 ± 7567
AUC <sub>0-24h</sub> , µg·hour/L	42.5 ± 7.3	18855 ± 3870	350 ± 13	79796 ± 21750
Chimpanzee (data presented as mean for 2 animals)				
C <sub>max</sub> , µg/L	5.5	703	Dose not administered	
T <sub>max</sub> , hours	0.8	1.0		
t <sub>1/2</sub> , hours	6.8	4.2		
AUC <sub>0-4h</sub> , µg·hour/L	13.3	2148		
AUC <sub>0-24h</sub> , µg·hour/L	33.1	6000		

<sup>a</sup>1 or 2 animals.

From Tominaga et al. (141).

### 2.1.2.3 Metabolism

Information is arranged in this section according to species. In rats, study summaries are arranged in order of those providing general or route-specific information on metabolites, specifics on organs or enzyme isoforms involved in metabolism, and pregnancy-, sex-, or age-related effects on metabolism.

Pottenger et al. (119) examined the effects of dose and route on toxicokinetics of bisphenol A in rats. Disposition of bisphenol A and its metabolites in urine and feces is primarily described in this section, while results of the toxicokinetics study are primarily described in Section 2.1.2.2. Five adult F344 rats/sex/group were dosed with <sup>14</sup>C-bisphenol A (99.3% radiochemical purity)/non-radiolabeled bisphenol A (99.7% purity) at doses of 10 or 100 mg/kg bw by oral gavage or ip or sc injection. Excreta were collected for 7 days. Samples were analyzed by HPLC or HPLC/electrospray ionization/MS. The percentage of radioactivity recovered from all groups was 84–98%. Fecal elimination represented the largest percentage of radioactivity in all exposure groups (52–83%). Eight peaks were identified in feces, and the largest peak (representing 86–93% of radioactivity) was for unchanged bisphenol A. Elimination of radioactivity through urine was ~2-fold higher in females (21–34%) than males (13–16%) in all dose groups. Fourteen different peaks were identified in urine. It was estimated that radioactivity in urine was represented by bisphenol A monoglucuronide (57–87%), bisphenol A (3–12%), and bisphenol A sulfate (2–7%). Some differences were noted for retention of radioactivity following dosing by gavage (0.03–0.26%), ip injection (0.65–0.85%), and sc injection (1.03–1.29%).

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1 Metabolites associated with bisphenol A exposure were examined in a second study by Pottenger et al.  
2 (119). Three rats/sex/dose/route/time point were dosed with <sup>14</sup>C-bisphenol A/non-radiolabeled bisphenol  
3 A at 10 or 100 mg/kg bw by oral gavage or ip or sc injection. Rats were killed at 2 different time points  
4 following dosing, T<sub>max</sub>, and the time when bisphenol A concentrations were no longer quantifiable. Times  
5 at which rats were killed were determined by data obtained during the first study. Plasma samples were  
6 pooled at each time period and examined by HPLC or HPLC/electrospray ionization/MS. Qualitative and  
7 quantitative differences were observed for parent compound and metabolites in plasma following  
8 exposure through different routes. Following oral exposure, bisphenol A glucuronide was the most  
9 abundant compound detected in plasma at both time periods (C<sub>max</sub> and time when parent compound was  
10 not quantifiable) and represented 68–100% of total radioactivity. Following ip or sc exposure,  
11 unmetabolized bisphenol A was the most abundant compound at T<sub>max</sub>; levels of radioactivity represented  
12 by unmetabolized bisphenol A were 27–51% following ip exposure and 65–76% following sc exposure.  
13 Only 2–8% of radioactivity was represented by bisphenol A following oral exposure. Some compounds  
14 observed following ip or sc exposure were not observed following oral exposure. A compound tentatively  
15 identified as a sulfate conjugate was observed following ip exposure and represented a small portion of  
16 radioactivity. An unresolved peak of 3 compounds was observed following ip or sc exposure, at the time  
17 when parent compound was not quantifiable and represented that major percent of radioactivity for that  
18 time point. Three additional unidentified, minor peaks were observed following ip or sc but not oral  
19 exposure. The major sex differences observed were higher C<sub>max</sub> values for bisphenol A and bisphenol A  
20 glucuronide in females than males, especially following ip administration. A review by the European  
21 Union (2) noted that the substantially higher concentrations of parent compound with ip and sc compared  
22 to oral exposure indicated the occurrence of first-pass metabolism following oral intake.

23  
24 Elsbey et al. (142) examined bisphenol A metabolism by rat hepatocytes. In the hepatocyte metabolism  
25 study, hepatocytes were isolated from livers of adult female Wistar rats and incubated in  
26 dimethylsulfoxide (DMSO) vehicle or bisphenol A 100 or 500 μM [23 or 114 mg/L] for 2 hours.  
27 Metabolites were identified by HPLC or LC/MS. Data were obtained from 4 experiments conducted in  
28 duplicate. At both concentrations, the major metabolite was identified as bisphenol A glucuronide, which  
29 was the only metabolite identified following incubation with 100 μM bisphenol A. Two additional minor  
30 metabolites identified at the 500 μM concentration included 5-hydroxy-bisphenol A-sulfate and bisphenol  
31 A sulfate. Another part of the study comparing metabolism of bisphenol A by rat and human metabolites  
32 is discussed in Section 2.1.1.3. Another study (143) comparing metabolism of bisphenol A in humans,  
33 rats, and mice is also summarized in Section 2.1.1.3.

34  
35 In neonatal rats gavaged with 1 or 10 mg/kg bw <sup>14</sup>C-bisphenol A on PND 4, 7, and 21 and adult rats  
36 gavaged with 10 mg/kg bw bisphenol A, the major compounds detected in plasma were bisphenol A  
37 glucuronide and bisphenol A (118). Up to 13 radioactive peaks were identified in neonatal rats dosed with  
38 10 mg/kg bw and 2 were identified in neonates dosed with 1 mg/kg bw/day. At the 10 mg/kg bw dose, the  
39 concentration of bisphenol A glucuronide detected in plasma increased with age. Metabolic profiles were  
40 generally similar in males and females. The study authors noted that metabolism of bisphenol A to its  
41 glucuronide conjugate occurs as early as PND 4 in rats. However, age-dependent differences were  
42 observed in neonatal rats, as noted by a larger fraction of the lower dose being metabolized to the  
43 glucuronide. More details from this study are included in Section 2.1.2.2.

44  
45 Kurebayashi et al. (127) used a thin layer chromatography technique to examine metabolite profiles in  
46 blood, urine, and feces of 3 male rats orally dosed with 0.5 mg/kg bw <sup>14</sup>C-bisphenol A. [The procedure  
47 did not identify metabolites.] Parent bisphenol A represented ~2% of the dose in plasma at 0.25 and 6  
48 hours post dosing and ~0.3% of the dose at 24 hours after exposure. Unmetabolized bisphenol A  
49 represented 1.6% of compounds in urine and 77.2% of compounds in feces collected over a 24-hour  
50 period. Free bisphenol A represented 47.1% of compounds in urine following β-glucuronidase hydrolysis  
51 of urine, and there was an almost equivalent decrease in a metabolite the study authors identified as

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“M2.” Therefore, the study authors stated that M2 was most likely bisphenol A glucuronide. M2 was the major metabolite identified in plasma (~74–77%) and urine (~40%).

The European Union (2) reviewed studies by Atkinson and Roy (144, 145) that reported two major and several minor adducts in DNA obtained from the liver of CD-1 rats dosed orally or ip with 200 mg/kg bw bisphenol A. Chromatographic mobility of the two major adducts was the same as that observed when bisphenol A was incubated with purified DNA and a peroxidase or microsomal P450 activation system. The profile closely matched that of adducts formed with the interaction between bisphenol O-quinone and purified rat DNA deoxyguanosine 3'-monophosphate. Formation of the adduct appeared to be inhibited by known inhibitors of cytochrome P (CYP) 450. It was concluded that bisphenol A is possibly metabolized to bisphenol O-quinone by CYP450.

Biliary excretion of bisphenol A and its metabolites following oral or iv dosing with bisphenol A was examined by Kurebayashi et al. (138). Bile ducts of 3 rats/sex/group were cannulated, and the rats were dosed with 0.1 mg/kg bw <sup>14</sup>C-bisphenol A (>99% radiochemical purity) in phosphate buffer vehicle by oral gavage or iv injection. Biliary fluid was collected every 2 hours over a 6-hour period to determine percent total biliary excretion and percent of dose represented by bisphenol A glucuronide. Results are summarized in Table 43. The study authors noted that the importance of biliary excretion following oral or iv dosing. <sup>14</sup>C-bisphenol A-glucuronide was the predominant metabolite in bile.

**Table 43. Biliary Excretion in Male and Female Rats Exposed to 0.1 mg/kg bw <sup>14</sup>C-Bisphenol A Through the Oral or iv Route**

Parameters	Male		Female	
	IV	Oral	IV	Oral
Biliary excretion, %				
0–2 hours	48	32	35	28
0–4 hours	61	44	50	39
0–6 hours	66	50	58	45
Radioactivity in bile represented by glucuronide, %	84	86	87	88
Dose excreted as glucuronide in bile, %	55	43	50	40

From Kurebayashi et al. (138).

In another study by Kurebayashi et al. (138), biliary, fecal, and urinary metabolites were examined in male rats gavaged with 100 mg/kg bw bisphenol A or D<sub>16</sub>-bisphenol A in corn oil. Bile was collected over an 18-hour period, and urine and feces were collected over a 72-hour period. The primary metabolite detected in urine was bisphenol A glucuronide, which represented 6.5% of the dose. Lower percentages of the dose (≤1.1%) were present in urine as bisphenol A and bisphenol A sulfate. In feces, the primary compound detected was bisphenol A, which represented 61% of the dose. No glucuronide or sulfate conjugated metabolites of bisphenol A were detected in feces. Most of the dose in bile consisted of bisphenol A glucuronide (41% of the dose). Bisphenol A represented 0.3% of the dose in bile. The study authors noted that as with oral or iv exposure to a smaller dose, feces was the main route of elimination for bisphenol A and bile was the main elimination route for bisphenol A glucuronide.

A study by Yokota et al. (146) examined the hepatic isoform of uridine diphosphate glucuronosyltransferase (UDPGT) involved in the metabolism of bisphenol A and distribution of the enzyme in organs of Wistar rats. Using yeast cells genetically engineered to express single rat UDPGT enzymes, it was determined that UGT2B1 was the only isoform capable of glucuronidating bisphenol A. Microsomal UDPGT activity towards bisphenol A was demonstrated in liver, kidney, and testis, but activity was minimal in lung and brain. [Minimal activity was also observed for intestine]. Northern blot analyses revealed high expression of UGTB1 only in liver. It was demonstrated that 65% of glucuronidation activity was absorbed by binding with anti-UGTB1, indicating that additional isoforms are likely involved in glucuronidation of bisphenol A.

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1 The intestine was determined to play a role in the metabolism of bisphenol A in rats. Nine-week-old male  
2 Sprague Dawley rats were orally administered 0.1 mL of a solution containing 50 g/L bisphenol A [**5 mg**  
3 **total or ~17 mg/kg bw assuming a body weight of ~0.3 kg (115)**] (147). Rats were killed at multiple  
4 time intervals between 15 minutes and 12 hours following exposure. The small intestine was removed and  
5 separated into upper and lower portions. Intestinal contents were removed from each section. Bisphenol A  
6 and metabolite concentrations were measured by HPLC. Activities and expression of  $\beta$ -glucuronidase  
7 were determined. A large amount of bisphenol A glucuronide was detected in the upper and lower  
8 portions of the small intestine, and a large amount of free bisphenol A was detected in the cecum. Less  
9 bisphenol A was detected in colon and feces. The observations lead the study authors to conclude that free  
10 bisphenol A generated in the cecum as a result of deconjugation was reabsorbed in the colon. The  
11 presence of large amounts of bisphenol A glucuronide in the small intestine at 12 hours following  
12 exposure suggested that bisphenol A was reabsorbed in the colon and re-excreted as the glucuronide. As  
13 determined in an assay using *p*-nitrophenol- $\beta$ -*d*-glucuronide as a substrate, ~70% of total  $\beta$ -glucuronidase  
14 activity was present in the cecum and 30% in the colon. Western blot analysis revealed a large amount of  
15 bacterial  $\beta$ -glucuronidase protein in cecum and colon contents.

16  
17 Glucuronidation and absorption of bisphenol A in rat intestine were studied by Inoue et al. (148).  
18 Intestines were obtained from 8-week-old male Sprague Dawley rats, and the small intestine was divided  
19 into 4 sections. Small intestine and colon were everted and exposed to 40 mL of a solution containing  
20 bisphenol A at 10, 50, or 100  $\mu$ M [**2.3, 11, or 23 mg/L, resulting in delivery of 91, 456, or 913  $\mu$ g**  
21 **bisphenol A to the everted intestine**]. Every 20 minutes during a 60-minute time period, reaction  
22 products were collected from serosal and mucosal sides and analyzed by HPLC. Optimal glucuronidation  
23 was observed at 50  $\mu$ M [**11 mg/L**]. At 60 minutes following exposure to 50  $\mu$ M bisphenol A, ~37% of  
24 bisphenol A was absorbed by the small intestine and ~83% was glucuronidated. Approximately 74.7% of  
25 the glucuronide was excreted on the mucosal side and ~25.3% transported to the serosal side of small  
26 intestine. Slightly greater absorption of bisphenol A in the colon (48.6%) compared to the proximal  
27 jejunum (37.5%) was observed at 60 minutes following exposure to the 50  $\mu$ M solution. Transport of both  
28 bisphenol A and bisphenol A glucuronide to the serosal side of intestine increased distally and was  
29 greatest in the colon. Minimal mucosal excretion was observed in the colon.

30  
31 Inoue et al. (149) compared glucuronidation of bisphenol A in pregnant, non-pregnant, and male rats.  
32 Livers of 4 male and non-pregnant Sprague Dawley rats/group were perfused via the portal vein for 1  
33 hour with solutions containing bisphenol A at 10 or 50  $\mu$ M [**2.3 or 11 mg/L**]. The total amount of  
34 bisphenol A infused into livers was 1.5 or 7.5  $\mu$ mol [**0.34 or 1.7 mg**]. On GD 20 or 21, livers of 4  
35 pregnant Sprague Dawley rats were perfused for 1 hour with 10  $\mu$ M [**2.3 mg/L**] bisphenol A. At the start  
36 of perfusion, excreted bile and perfusate in the vein were collected every 5 minutes for 1 hour. Samples  
37 were analyzed by HPLC. Statistical analyses were conducted by Student *t*-test and ANOVA. Bisphenol A  
38 glucuronidation in the liver was 59% in male rats and 84% in non-pregnant female rats perfused with the  
39 10  $\mu$ M solution. The glucuronide was excreted primarily through bile in both males and females, but a  
40 significantly higher amount was excreted through bile in non-pregnant females than in males. The total  
41 amount of glucuronide excreted into bile and vein was ~1.4-fold higher in females than males following  
42 perfusion with the 10  $\mu$ M [**2.3 mg/L**] solution. At the 50  $\mu$ M [**11 mg/L**] concentration, bisphenol A  
43 glucuronidated within liver was 66% in males and 91% in females. In males the glucuronide was excreted  
44 mainly in bile, and in females, a higher amount of glucuronide was excreted in the vein. In livers of  
45 pregnant rats perfused with the 10  $\mu$ M [**2.3 mg/L**] solution, 69% of bisphenol A was glucuronidated in  
46 the liver. Percentages of glucuronide excretion were 54.5% through bile and 45.5% through the vein in  
47 pregnant rats. In a comparison of pregnant rats and non-pregnant rats perfused with 10  $\mu$ M [**2.3 mg/L**]  
48 bisphenol A, biliary excretion in pregnant rats was half that observed in non-pregnant rats, and venous  
49 excretion in pregnant rats was 3-fold higher than in non-pregnant rats. To determine the pathway of  
50 bisphenol A glucuronide excretion, livers of 4 male Eisai hyperbilirubinemic rats, a strain deficient in  
51 multidrug resistance-associated protein, were perfused with 50  $\mu$ M [**11 mg/L**] bisphenol A. During and  
52 after perfusion, nearly all of the bisphenol A was excreted into the vein, thus indicating that multidrug

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1 resistance-associated protein mediates biliary excretion of bisphenol A glucuronide. The study authors  
2 concluded that bisphenol A is highly glucuronidated and excreted into bile using a multidrug resistance-  
3 associated protein-dependent mechanism, and that venous excretion increases and biliary excretion  
4 decreases during pregnancy.

5  
6 Miyakoda et al. (150) examined the production of bisphenol A glucuronide in fetal and adult rats.  
7 Bisphenol A was orally administered at 10 mg/kg bw to pregnant Wistar rats on GD 19 and to 10-week-  
8 old adult male Wistar rats. **[The number of animals exposed was not reported. In some legends for  
9 study figures, it was stated that the data were from 4 experiments, suggesting that 4 pregnant rats  
10 and adult males may have been exposed.]** Fetuses were removed at 1 hour following dosing. Blood was  
11 drawn and testes were removed from adult males at 1, 3, and 8 hours following dosing. GC/MS was used  
12 to measure bisphenol A concentrations in 19 fetuses and in testis of adult rats prior to and following  
13 homogenization with  $\beta$ -glucuronidase. In fetal extracts, there were no differences in bisphenol A  
14 concentrations before or after treatment with  $\beta$ -glucuronidase, suggesting that bisphenol A glucuronide  
15 was not present at detectable concentrations. The study authors noted the possibility that bisphenol A  
16 glucuronide was not transferred from dams to fetuses and stated that glucuronidation by the rat fetus is  
17 unlikely. At 1 hour following dosing of adult male rats, 90% of bisphenol A was detected as glucuronide  
18 in plasma and testis. Bisphenol A glucuronide concentrations gradually decreased and bisphenol A  
19 concentrations increased slightly in testis over the 8-hour sampling period. In plasma, bisphenol A-  
20 glucuronide decreased to 55% of the maximum observed concentration at 3 hours following dosing and  
21 increased to 100% of maximum observed concentration at 8 hours following dosing. Based on  
22 concentrations of bisphenol A glucuronide in testis and blood (40 ppb [ $\mu\text{g}/\text{kg}$ ] and 600 ppb [ $\mu\text{g}/\text{L}$ ]) at 8  
23 hours, the study authors concluded that bisphenol A glucuronide passage through the testicular barrier  
24 was unlikely. It was thought that bisphenol A passed through the testicular barrier, was converted to the  
25 glucuronide within the testis, and was then gradually released following digestion of the glucuronide by  
26  $\beta$ -glucuronidase.

27  
28 Matsumoto et al. (151), studied developmental changes in expression and activity of the UDPGT isoform  
29 UGT2B towards bisphenol A in Wistar rats. Activity towards other compounds was also examined but  
30 this summary focuses on bisphenol A. Microsomes were prepared from livers of fetuses, neonates on  
31 PND 3, 7, 14, and 21, and pregnant rats on GD 10, 15, and 19. Activity towards the bisphenol A substrate  
32 was measured using an HPLC method. Expression of UGT2B1 protein was examined by Western blot  
33 and messenger ribonucleic acid (mRNA) expression was examined by Northern blot. Little-to-no UGT2B  
34 activity towards bisphenol A was detected in microsomes of fetuses. Activity increased linearly following  
35 birth and reached adult concentrations by PND 21. **[No data on UGT2B activity for non-pregnant  
36 adult rats were shown and it was not clear if activity in adults was examined in this study.]** The  
37 same developmental patterns were observed for expression of UGT2B1 protein and mRNA. Activity and  
38 protein expression of UGT2B1 were also found to be reduced in pregnant rats.

39  
40 The European Union (2) reviewed an unpublished study by Sipes that compared clearance of bisphenol A  
41 by hepatic microsome from fetal (n = 8/sex), immature (n = 4/sex), and adult (n = 4) rats. The clearance  
42 rate in microsomes from male and female GD 19 rat fetuses (0.7–0.9 mL/minute/mg) was lower than  
43 clearance rates in microsomes from 4-day-old males and females (1.2–2.6 mL/minute/mg), 21-day-old  
44 males and females (2.4–2.7 mL/minute/mg), and their dams (2.6 mL/minute/mg). The European Union  
45 concluded that clearance rate was lower in fetuses but reached adult concentrations by 4 days of age.

46  
47 In a qualitative study of bisphenol A metabolites in pregnant mice injected with 0.025 mg/kg bw  
48 bisphenol A, 10 radioactive peaks were observed in urine by Zalko et al. (135). The major metabolites  
49 detected in urine were bisphenol A glucuronide and a hydroxylated bisphenol A glucuronide. Unchanged  
50 bisphenol A was the major compound detected in feces (>95%). Bisphenol A glucuronide represented  
51 more than 90% of the compounds detected in bile. Additional compounds detected in urine, feces,  
52 digestive tract, or liver included a double glucuronide of bisphenol A and sulfate conjugates. Unchanged

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1 bisphenol A, bisphenol A glucuronide, and “metabolite F” (disaccharide conjugate of BPA) were the  
2 major compounds detected in all tissues. **[Authors state that formation of glucuronic acid conjugate of**  
3 **BPA, several double conjugates, and conjugated methoxylated compounds, demonstrate the**  
4 **formation of potentially reactive intermediates]** The most abundant compound in all tissues was  
5 bisphenol A glucuronide, except in placenta where bisphenol A and metabolite F were the major  
6 compounds detected. Concentrations of bisphenol A decreased rapidly in all tissues. It was determined  
7 that metabolite F was most likely bisphenol A glucuronide conjugated to acetylated galactosamine or  
8 glucosamine. Distribution of bisphenol A and its metabolites in maternal and fetal tissues is summarized  
9 in [Table 31](#). Additional details of this study are included in Section 2.1.2.2.

10  
11 Jaeg et al. (152) reported metabolites observed following incubation of CD-1 mouse liver microsomes or  
12 S9 fractions with bisphenol A at 20–500  $\mu\text{M}$  **[4.6–114 mg/L]**. The metabolites included isopropyl-  
13 hydroxyphenol, bisphenol A glutathione conjugate, glutathionyl-phenol, glutathionyl 4-isopropylphenol,  
14 2,2-bis-(4-hydroxyphenyl)1-propanol, 5-hydroxy bisphenol A, and bisphenol A dimers. It was postulated  
15 that bisphenol A-ortho-quinone, produced from 5-hydroxy bisphenol A (catechol), may be the reactive  
16 intermediate leading to the formation of these metabolites..

17  
18 Kurebayshi et al. (124) examined metabolism of bisphenol A in monkeys. Three adult male and female  
19 Cynomolgus monkeys were dosed with 0.1 mg/kg bw  $^{14}\text{C}$ -bisphenol A/non-radiolabeled bisphenol A by  
20 iv injection on study day 1 and by gavage on study day 15 (124). Additional details of the study are  
21 included in Section 2.1.2.2. Up to five peaks were identified in urine. Analysis by radio-HPLC suggested  
22 that the major peaks in both sexes treated by either exposure route were mono- and diglucuronides. Five  
23 peaks were identified in plasma, and some differences were noted in comparisons of iv to oral exposure.  
24 In the 2 hours following dosing, most of the radioactivity in plasma was represented by bisphenol A  
25 glucuronide after iv dosing (57–82%) and oral dosing (89–100%). The percentage of radioactivity  
26 represented by unchanged bisphenol A was higher following iv (5–29%) than oral (0–1%) dosing.

27  
28 Kang et al. (153) reviewed studies that provided some information about metabolism of bisphenol A in  
29 fish and birds. One study reported bisphenol A sulfate and bisphenol A glucuronide as the major  
30 metabolites detected in zebrafish exposed to bisphenol A. A second study conducted in carp reported an  
31 increase in UDPGT activity for bisphenol A in microsomes and metabolism of bisphenol A to bisphenol  
32 A glucuronide in intestine. In quail embryos, metabolism and excretion of bisphenol A was reported, but  
33 specific metabolites were not indicated. Another study reported that  $^{14}\text{C}$ -bisphenol A administered orally  
34 or iv to laying quail was rapidly removed via bile and excreted through feces.

### 35 2.1.2.4 Elimination

36 Elimination of bisphenol A and its metabolites was examined in Sprague Dawley rats that were gavaged  
37 with bisphenol A and  $^{14}\text{C}$ -bisphenol A at 10 mg/kg bw (126). One group of rats was not pregnant, and 3  
38 additional groups were treated on either GD 6 (early gestation), 14 (mid gestation), or 17 (late gestation).  
39 More details of this study are available in Section 2.1.2.2. Most of the radioactivity (65–78%) was  
40 eliminated in feces. Elimination in urine accounted for 14–22% of the dose, and considerable variability  
41 for urinary elimination among animals was evident by the large standard deviations, which were 50% of  
42 means. The authors stated that bisphenol A glucuronide represented 62–70% of radioactivity in urine and  
43 bisphenol A represented 19–23% of radioactivity in urine **[data were not shown by authors]**. A total of  
44 9 peaks were identified in urine. In feces, 83–89% of radioactivity was represented by bisphenol A and 2–  
45 3% was represented by bisphenol A glucuronide; 7 peaks were identified in feces. The study authors  
46 concluded that urinary elimination and fecal elimination of radioactivity were similar in pregnant and  
47 non-pregnant rats.

48  
49  
50 Difference in excretion following oral or iv exposure of rats to a low bisphenol A dose was examined by  
51 Kurebayashi et al. (138). Three male rats/group were exposed to 0.1 mg/kg bw  $^{14}\text{C}$ -bisphenol A (> 99%  
52 radiochemical purity) in phosphate buffer vehicle by oral gavage or iv injection. Radioactivity levels were

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1 measured in urine and feces, which were collected over a 48-hour period. Additional details of the study  
2 are included in Section 2.1.2.2. Results of that study are summarized in Table 44. With both oral and IV  
3 dosing, fecal excretion was the main route of elimination.

4  
5 **Table 44. Excretion of Radioactivity Following Oral or iv Dosing of Rats with 0.1 mg/kg bw <sup>14</sup>C-**  
6 **Bisphenol A**

Time post dosing, hours	Percent radioactive dose excreted		
	Urine	Feces	Total
Oral			
0–24	6.3 ± 1.1	49.3 ± 2.1	55.7 ± 2.8
24–48	3.8 ± 1.0	32.3 ± 2.1	36.1 ± 3.0
Total	10.1 ± 1.6	81.6 ± 3.7	91.8 ± 5.0
iv			
0–24	8.4 ± 1.8	46.2 ± 1.8	54.6 ± 3.4
24–48	4.1 ± 0.9	31.4 ± 1.5	35.4 ± 1.8
Total	12.5 ± 0.9	77.6 ± 1.8	90.1 ± 2.7

Values presented as mean ± SD.  
From Kurebayashi et al. (138)

7  
8 Kurebayashi et al. (127) examined elimination of radioactivity in 3 adult male and female F344 rats that  
9 were orally dosed with 0.1 mg/kg bw <sup>14</sup>C-bisphenol A. Urine and feces were collected over a 168-hour  
10 period and analyzed by liquid scintillation counting. Total radioactivity excreted in urine and feces over  
11 the 168-hour period was ~98% in males and females. In male rats, ~10% was excreted in urine and ~88%  
12 was excreted in feces. Female rats excreted ~34% of the radioactivity in urine and ~64% in feces. **[The**  
13 **majority of radioactivity, ~90%, was excreted over 48 hours by males and 72 hours by females.]**  
14

15 Snyder et al. (129) compared toxicokinetics of bisphenol A in CD and F344 rats. Four CD and F344 rats  
16 were gavaged with 100 mg/kg bw <sup>14</sup>C-bisphenol A in propylene glycol vehicle. Disposition of  
17 radioactivity in urine, feces, and carcass was examined over a 144-hour period. Samples were analyzed by  
18 scintillation counting, HPLC, or nuclear magnetic resonance. Data were analyzed by ArcSin  
19 transformation of the square root of the mean and using two-sample *t*-test. Recovery of radioactivity was  
20 93% in both strains. The highest concentrations of radioactivity were detected in feces (70% of dose in  
21 CD rat and 50% of dose in F344 rats) followed by urine (21% of dose in CD rat and 42% of dose in F344  
22 rats). The percentages of the dose excreted in urine and feces differed significantly by strain. Much lower  
23 percentages of radioactivity were detected in the carcass (~1%). Bisphenol A glucuronide, representing  
24 81–89% of the dose, was the major urinary metabolite detected in both strains. A much lower percentage  
25 (2.2–10%) of the dose was represented by urinary bisphenol A.  
26

27 Kim et al. (154) reported urinary excretion of bisphenol A in 4-week-old male F344 rats given bisphenol  
28 A in drinking water at 0 (ethanol vehicle), 0.1, 1, 10, or 100 ppm (equivalent to 0.011, 0.116, 1.094, or  
29 11.846 mg/kg bw/day) for 13 weeks. Urine samples were collected for 24 hours following administration  
30 of the last dose and analyzed by HPLC before and after digestion with β-glucuronidase. The focus of the  
31 study was male reproductive toxicity; the study is described in detail in Section 4.2.2.1. Bisphenol A was  
32 not detected in the urine of rats from the control and 2 lowest dose groups. **[At the 2 highest doses, free**  
33 **bisphenol A represented 60 and 30% of the total urinary bisphenol A concentrations.]**  
34

35 In rats exposed to 10 or 100 mg/kg bw/day <sup>14</sup>C-bisphenol A through the oral, ip, or sc routes, fecal  
36 elimination represented the highest percentage of radioactivity in all exposure groups (52–83%) (119).  
37 Elimination of radioactivity through urine was ~2-fold higher in females (21–34%) than males (13–16%)  
38 in all dose groups. Additional details of this study are included in Section 2.1.2.3.  
39

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1 Elimination of bisphenol A and metabolites was examined in 3 adult male and female Cynomolgus  
 2 monkeys dosed with 0.1 mg/kg bw <sup>14</sup>C-bisphenol A/non-radiolabeled bisphenol A by iv injection on  
 3 study day 1 and by gavage on study day 15 (124). Additional details of the study are included in Section  
 4 2.1.2.2. Following oral or iv exposure, the percentage of radioactivity recovered in excreta and cage  
 5 washes was 81–88% over a 1-week period. Most of the radioactivity was recovered in urine (combination  
 6 of urine and cage washes), with most of the radioactivity excreted in urine within 12 hours and essentially  
 7 all of the dose excreted within 24 hours following treatment. Percentages of radioactive doses recovered  
 8 in urine within 1 week after dosing were ~79–86% following iv dosing and 82–85% following oral  
 9 dosing. Much smaller amounts were recovered in feces during the week following iv or oral exposure  
 10 (~2–3%). The study authors concluded that because fecal excretion was very low following oral exposure,  
 11 absorption was considered to be complete. The authors also noted that there were no obvious route or sex  
 12 differences in excretion of radioactivity. The study authors concluded that terminal elimination half-lives  
 13 were longer following iv than oral exposure. A limited amount of information was presented for the fast  
 14 phase, defined as the 2 hours following iv injection. Fast-phase elimination half-life of bisphenol A  
 15 following iv exposure was significantly lower in females (0.39 hours) than males (0.57 hours). There  
 16 were no sex-related differences in fast-phase half-life for bisphenol A glucuronide (0.79–0.82 hours) or  
 17 total radioactivity (0.61–0.67 hours).

### 19 2.1.3 Comparison of humans and experimental animals

20 Studies comparing toxicokinetics and metabolism of bisphenol A in humans and laboratory animals were  
 21 reviewed and are summarized below. In most cases the data were from original sources, but information  
 22 from secondary sources was included if the information was not new or critical to the evaluation of  
 23 developmental or reproductive toxicity.

25 Elsby et al. (142) compared bisphenol A metabolism by rat and human microsomes. Microsomes were  
 26 obtained from 8 immature Wistar rats (21–25 days old) and histologically normal livers from 4 male (25–  
 27 57 years old) and 4 female (35–65 years old) Caucasian donors who were killed in accidents. Human  
 28 microsomes were pooled according to sex of the donor. Glucuronidation was examined following  
 29 exposure of microsomes to bisphenol A concentrations of 0–1000 μM [0–228 mg/L] for 30 minutes with  
 30 human microsomes and 10 minutes with rat microsomes. Metabolites were identified by HPLC or  
 31 LC/MS. Data were obtained from 4 experiments conducted in duplicate. Data were analyzed by Mann-  
 32 Whitney test. Maximum velocity ( $V_{max}$ ) and the rate constant ( $K_m$ ) values are summarized in Table 45.  
 33 The study authors reported a significant difference between the  $V_{max}$  for glucuronidation in immature rats  
 34 and humans. No sex-related difference was reported for glucuronidation by human microsomes. As a  
 35 result of less extensive glucuronidation by human than rat microsomes, the study authors noted that  
 36 estrogen target tissues in humans may receive higher exposure to bisphenol A than tissues of immature  
 37 female rats used in estrogenicity studies. Lastly, oxidation of bisphenol A by female rat or human  
 38 microsomes was examined following incubation with 200 μM [46 mg/L] bisphenol A and NADPH. The  
 39 only metabolite identified was 5-hydroxybisphenol A.

41 **Table 45. Glucuronidation Kinetics in Microsomes From Immature Rats and Adult Humans**

Sex/Species	$V_{max}$ , nmol/minute/mg protein	$K_m$ , μM
Male/human	5.9 ± 0.4	77.5 ± 8.3
Female/human	5.2 ± 0.3	66.3 ± 7.5
Female/immature rat	31.6 ± 8.1	27.0 ± 1.2

Data presented as mean ± SEM.

From Elsby et al. (142).

42  
 43 The European Union (2) reviewed a series of studies by Sipes that compared metabolism of bisphenol A  
 44 in microsomes from male and female humans (15 pooled samples/sex and 3–5 individual samples/sex),  
 45 rats (4/sex), and mice (4/sex). It was concluded that the studies generally agreed with the findings of  
 46 Elsby et al. (142). Clearance rates ( $V_{max}/K_m$ ) in human microsomes (0.4–0.9 mL/minute/mg for pooled

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1 samples and 0.3–0.5 mL/minute/mg in individual samples) were lower than those observed in rats (1.0–  
2 1.7 mL/minute/mg) and mice (1.3–3.0 mL/minute/mg).  
3

4 Pritchett et al. (143) compared metabolism of bisphenol A in hepatocyte cultures from humans, rats, and  
5 mice. Cell cultures were prepared from adult male and female F344 rats, Sprague Dawley rats, and CF1  
6 mice. Human hepatocyte cultures were obtained from 3 females and 2 males. [No information was  
7 provided about the age of human donors.] Cells were exposed to <sup>14</sup>C-bisphenol A (99.3% purity)/  
8 bisphenol A (>99% purity) in a DMSO vehicle. In a cytotoxicity assessment, lactate dehydrogenase  
9 activity was measured in rat cells following incubation for 18 hours in 5–100 μM [1.1–23 mg/L]  
10 bisphenol A, and cytotoxicity was observed at ≥75 μM bisphenol A. Bisphenol A concentrations tested  
11 and times of exposure were 5–20 μM [1.1–4.6 mg/L] for up to 6 hours in time-dependent metabolism  
12 studies and 2.5–30 μM [0.57–6.8 mg/L] for 10 minutes in concentration-dependent metabolism studies.  
13 Metabolites in cell media were analyzed by HPLC and LC-MS/MS.  
14

15 Analysis of media from human hepatocytes incubated with bisphenol A indicated that the major  
16 metabolite was bisphenol A glucuronide, and compounds found at lower concentrations were bisphenol A  
17 glucuronide/sulfate diconjugate, and bisphenol A sulfate conjugate. Table 46 summarizes percentages of  
18 each type of metabolite detected in media following incubation with 20 μM [4.6 mg/L] bisphenol A for 3  
19 hours in human cells and 6 hours in rodent cells. In cells from all sexes and species except male F344 rats,  
20 bisphenol A glucuronide was the major metabolite detected. The glucuronide/sulfate diconjugate was the  
21 major metabolite detected in cells from male F344 rats. In concentration-dependent studies conducted in  
22 F344 rat hepatocytes, a biphasic curve was obtained following a 10-minute incubation, with a V<sub>max</sub> of  
23 0.36 nmol/min at bisphenol A concentrations of 20–30 μM [4.6–6.8 mg/L] and a V<sub>max</sub> of ~0.15 nmol/min  
24 at bisphenol A concentrations of 2.5–10 nM [0.57–2.3 mg/L]. Table 47 summarizes the higher V<sub>max</sub>  
25 values obtained with cells from human, rat, and mouse livers. Total hepatic capacity was determined by  
26 multiplying V<sub>max</sub> by total number of hepatocytes/liver in vivo. [The only graphical data presented were  
27 for male F344 rats]. The authors noted that V<sub>max</sub> values were highest in mice > rats > humans. However,  
28 when adjusted for total hepatocyte number in vivo, the values were predicted to be highest in humans >  
29 rats > mice.  
30

31 **Table 46. Metabolites Obtained from Incubation of Human, Rat, and Mouse Hepatocyte Cultures**  
32 **with 20 μM [4.6 mg/L] Bisphenol A**

Sex and species	Percentage of parent compound or metabolites			
	Glucuronide/sulfate	Sulfate	Glucuronide	Bisphenol A
<b>Human samples</b>				
Female-1	4	0	93	0
Female-2	2	0	84	2
Female-3	43	2	55	0
Male-1	1	0	85	0
Male-2	0	7.5	75	0
<b>Rodent samples</b>				
Male F344 rat	70	0	30	0
Female F344 rat	10	0	86	0
Male Sprague Dawley rat	30	2	58	0
Female Sprague Dawley rat	0	0	100	0
Male CF1 Mouse	0	0	100	0
Female CF1 mouse	0	0	93	0

Human cells were incubated for 3 hours, and animal cells were incubated for 6 hours.  
From Pritchett et al. (143).

1 **Table 47. Rates of Bisphenol A Glucuronide Formation Following Incubation of Human, Rat, and**  
 2 **Mouse Hepatocytes with Bisphenol A**

Species and sex	$V_{\max}$ , nmol/min/ $0.5 \times 10^6$ hepatocytes	Hepatic capacity, $\mu\text{mol/hours}^a$
Human female	0.27	8000
F344 rat female	0.46	46.5
F344 rat male	0.36	61.8
Sprague Dawley female	0.39	54.5
Sprague Dawley male	0.45	79.9
CF1 mouse female	0.50	13.8
CF1 mouse male	0.82	23.6

<sup>a</sup>Hepatic capacity was estimated by multiplying  $V_{\max}$  by total numbers of hepatic cells in vivo. From Pritchett et al. (143).

3  
 4 Data from Pritchett et al. (143) appeared to be included in a series of unpublished studies by Sipes that  
 5 were reviewed by the European Union (2). In their review, the European Union noted that metabolic  
 6 patterns appear to be similar in humans, rats, and mice. It was stated that the biphasic kinetic profile  
 7 indicated involvement of a high-affinity glucuronidase enzyme at low concentrations and a high-capacity  
 8 enzyme at high concentrations. In the interpretation of kinetic profiles in humans and experimental  
 9 animals, the authors of the European Union report noted that the study calculations did not consider in  
 10 vivo conditions such as varying metabolic capacity of hepatic cells, relationship of hepatic size to body  
 11 size, and possibly important physiological endpoints such as blood flow. In addition, it was noted that  
 12 calculations were based on limited data that did not address inter-individual variability in enzyme  
 13 expression.

14  
 15 Cho et al. (155) examined toxicokinetics of bisphenol A in mouse, rat, rabbit, and dog and used that  
 16 information to predict toxicokinetic values in humans. Bisphenol A was administered by iv injection at 2  
 17 mg/kg bw to 5 male ICR mice and at 1 mg/kg bw to 7 male Sprague Dawley rats, 7 male New Zealand  
 18 White rabbits, and 5 male beagle dogs. Blood samples were drawn before dosing and at multiple time  
 19 points between 2 minutes and 6 hours following injection. Serum bisphenol A concentrations were  
 20 measured by HPLC. Toxicokinetic endpoints in animals are summarized in Table 48. The study authors  
 21 noted that clearance and volume of distribution increased with increasing animal weight but that terminal  
 22 half-life remained relatively constant across the different species. Simple allometric scaling and species-  
 23 invariant time methods were used to predict values for a 70 kg human, and those values are summarized  
 24 in Table 49. Regression analyses of estimates using the species-invariant time methods demonstrated that  
 25 data from the 4 animal species were superimposable ( $r = 0.94\text{--}0.949$ ).

26  
 27 **Table 48. Toxicokinetic Endpoints for Bisphenol A in Mice, Rats, Rabbits, and Dogs iv Dosed with**  
 28 **2 mg/kg bw Bisphenol A**

Endpoint	Mouse <sup>a</sup>	Rat	Rabbit	Dog
Systemic clearance, L/hour	0.3	$1.9 \pm 0.4$	$12.6 \pm 4.9$	$27.1 \pm 8.0$
Volume of distribution, L	0.1	$1.3 \pm 0.4$	$7.1 \pm 2.3$	$20.0 \pm 5.4$
Half-life, minute	39.9	$37.6 \pm 12.8$	$40.8 \pm 17.1$	$43.7 \pm 21.9$

Data are presented as mean  $\pm$  SD.

<sup>a</sup>Variances not reported.

From Cho et al. (155).

1 **Table 49. Predicted Bisphenol A Toxicokinetic Endpoints in Humans Based on Results from**  
 2 **Experimental Animal Studies**

Endpoint	Prediction method			
	Allometric scaling	Kallynochrons	Apolysichrons	Dienetichrons
Systemic clearance, L/hour	127.1	123	120.7	46.0
Volume of distribution, L	125.3	229.7	138.0	149.3
Half-life, minute	43.6	110.4	67.8	196.2

From Cho et al. (155).

3  
 4 Teeguarden et al. (139) developed a physiologically based pharmacokinetic (PBPK) model for bisphenol  
 5 A. Rat toxicokinetic data for the model were obtained from the studies by Pottenger et al. (119) and  
 6 Upmeier et al. (123). Human toxicokinetic data were obtained from the study by Völkel et al. (109). The  
 7 model was developed to simulate blood and uterine concentrations of bisphenol A following exposure of  
 8 humans through relevant routes. Correlations were determined for simulated bisphenol A binding to  
 9 uterine receptors and increases in uterine wet weight, as determined by an unpublished study by Twomey.  
 10 Although intestinal metabolism of bisphenol A to the glucuronide metabolite had been recently  
 11 demonstrated, the model attributed bisphenol A metabolism entirely to the liver. Plasma protein binding  
 12 was considered in both the rat and human model. The model accurately simulated plasma bisphenol A  
 13 glucuronide concentrations in humans orally administered 5 mg bisphenol A, with the exception of  
 14 underpredicting bisphenol A glucuronide concentrations at the 24–48 hour period following exposures.  
 15 Cumulative urinary elimination of bisphenol A glucuronide in human males and females was accurately  
 16 simulated. Less accurate simulations were observed for toxicokinetics in orally exposed rats, and the  
 17 study authors indicated that a likely cause was oversimplification of the rat gastrointestinal compartment.  
 18 Comparisons in metabolic clearance rates for iv and oral exposure suggested significant intestinal  
 19 glucuronidation of bisphenol A. Enterohepatic recirculation strongly affected terminal elimination in rats  
 20 but not humans. Consideration of bound versus unbound bisphenol A was found to be important in  
 21 simulating occupancy of the estrogen receptor (ER) and uterine weight response. No increase in uterine  
 22 weight was reported with simulated receptor occupancy of ~1–15%. An increase in uterine weight was  
 23 reported with ~25% receptor occupancy, and doubling of uterine weight was reported with 63% receptor  
 24 occupancy.

25  
 26 Shin et al. (156) developed a PBPK model to predict the tissue distribution (lung, liver, spleen, kidneys,  
 27 heart, testes, muscle, brain, adipose tissue, stomach, and small intestine) and blood pharmacokinetics of  
 28 bisphenol A in rats and humans. The model was based on experimentally determined steady state blood-  
 29 to-serum and tissue-to-blood partition ratios and does not include parameters to account for elimination  
 30 via glucuronidation or differences in metabolism between rats and humans (e.g., enterohepatic  
 31 circulation). Predicted concentration-time profiles were then compared to actual rat toxicokinetic data and  
 32 to a profile for a simulated 70-kg human. Rat toxicokinetic information was obtained by administering  
 33 multiple iv injections of bisphenol A (0.5 mg/kg) to adult male rats to achieve steady state. Bisphenol A  
 34 concentrations were determined by a modified HPLC method with fluorescence detection. The authors  
 35 noted good agreement between predicted and observed concentration-time profiles for blood and all  
 36 tissues but did not present any statistical analysis or evaluate the performance of alternative models in  
 37 order to establish goodness of fit. Based on the figures presented in the article, the PBPK model appeared  
 38 to more accurately predict concentrations of bisphenol A in some tissues (e.g., blood, lung and liver)  
 39 better than others such as the small intestine and adipose tissue. The model was then applied to predict  
 40 blood and tissue levels of bisphenol A in a 70 kg human after single iv injection (5-mg dose) and multiple  
 41 oral administrations to steady state (100-mg doses every 24 h). Tissue volumes and blood flow rates for a  
 42 70 kg human were taken from the literature. The authors concluded that simulated steady-state human  
 43 blood levels (0.9 – 1.6 ng/ml) were comparable to blood levels of bisphenol A reported in the literature  
 44 (1.49 ng/ml). In addition, the authors noted the similarity of predicted toxicokinetic endpoints obtained

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1 from their PBPK model to those predicted by Cho et al (155) based on simple allometric scaling on rat  
2 data.

### 4 **2.2 General Toxicity, Estrogenicity, and Androgenicity**

5 This section includes information on general toxicity as well as information on estrogenicity and  
6 androgenicity; however, results of estrogenicity and androgenicity testing are not considered a priori  
7 evidence of toxicity.

#### 9 *2.2.1 General toxicity*

10 The European Union (2) reported there were no adequate studies for assessing acute toxicity of bisphenol  
11 A in humans.

13 In an acute toxicity study in rats orally dosed with bisphenol A at  $\geq 2000$  mg/kg bw, clinical signs  
14 included lethargy, prostration, hunched posture, and piloerection [reviewed by the European Union (2)].  
15 Gross signs in animals that died included pale livers and hemorrhage in the gastrointestinal tract. In a  
16 study in which male and female rats were subjected to whole body inhalation exposure to  $170 \text{ mg/m}^3$   
17 bisphenol A dust for 6 hours, there were no gross signs of toxicity [reviewed by the European Union (2)].  
18 Effects observed in the respiratory tract at 2 but not 14 days following exposure included slight  
19 inflammation of nasal epithelium and slight ulceration of the oronasal duct.  $\text{LD}_{50}$ s reported in studies with  
20 oral, dermal, inhalation, or ip exposure are summarized in Table 50. The European Union (2) concluded  
21 that bisphenol A is of low acute toxicity through all exposure routes relevant to humans.

22 **Table 50.  $\text{LD}_{50}$ s for Bisphenol A**

Species	Exposure route	$\text{LD}_{50}$ (mg/kg bw)
Rat	Oral	3300–4100 <sup>a</sup>
		5000 <sup>b</sup>
		3250 <sup>c</sup>
Mouse	Inhalation	$>170 \text{ mg/m}^3$ <sup>b</sup>
	Oral	4100–5200 <sup>a</sup>
Guinea pig	ip	2400 <sup>c</sup>
	Oral	150 <sup>c</sup>
Rabbit	Oral	4000 <sup>c</sup>
	Oral	2230 <sup>b,c</sup>
	Dermal	$> 2000$ <sup>b</sup>
		3 mL/kg <sup>c</sup>

<sup>a</sup>National Toxicology Program (NTP) (157).

<sup>b</sup>Reviewed by the European Union (2).

<sup>c</sup>Reviewed in ChemIDplus (1).

24  
25 The European Union (2) noted limited anecdotal data reporting skin, eye, and respiratory tract irritation in  
26 workers exposed to bisphenol A, but concluded that the reports were of uncertain reliability. It was noted  
27 that a recent, well-conducted study in rabbits demonstrated that bisphenol A is not a skin irritant. Other  
28 studies conducted in rabbits demonstrated eye irritation and damage, and it was concluded the bisphenol  
29 A can potentially cause serious eye damage. Slight respiratory tract inflammation occurred in rats  
30 inhaling  $\geq 50 \text{ mg/m}^3$  bisphenol A, and it was concluded that bisphenol A had limited potential for  
31 respiratory irritation. Based on the results of the studies described above, the European Union concluded  
32 that bisphenol A is not corrosive.

33  
34 The European Union (2) reviewed studies examining possible sensitization reactions in humans exposed  
35 to products containing bisphenol A, and those studies reported mixed results. In studies reporting positive  
36 findings, it was unclear if bisphenol A or epoxy resins were the cause of hypersensitivity. Cross-  
37 sensitization responses in individuals exposed to compounds similar to bisphenol A were also reported.

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1 Animal studies were determined unreliable for assessing sensitization. Based on the results of human  
2 studies, it was concluded that bisphenol A may have potential for sensitization in individuals exposed to  
3 resins. Human studies suggested that bisphenol A can induce dermal photosensitization responses.  
4 Photosensitization studies in mice resulted in reproducible positive results. Mechanistic studies in mice  
5 suggested that sensitization occurs through an immune-mediated process. The overall conclusion of the  
6 European Union was that it was somewhat unclear if bisphenol A induces orthodox skin sensitization,  
7 photosensitization, or responses in individuals previously sensitized to another substance, such as epoxy  
8 resins. No information was available on potential respiratory sensitization by bisphenol A.  
9

10 The European Union (2) summarized systemic toxicity reported in subchronic, chronic, and reproductive  
11 toxicity studies of rats, mice, and dogs. CERHR also reviewed the studies that examined reproductive  
12 organs, and those studies are summarized in detail in the appropriate section of this report. A relevant  
13 study by Yamasaki et al. (158) was published subsequent to the European Union review and was  
14 reviewed in detail by CERHR.  
15

16 In studies reviewed by the European Union (2) and in a study by Yamasaki et al. (158), rats were orally  
17 exposed to bisphenol A for periods of 28 days to 2 years. Cecal enlargement occurring at doses  $\geq 25$   
18 mg/kg bw/day was the most frequently observed effect in those studies but was not considered  
19 toxicologically significant by the European Union. Histological alteration in the cecum consisting of  
20 mucosal hyperplasia was only reported in one study at doses  $\geq 200$  mg/kg bw/day. Histopathological  
21 changes in liver and kidney were reported at doses  $\geq 500$  mg/kg bw/day. The changes in liver were  
22 characterized by prominent hepatocyte nuclei or inflammation. Histopathology in kidney was  
23 characterized by renal tubule degeneration or necrosis. Testicular toxicity (degeneration of seminiferous  
24 tubules and arrested spermatogenesis) was observed in 1 study at doses  $\geq 235$  mg/kg bw/day.  
25

26 The European Union (2) found subchronic and chronic studies conducted by the NTP (157) to be the only  
27 reliable studies for assessing systemic toxicity in mice orally exposed to bisphenol A. The liver was found  
28 to be the target organ of toxicity, with multinucleated giant hepatocytes observed in male mice exposed to  
29  $\geq 120$  mg/kg bw/day and female mice exposed to 650 mg/kg bw/day.  
30

31 In a 90-day dietary study in dogs reviewed by the European Union (2), an increase in relative liver weight  
32 with no accompanying histopathological alterations was found to be the only effect at doses  $\geq 270$  mg/kg  
33 bw/day. This finding was considered by the European Union to be of doubtful toxicological significance.  
34

35 In a subchronic inhalation exposure study in rats reviewed by the European Union (2), cecal enlargement  
36 as a result of distention by food was observed at  $\geq 50$  mg/m<sup>3</sup>. Also observed at  $\geq 50$  mg/m<sup>3</sup> were slight  
37 hyperplasia and inflammation of epithelium in the anterior nasal cavity.  
38

39 A limited number of repeat-dose systemic toxicity studies were summarized in detail by CERHR because  
40 they included examination of reproductive organs. Those studies are summarized in detail below.  
41

42 NTP (157), conducted acute, subacute, and subchronic bisphenol A toxicity studies in F344 rats and  
43 B6C3F<sub>1</sub> mice. Animals were randomly assigned to treatment groups. Purity of bisphenol A was <98.2%.  
44 Concentration and stability of bisphenol A in feed were verified. In acute studies, single doses of  
45 bisphenol A in a 1.5% acacia vehicle were administered by gavage to 5 rats/group/sex at doses of 2150,  
46 3160, 4640, or 6810 mg/kg bw/day and 5 mice/group/sex at 1470, 2150, 3160, 4640, 6810, or 10,000  
47 mg/kg bw. LD<sub>50</sub> values for that study are summarized in [Table 50](#).  
48

49 In a 14-day repeat dose study, survival and body weight gain were evaluated in 5 rats and mice/sex/group  
50 that were fed diets containing bisphenol A at 0, 500, 1000, 2500, 5000, or 10,000 ppm. Survival was  
51 unaffected by treatment. Weight gain was reduced by 60% or more in male rats exposed to  $\geq 2500$  ppm

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1 and 40% or more in female rats exposed to  $\geq 5000$  ppm bisphenol A. Survival and weight gain in mice  
2 were not affected by Bisphenol A exposure.

3  
4 In subchronic studies, 10 rats and mice/sex/group were exposed to bisphenol A in diet for 13 weeks.  
5 Dietary doses were 0, 250, 500, 1000, 2000, or 4000 ppm for rats and 0, 5000, 10,000, 15,000, 20,000, or  
6 25,000 ppm for mice. A review by the European Union (2) estimated bisphenol A intake at 0, 25, 50, 100,  
7 200, and 400 mg/kg bw/day for rats, 0, 600, 1200, 1800, 2400, and 3000 mg/kg bw in male mice, and 0,  
8 650, 1300, 1950, 2600, and 3250 mg/kg bw/day in female mice. Animals were observed and weighed  
9 during the study and killed and necropsied on the 91<sup>st</sup> day of the study. **[Histopathological evaluations  
10 were conducted but it was not clear if all dose groups and all animals/dose group were examined.  
11 There was no mention of statistical analyses.]** In rats, the only deaths occurred in 2/10 males of the  
12 1000 ppm group. Weight gain was reduced by 18% or more in male rats and 10% or more in female rats  
13 exposed to  $\geq 1000$  ppm. There were no effects on feed intake. Hyaline masses in the bladder lumen were  
14 not observed in control male rats but were observed in 5 of 10 males exposed to 250 ppm, 3 of 10  
15 exposed to 500 ppm, 3 of 10 exposed to 1000 ppm, 6 of 10 exposed to 2000 ppm, and 4 of 10 exposed to  
16 4000 ppm. Cecal enlargement, which was observed in rats at a rate of 60–100% in each dose group with  
17 the exception of females exposed to 250 ppm was considered to be treatment-related. No histological  
18 alterations were observed in the cecum. Death in mice was limited to 2 of 10 females in the 5000 ppm  
19 group. Weight gain was reduced by at least 14% in male mice exposed to  $\geq 15,000$  ppm. Non-dose-related  
20 decreases in weight gain of 17% or more occurred in female mice of all dose groups. A dose-related  
21 increase in multinucleated giant hepatocytes was observed in all dose groups of male mice; the only  
22 incidence data reported for multinucleated giant hepatocytes were 0 of 10 female controls and 9 of 10  
23 male mice of the 25,000 ppm group. **[A complete set of data for histopathological findings was not  
24 presented for rats or mice.]**

25  
26 Yamasaki et al. (158) examined the effects of bisphenol A exposure on male and female CD rats in a  
27 study conducted according to Good Laboratory Practices (GLP). **[Because this study included a  
28 number of reproductive organ and hormone endpoints, it is also discussed in Sections 4.2.1.1 and  
29 4.2.2.1.]** Rats were fed a commercial diet (MF Oriental Yeast Co.) and housed in stainless steel wire-  
30 mesh cages. Rats were grouped according to body weight and then randomly assigned to treatment groups.  
31 Ten 7-week-old rats/sex/group were gavaged with bisphenol A at 0 (olive oil vehicle), 40, 200, or 1000  
32 mg/kg bw/day for 28 days. Due to the death of 1 animal exhibiting clinical signs in the 1000 mg/kg  
33 bw/day group, the high dose was reduced to 600 mg/kg bw/day on study day 8. In an additional study,  
34 rats were exposed to ethinyl estradiol at 0, 10, 50, or 200  $\mu\text{g}/\text{kg}$  bw/day for 28 days. Endpoints examined  
35 during the study were clinical signs, body weight gain, and food intake. Estrous cyclicity was examined in  
36 females for 2 weeks beginning on study day 15. Males were killed on study day 29 and females were  
37 killed in diestrus on study day 30, 31, or 32. Hematology and clinical chemistry endpoints were assessed,  
38 and blood hormone concentrations were measured by immunoassay systems. Sperm motility and viability  
39 were evaluated. Organs, including those of the reproductive system, were weighed and subjected to  
40 histopathological evaluation. With the exception of the testis and epididymis, which were fixed in Bouin  
41 solution, the organs were fixed in 10% neutral buffered formalin. Statistical analyses included Bartlett test  
42 for homogeneity of variance, ANOVA, Dunnett test, and/or Kruskal-Wallis test.

43  
44 One female and 3 males from the high-dose group died; clinical signs observed in those animals included  
45 soft stools, decreased mobility, reduced respiration rate, and decreased body temperature. Soft stools were  
46 also observed in surviving males and females of the mid- and high-dose groups. Results of the study are  
47 summarized in Table 51. Terminal body weights were lower in females of the mid- and high-dose groups  
48 and males of the high-dose group. During the first week of study, food intake was decreased in both sexes  
49 of the mid- and high-dose group. **[Data were not shown by study authors.]** As noted in Table 51,  
50 some alterations in hematological and clinical chemistry endpoints were observed, mainly at the high  
51 dose. **[Data were not shown by study authors.]** There were no treatment-related abnormalities in sperm  
52 or alterations in blood concentrations of thyroid hormones, follicle stimulating hormone (FSH),

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1 luteinizing hormone (LH), 17 $\beta$ -estradiol, prolactin, or testosterone. Number of females with diestrus  
 2 lasting 4 or more days was increased in the high-dose group. Changes in relative organ weights [**assumed**  
 3 **to be relative to body weight**] included decreased heart weight in females from the mid- and high-dose  
 4 groups. At the high dose, there were decreases in relative weight of ventral prostate and increases in  
 5 relative weights of testis and adrenals in males and thyroid and liver in females. Gross signs observed in  
 6 animals that died included enlarged kidney, elevated mucosa in the forestomach, and atrophied spleen and  
 7 thymus. In surviving animals, the cecum was enlarged in the mid- and high-dose group and forestomach  
 8 mucosa was elevated in the high-dose group. As described in more detail in [Table 51](#), histopathological  
 9 alterations were observed in the intestine, cecum, and colon of males and intestine and cecum of females  
 10 in the mid and high dose groups. Additional histopathological alterations were observed in the high-dose  
 11 group in the kidney, forestomach, and adrenals of males and females and livers of females.  
 12 Male rats from the mid- and high-dose ethinyl estradiol groups experienced decreased prostate, seminal  
 13 vesicle, and pituitary weights, increased testis weight, and histopathological alterations in prostate,  
 14 seminal vesicle, mammary gland, and testis. Females from the mid- and high-dose ethinyl estradiol group  
 15 experienced alterations in estrous cyclicity. Females from the high-dose group experienced decreased  
 16 ovary weight, increased uterine weight, and histopathological changes in ovary, uterus, and vagina.

17  
 18 **Table 51. Toxicological Effects in Rats Gavaged With Bisphenol A for 28 Days**

Endpoint	Bisphenol A dose (mg/kg bw/day)		
	40	200	600–1000 <sup>b</sup>
Males			
Terminal body weight	↔	↔	↓17%
Relative testes weight	↔	↔	↑21%
Relative ventral prostate weight	↔	↔	↓28%
Relative adrenal weight	↔	↔	↑19%
Feed intake <sup>a</sup>	↔	↓	↓
Prothrombin time <sup>a</sup>	↔	↔	↑
Glutamic-oxaloacetic transaminase <sup>a</sup>	↔	↑	↑
Triglyceride <sup>a</sup>	↔	↔	↓
Alkaline phosphatase <sup>a</sup>	↔	↔	↑
$\gamma$ -Glutamyl transpeptidase <sup>a</sup>	↔	↔	↑
Chloride <sup>a</sup>	↔	↔	↑
Renal tubular degeneration and necrosis	0/10	0/10	7/7
Forestomach squamous epithelial cell hyperplasia	0/10	0/10	6/7
Lacteal dilatation in duodenum	0/10	10/10	2/7
Lacteal dilation in jejunum	0/10	0/10	2/7
Mucosal hyperplasia in cecum	0/10	3/10	6/7
Mucosal hyperplasia in colon	0/10	2/10	7/7
Adrenal cortical vacuolization	0/10	0/10	3/7
Females			
Terminal body weight	↔	↓7%	↓5%
Relative thyroid weight	↔	↔	↑22%
Relative liver weight	↔	↔	↑10%
Relative heart weight	↔	↓9%	↓15%
Feed intake <sup>a</sup>	↔	↓	↓
Hemoglobin and hematocrit values <sup>a</sup>	↔	↔	↓
Cholinesterase <sup>a</sup>	↔	↓	↓
Glutamic-oxaloacetic transaminase <sup>a</sup>	↔	↔	↑
Albumin and albumin:globulin rats <sup>a</sup>	↔	↔	↓
Diestrus $\geq$ 4 days	0/10	0/10	3/9
Prominent hepatocyte nuclei	0/10	0/10	4/9
Renal tubular degeneration and necrosis	0/10	0/10	9/9

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Endpoint	Bisphenol A dose (mg/kg bw/day)		
	40	200	600–1000 <sup>b</sup>
Forestomach squamous epithelial cell hyperplasia	0/10	0/10	5/9
Lacteal dilatation in duodenum	0/10	7/10	6/9
Mucosal hyperplasia in cecum	0/10	6/10	4/9
Adrenal cortical vacuolization	0/10	0/10	3/9

↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

<sup>a</sup>Data were not shown by study authors.

<sup>b</sup>The dose was 1000 mg/kg bw/day at the beginning of the study, but was decreased to 600 mg/kg bw/day in the second week of the study due to excessive toxicity.

From Yamasaki et al. (158).

1  
2 General Electric (159) conducted a subchronic toxicity study in Beagle dogs orally dosed with bisphenol  
3 A [**purity not reported**]. Dogs weighing 6.5–13.4 kg were housed in metal metabolism cages and fed  
4 Purina Dog Chow. During a 90-day period, 4 dogs/sex/group were given feed containing bisphenol A at  
5 0, 1000, 3000, or 9000 ppm. The European Union (2) estimated bisphenol A intake at 0, 28, 74, or 261  
6 mg/kg bw/day in males and 0, 31, 87, or 286 mg/kg bw/day in females. Dogs were observed for body  
7 weight gain, food, intake, and clinical signs. Ophthalmoscopic examination was conducted prior to and  
8 following the treatment period. Hematology, clinical chemistry, and urinalysis evaluations were  
9 conducted prior to treatment and at 1, 2, and 3 months into the study. Dogs were killed at the end of the  
10 treatment period. Organs were weighed and fixed in 10% neutral buffered formalin. Histopathological  
11 evaluations were conducted in organs from the control and high-dose groups; prostate, uterus, testis, and  
12 ovary were among organs evaluated. [**Procedures for statistical analyses were not described.**] No  
13 treatment-related clinical signs (conducted monthly), ophthalmological changes, or death were observed  
14 during the study. Bisphenol A treatment did not affect body weight gain or food intake. There were no  
15 treatment-related effects on hematology, biochemistry, or urinalysis. Relative liver weight was  
16 significantly increased [**by 18% in males and 26% in females**] in the high-dose group, and the study  
17 authors considered the effect to be treatment-related. No treatment-related gross or histopathological  
18 lesions were observed in the high-dose group.  
19  
20 Nitschke et al. (160) conducted a subchronic inhalation toxicity test with bisphenol A in F344 rats. Rats  
21 were fed Purina Certified Rodent Chow #5002 and housed in stainless steel wire cages. At 7 weeks of  
22 age, rats were stratified according to body weight and randomly assigned to treatment groups. Thirty  
23 rats/sex/group received whole-body exposures to polycarbonate grade bisphenol A dust (99.7% purity) at  
24 0, 10, 50, or 150 mg/m<sup>3</sup> for 6 hours/day, 5 days/week, for 13 weeks. Mass median aerodynamic diameter  
25 of bisphenol A dust was measured at ≤5.2 microns. Stability and concentrations of bisphenol A were  
26 verified. Rats were observed for clinical signs, body weight gain, and food intake. Ten rats/sex/group in  
27 each time period were killed and necropsied on the day following and at 4 and 12 weeks following  
28 exposure. At each necropsy period, hematological and clinical chemistry endpoints were examined. The  
29 lungs, brain, kidneys, and testes were weighed. Numerous organs were preserved in 10% phosphate-  
30 buffered formalin. In most cases, histological examinations were conducted in organs from the control  
31 and high-dose groups. Respiratory organs and organs with lesions or signs of toxicity were histologically  
32 examined at all dose levels. Included among organs undergoing histopathological examination  
33 immediately after the exposure period were the epididymis, mammary gland, ovary, oviduct, prostate,  
34 seminal vesicles, testis, uterus, and vagina. No reproductive organs were examined following the recovery  
35 periods. Statistical analyses included Bartlett's test, ANOVA, Dunnett test, Wilcoxon Rank-Sum test, and  
36 Bonferroni correction for multiple comparisons. Gross pathology and histopathology data did not appear  
37 to have been statistically analyzed.  
38  
39 During the exposure period, a reddish material around the nose (most likely porphyrin) was observed in  
40 2–10 of 10 animals/sex in the 50 and 150 mg/m<sup>3</sup> groups. Perineal soiling was observed in 2 of 10 females  
41 in the 10 mg/m<sup>3</sup> group and 9–10 of 10 animals/sex in the 50 and 150 mg/m<sup>3</sup> groups. Decreased body

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1 weight gain during treatment was observed in males from all dose groups and females in the 50 and 150  
2 mg/m<sup>3</sup> groups. Immediately following the treatment period, terminal body weights were reduced by ~5%  
3 in males and ~11% in females from the 150 mg/m<sup>3</sup> group. **[Body weights were ~4% lower in males**  
4 **from the 50 mg/m<sup>3</sup> group.]** No differences in feed intake were observed at this or any other time period  
5 in the study. The only hematological effect observed was slightly increased hemoglobin in males exposed  
6 to 10 mg/m<sup>3</sup>, but the study authors did not consider the effect to be biologically significant. Clinical  
7 chemistry observations in the 150 mg/m<sup>3</sup> group included decreased serum glutamic pyruvic transaminase  
8 activity, serum glutamic oxaloacetic transaminase activity, and glucose in males and decreased total  
9 protein and albumin and increased alkaline phosphatase activity in females. Alkaline phosphatase activity  
10 was also increased in females exposed to 50 mg/m<sup>3</sup>. The study authors did not consider any of the clinical  
11 chemistry changes to be biologically **[toxicologically]** significant. Absolute liver weight was decreased in  
12 males exposed to ≥10 and 150 mg/m<sup>3</sup>, and relative brain weight was increased in females exposed to ≥50  
13 mg/m<sup>3</sup>. Additional organ weight changes observed in females from the 150 mg/m<sup>3</sup> group included  
14 decreased absolute liver and kidney weights and increased relative lung weights. Because the organ  
15 weight changes were not associated with microscopic changes in organs, the study authors concluded that  
16 the effects reflected decreases in body weight and were not toxicologically significant. Cecal size was  
17 increased as a result of distention by food in all (10/dose/sex) males and females exposed to ≥50 mg/m<sup>3</sup>,  
18 and the effect was considered to be treatment-related. No histopathological alterations were observed for  
19 cecal wall morphology. Hemolyzed blood was observed in the stomachs of 3–7 of 10 males/group  
20 exposed to 50 and 150 mg/m<sup>3</sup>, but there were no signs of histopathological alterations in the  
21 gastrointestinal tract. Slight histopathological alterations, consisting of hyperplasia in stratified squamous  
22 and ciliated epithelium lining and inflammation of submucosal tissues was observed in the anterior nasal  
23 cavities of all (10/dose/sex) males and females exposed to ≥50 mg/m<sup>3</sup>. Slight-to-moderate hyperplasia of  
24 goblet cells was also observed in the lateral nasal wall. No other treatment-related histopathological  
25 alterations were observed, including in reproductive organs.

26  
27 During the 4-week recovery period, body weights remained lower in males and females of the 50 and 150  
28 mg/m<sup>3</sup> groups. At 4 weeks following exposure, terminal body weights of males and females in the 150  
29 mg/m<sup>3</sup> group were ~6% lower than control values. A decrease in white blood cell count in females from  
30 the 10 and 150 mg/m<sup>3</sup> groups was the only hematological effect observed. The clinical chemistry effects  
31 that were somewhat consistent with effects observed immediately following treatment were increased  
32 alkaline phosphatase activity in females exposed to 10 and 150 mg/m<sup>3</sup> and decreased serum glutamic  
33 pyruvic activity transaminase activity in females exposed to 150 mg/m<sup>3</sup>; the study authors did not  
34 consider the clinical chemistry changes to be treatment related. The study authors concluded that an  
35 increase in relative brain weight in males of the 150 mg/m<sup>3</sup> group was related to decreased body weights  
36 in those animals. Enlarged cecal size was observed in 5 of 10 males of the 150 mg/m<sup>3</sup> group, a decreased  
37 incidence compared to the period immediately following treatment. Nasal histopathology was observed in  
38 the 150 mg/m<sup>3</sup> but was reduced in magnitude and severity compared to rats observed immediately  
39 following exposure.

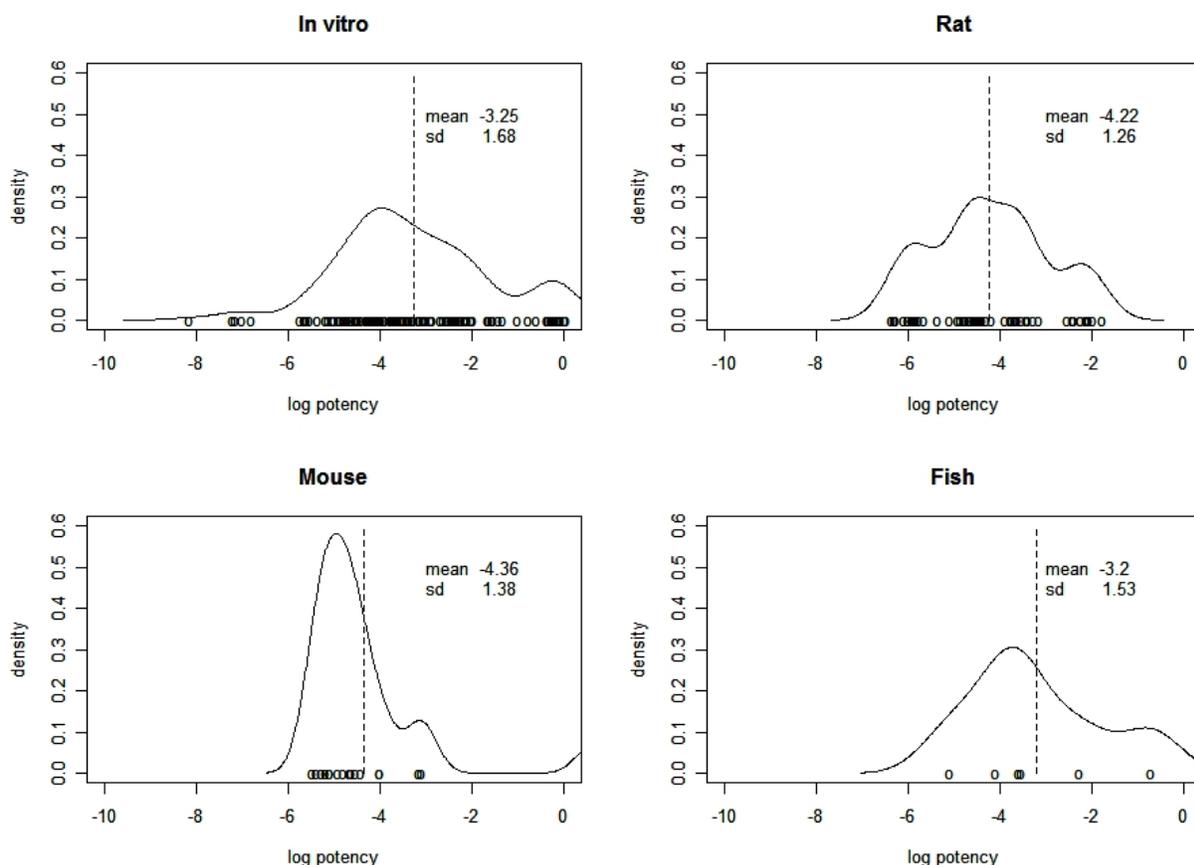
40  
41 In rats examined following 12 weeks of recovery, body weights of males in the 150 mg/m<sup>3</sup> group  
42 remained lower than controls, and terminal body weight was decreased by ~6%. An increase in white  
43 blood cell counts but not differential counts was observed in male rats of the 10 and 150 mg/m<sup>3</sup> group.  
44 The only clinical chemistry finding consistent with earlier observations was decreased total protein and  
45 globulin in females from the 150 mg/m<sup>3</sup> group, but the study authors did not consider the effect to be  
46 biologically significant. Organ weight changes in the 150 mg/m<sup>3</sup> group included decreased absolute  
47 kidney and lung weights in males and decreased absolute and relative kidney weights in females. No  
48 histopathological alterations were observed in kidney or lung. No other gross or histopathological  
49 alterations were observed, including cecal enlargement and nasal histopathology, which were observed at  
50 earlier time periods.

51

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### 2.2.2 Estrogenicity

The first identification of bisphenol A as an estrogen has been attributed to Dodds and Lawson (161), who reported that 100 mg injected by an unspecified route twice daily for 3 days resulted in maintenance of 5 of 5 rats in vaginal estrus for 40 days. The estrogenicity of bisphenol A has since been evaluated using several different kinds of assays. In vitro studies are summarized in Table 52, and in vivo studies are summarized in Table 53 using comparisons with 17 $\beta$ -estradiol, ethinyl estradiol, diethylstilbestrol, and, in one study, estrone. There is considerable variability in the results of these studies with the estrogenic potency of bisphenol A ranging over about 8 orders of magnitude, but similar means (Figure 2).



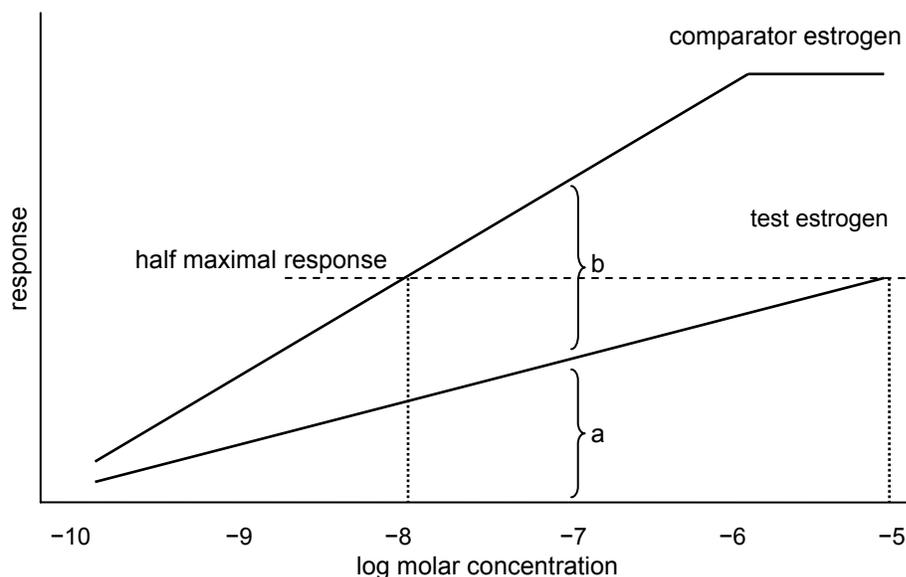
**Figure 2. In vitro Estrogenic Potency ( $\log_{10}$ ) in ER alpha and beta binding and transcriptional assays and estrogen-dependent cell proliferation assays) distributions of bisphenol A and estrogen responses in vivo in rats, mice and fish.**

Each data point represents 1 bisphenol A study in which bisphenol A was compared to a reference estrogen in rats, mice, fish, or in vitro. Data summarized from Table 52 and Table 53, midrange values used when a range is given in the table.

The most common method of comparing potency is to test responses over a range of concentrations and to compare the concentrations producing the half-maximal (or other fractional) response of the comparator estrogen. An alternative is to compare the magnitude of the response at an equimolar concentration of the 2 estrogens. The difference in these two methods is illustrated in Figure 3. An example of the difference in potency estimations according to comparison method is the study of Vivacqua et al. (71), in which the fold-increase in reporter activity for an estrogen-responsive gene was compared over a range of concentrations for bisphenol A and for 17 $\beta$ -estradiol. This study's Figure 3 presents curves analogous to Figure 3, but also presents a bar graph comparing response of the reporter at a  $10^{-7}$  M concentration of each estrogen. Based on the half-maximal response to 17 $\beta$ -estradiol, bisphenol-

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1 A appeared 1000 times less potent than 17 $\beta$ -estradiol, but based on the fold-difference in reporter activity  
2 at 10<sup>-7</sup> M, bisphenol A was about half as potent. Data for other estrogenicity comparisons in this paper  
3 and in many other papers are presented only using bar graphs comparing responses at the same molar  
4 concentrations of the 2 estrogens, thereby overestimating the estrogenic potency of bisphenol A compared  
5 to studies in which comparisons are based on the half-maximal response.  
6  
7



8  
9 **Figure 3. Alternative Approaches to Comparing Estrogenic Potency**

10 In this example, the half-maximal response to the comparator estrogen occurs at 10<sup>-8</sup> M. A  
11 similar response occurs with the test estrogen at 10<sup>-5</sup> M, suggesting a 1000-fold difference  
12 in potency. If the magnitudes of response at equimolar concentrations are compared, the  
13 apparent potency may be much different. The response to the test estrogen at 10<sup>-7</sup> M (a)  
14 is about half the response to the comparator estrogen at 10<sup>-7</sup> M (a + b).  
15

16 Competitive binding assays, which evaluate the concentration at which bisphenol A displaces labeled  
17 17 $\beta$ -estradiol from ER, are summarized in the top part of [Table 52](#). The receptor binding of bisphenol A  
18 in these assays varies over 3 orders of magnitude. Bisphenol A competes for human ER binding at molar  
19 concentrations 20–10,000 times that of the native ligand. When bisphenol A binding to ER $\alpha$  and ER $\beta$   
20 was compared in the same study, 3 reports found little difference by receptor subtype (162-164), and 3  
21 studies found binding to ER $\beta$  to be 4, 10, 47, and 254 times greater than binding to ER $\alpha$  (165-169). Yeast  
22 reporter systems, which reflect activation of post-receptor pathways, show less variability; these studies  
23 show bisphenol A activity to be 10,000–26,000 times less than that of 17 $\beta$ -estradiol.  
24

25 Some variability in estimating bisphenol A potency appears to be due to differences between laboratories.  
26 Andersen et al. (170) reported results from 3 laboratories that evaluated the proliferative response of  
27 MCF-7 breast cancer cells to bisphenol A. The laboratories, which were in the US, Spain, and Denmark,  
28 were sent samples of the same stock of bisphenol A, 17 $\beta$ -estradiol, and MCF-7 cells. Procedures were  
29 similar in the labs, although 2 different counting methods were used. The bisphenol A potencies relative  
30 to 17 $\beta$ -estradiol were  $5 \times 10^{-7}$ ,  $3 \times 10^{-6}$ , and  $1 \times 10^{-5}$ . Laboratory variability may underlie some of the  
31 large differences in cell-based assays for ER activation; in those studies bisphenol A molar potency  
32 compared to 17 $\beta$ -estradiol were reported to vary by over 7 orders of magnitude ([Table 52](#)). Another  
33 explanation for this wide range of reported values is the difference in defining relative potency in some  
34 assays, as discussed above. [According to a study author, the wide variability in relative bisphenol A

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1 potency was due to a wide fluctuation in the 17 $\beta$ -estradiol dose at which half-maximal proliferation was  
2 achieved (0.1–70 pM; A. Soto, personal communication, March 2, 2007).]

3  
4 A study using ER $\alpha$ - and ER $\beta$ -reporting systems in 3 human cell lines found that bisphenol A had a small  
5 antagonistic effect on ER $\alpha$  activation in the presence of 17 $\beta$ -estradiol in human embryonal kidney and  
6 endometrial carcinoma cells (171). There were no significant interactions between bisphenol A and 17 $\beta$ -  
7 estradiol on ER $\alpha$  activation in human osteosarcoma cells or on ER $\beta$  activation in any tested cell type. By  
8 contrast, a study using a recombinant yeast assay for ER $\alpha$  activation found 17 $\beta$ -estradiol and bisphenol A  
9 to have additive effects (172), and a study using MCF-7 cell proliferation found 17 $\beta$ -estradiol and  
10 bisphenol A to have synergistic effects (173).

11  
12 The data in Table 52 are applicable only to unconjugated bisphenol A. Estrogenic activity has not been  
13 identified for bisphenol A glucuronide (169) or sulfate (174).

14  
15 **Table 52. In Vitro Estrogenicity Testing of Bisphenol A**

Endpoint	Molar potency relative to 17 $\beta$ -estradiol	Reference
<i>Binding assays</i>		
Frog liver cytosol binding	$[1.4 \times 10^{-3}]$	Lutz and Kloas (175)
Carp liver cytosol binding	$[1.3 \times 10^{-3}]$	Segner et al. (176)
Rainbow trout ER binding	$5.8 \times 10^{-5}$	Olsen et al. (177)
Rainbow trout ER binding	$2.1 \times 10^{-3}$	Matthews et al. (178)
Anole ER binding	$1.3 \times 10^{-3}$	Matthews et al. (178)
Chicken ER binding	$4.4 \times 10^{-4}$	Matthews et al. (178)
Mouse ER $\alpha$ binding	$8.6 \times 10^{-5}$	Matthews et al. (178)
Mouse uterine cytosol binding	$[1.2 \times 10^{-4}]$	Matthews et al. (169)
Rabbit uterine ER binding	$[1.3 \times 10^{-5}]$	Andersen et al. (170)
Rat uterine cytosol binding	$\sim 5 \times 10^{-4}$	Krishnan et al. (179)
Rat uterine cytosol binding	$8 \times 10^{-5}$	Blair et al. (180)
Rat uterine cytosol binding	$1-2 \times 10^{-4}$	Kim et al. (181)
Rat ER $\alpha$ binding	$[2.5 \times 10^{-4}]$	Strunck et al. (182)
ER binding in rat lactotrophs	$1-10 \times 10^{-5}$	Chun and Gorski (183)
Rat ER $\alpha$ binding	$5 \times 10^{-4}$	Kuiper et al. (184)
Rat ER $\beta$ binding	$3.3 \times 10^{-4}$	Kuiper et al. (184)
Rat uterine ER $\alpha$ and $\beta$ binding	$6.2 \times 10^{-5}$	Washington et al. (185)
Rat uterine Type II estrogen-binding site	$4 \times 10^{-3}$	Washington et al. (185)
ER binding in MCF-7 lysates	$1 \times 10^{-2}$	Dodge et al. (186)
Human ER $\alpha$ binding	$4 \times 10^{-4}$	Bolger et al. (187)
Human ER $\alpha$ binding	$1 \times 10^{-4}$	Kuiper et al. (162)
Human ER $\beta$ binding	$1 \times 10^{-4}$	Kuiper et al. (162)
Human ER binding	$5.6 \times 10^{-4}$	Perez et al. (188)
Human ER binding	$[1.3 \times 10^{-4}]$	Andersen et al. (170)
ER binding in ECC-1 cells	$3 \times 10^{-3}$	Bergeron et al. (189)
Human ER $\alpha$ binding	$8 \times 10^{-5}$	Matthews et al. (178)
Human ER $\alpha$ binding	$[2.5 \times 10^{-3}$ diethylstilbestrol]	Nakagawa and Suzuki (190)
Human ER $\alpha$ binding	$7.3 \times 10^{-4}$	Routledge et al. (166)
Human ER $\beta$ binding	$7.5 \times 10^{-3}$	Routledge et al. (166)
Human ER binding	$[7.1 \times 10^{-5}]$	Sheeler et al. (191)
Human ER $\alpha$ binding	$[8 \times 10^{-5}]$	Matthews et al. (169)
Human ER $\beta$ binding	$[3.8 \times 10^{-3}]$	Matthews et al. (169)

## 2.0 General Toxicology and Biological Effects

Endpoint	Molar potency relative to 17 $\beta$ -estradiol	Reference
Human ER $\alpha$ binding	$5 \times 10^{-2}$	Paris et al. (163)
Human ER $\beta$ binding	$4 \times 10^{-2}$	Paris et al. (163)
Human ER binding	$[3 \times 10^{-4}]$	Strohecker et al. (192)
Human ER $\alpha$ binding	$[2.4 \times 10^{-4}]$	Seidlová-Wuttke et al. (167, 168)
Human ER $\beta$ binding	$[2.8 \times 10^{-2}]$	
Human ER $\alpha$ binding	$[1.1 \times 10^{-4}]$	Takemura et al. (165)
Human ER $\beta$ binding	$[4.4 \times 10^{-4}]$	Takemura et al. (165)
Human ER binding	$3.15 \times 10^{-3}$	Olsen et al. (177)
ER $\alpha$ binding	$[9.4 \times 10^{-4}]$	Takayanagi et al. (164)
ER $\beta$ binding	$[9.6 \times 10^{-4}]$	Takayanagi et al. (164)
<i>Recombinant yeast reporter systems</i>		
Human ER activation	$5 \times 10^{-5}$	Coldham et al. (193)
Human ER activation	$6.7 \times 10^{-5}$	Gaido et al. (194)
Human ER activation	$[2.5 \times 10^{-5}]$	Harris et al. (195)
Human ER activation	$[4-8 \times 10^{-5}]$	Andersen et al. (170)
Human ER activation	$[3.9 \times 10^{-5}]$	Sheeler et al. (191)
Human ER activation	$\sim 1 \times 10^{-4}$	Sohoni and Sumpter (196)
Human ER activation	$3.7 \times 10^{-5}$	Metcalfe et al. (197)
ER $\alpha$ activation	$6.2 \times 10^{-5}$	Silva et al. (198)
ER $\alpha$ activation	$[1 \times 10^{-4}]$	Nishihara et al. (199)
ER $\alpha$ activation	$[\sim 1 \times 10^{-4}]$	Beresford et al. (200)
Human ER $\alpha$	$[3.3 \times 10^{-5}]$	Rajapakse et al. (172)
Human ER $\alpha$ , no microsomes	$[5.5 \times 10^{-5}]$	Elsby et al. (142)
Human ER $\alpha$ , human liver microsomes	$[6.6 \times 10^{-6}]$	Elsby et al. (142)
ER activation	$\sim 10^{-5}$	Chen et al. (201)
Human ER activation	$[8.1 \times 10^{-5}]$	Segner et al. (176)
Human ER activation	$9 \times 10^{-5}$	Li et al. (202)
ER $\alpha$ activation	$[4 \times 10^{-5}]$	Singleton et al. (203)
Human ER $\alpha$ , with denatured rat S9	$[2.4 \times 10^{-6}]$	Yoshihara et al. (204)
Human ER $\alpha$ , with active rat S9	$[9.2 \times 10^{-6}]$	Yoshihara et al. (204)
Human ER $\alpha$ , with denatured mouse S9	$[3.0 \times 10^{-6}]$	Yoshihara et al. (204)
Human ER $\alpha$ , with active mouse S9	$[7.8 \times 10^{-6}]$	Yoshihara et al. (204)
Human ER $\alpha$ , with denatured monkey S9	$[2.4 \times 10^{-6}]$	Yoshihara et al. (204)
Human ER $\alpha$ , with active monkey S9	$[6.0 \times 10^{-6}]$	Yoshihara et al. (204)
Human ER $\alpha$ , with denatured human S9	$[2.2 \times 10^{-6}]$	Yoshihara et al. (204)
Human ER $\alpha$ , with active human S9	$[4.6 \times 10^{-6}]$	Yoshihara et al. (204)
Human ER $\alpha$ activity	$[2.3 \times 10^{-5}]$	Terasaki et al. (5)
Medaka ER $\alpha$ activity	$[3.3 \times 10^{-4}]$	Terasaki et al. (5)
“Estrogenic activity”	$3.4 \times 10^{-5}$	Kawagoshi et al. (28)
ER $\alpha$ activation	$[2.3 \times 10^{-4}]$	Singleton et al. (203)
Fish ER $\alpha$ activation	$4.1 \times 10^{-4}$	Fu et al. (205)
Fish ER $\beta$ 2 activation	$3.2 \times 10^{-5}$	Fu et al. (205)
<i>Other cell-based recombinant reporter systems</i>		
ER activation in trout gonad cell line	$5.4 \times 10^{-3}$	Ackerman et al. (206)
Mouse ER $\alpha$ in HeLa cells	$[<1 \times 10^{-5}]$	Ranhotra et al. (207)
Mouse ER $\beta$ in HeLa cells	$[\sim 1 \times 10^{-2}]$	Ranhotra et al. (207)
HepG2 cells, human ER $\alpha$	$[3.0 \times 10^{-3}]$	Snyder et al. (129)
HepG2 cells, human ER $\beta$	$[1.1 \times 10^{-2}]$	Snyder et al. (129)

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Endpoint	Molar potency relative to 17 $\beta$ -estradiol	Reference
Rat ER $\alpha$ in HeLa cells	[1.6 $\times 10^{-7}$ ]	Yamasaki et al. (208)
ER activation in HeLa cells	[8.8 $\times 10^{-4}$ ]	Takahashi et al. (209)
ER $\alpha$ activation in HeLa cells	[2.5 $\times 10^{-2}$ ]	Hiroi et al. (210)
ER $\beta$ activation in HeLa cells	[2.3 $\times 10^{-2}$ ]	Hiroi et al. (210)
ER $\alpha$ activation in HeLa cells	[6.1 $\times 10^{-1}$ ]	Vivacqua et al. (71)
ER $\beta$ activation in HeLa cells	[5.6 $\times 10^{-1}$ ]	Vivacqua et al. (71)
ER $\alpha$ activation in HeLa cells	[7.7 $\times 10^{-1}$ ]	Recchia et al. (211)
ER $\beta$ activation in HeLa cells	[1.2]	Recchia et al. (211)
ER $\alpha$ activation in T47D cells	[6.2–7.9 $\times 10^{-1}$ ]	Recchia et al. (211)
Proliferation in T47D cells	[6.6 $\times 10^{-1}$ ]	Recchia et al. (211)
Human ER in hepatoma cells	[3 $\times 10^{-2}$ ]	Gould et al. (212)
Human ER $\alpha$ , human embryonal kidney	[4.8 $\times 10^{-3}$ ]	Kurosawa et al. (171)
Human ER $\beta$ , human embryonal kidney	[4.6 $\times 10^{-3}$ ]	Kurosawa et al. (171)
Human ER $\alpha$ , endometrial carcinoma	[5.4 $\times 10^{-3}$ ]	Kurosawa et al. (171)
Human ER $\beta$ , endometrial carcinoma	[4.9 $\times 10^{-3}$ ]	Kurosawa et al. (171)
Human ER $\alpha$ , osteosarcoma	[7.3 $\times 10^{-3}$ ]	Kurosawa et al. (171)
Human ER $\beta$ , osteosarcoma	[7.7 $\times 10^{-3}$ ]	Kurosawa et al. (171)
Human ER $\alpha$ , human hepatoma cells	[2.7 $\times 10^{-1}$ ]	Gaido et al. (213)
Human ER $\beta$ , human hepatoma cells	[1.8 $\times 10^{-1}$ ]	Gaido et al. (213)
Human ER $\alpha$ , 239HEK cells	2 $\times 10^{-4}$ diethylstilbestrol	Lemmen et al. (214)
Human ER $\beta$ , 239HEK cells	7 $\times 10^{-4}$ diethylstilbestrol	Lemmen et al. (214)
Human ER $\alpha$ , endometrial carcinoma	[6.1 $\times 10^{-3}$ ]	Singleton et al. (203)
<i>MCF-7 cells</i>		
G6PD activity	[1 $\times 10^{-1}$ ]	Kim et al. (215)
Expression of proteins	[1 $\times 10^{-3}$ ]	Perez et al. (188)
Progesterone receptor mRNA	not increased at 10 $^{-6}$ M <sup>a</sup>	Diel et al. (216)
Androgen receptor mRNA	not decreased at 10 $^{-6}$ M <sup>a</sup>	Diel et al. (216)
Progesterone receptor	$\sim 2 \times 10^{-4}$	Krishnan et al. (179)
ER binding, serum-free	3.3 $\times 10^{-4}$	Samuelsen et al. (217)
ER binding, 100% human serum	1.7 $\times 10^{-4}$	Samuelsen et al. (217)
ER binding	3.2 $\times 10^{-3}$	Olsen et al. (218)
ER activation	[1.4 $\times 10^{-5}$ ]	Kitamura et al. (219)
ER $\alpha$ expression	[7.5 $\times 10^{-5}$ ]	Matthews et al. (169)
ER $\beta$ expression	[1.8 $\times 10^{-4}$ ]	Matthews et al. (169)
ER $\alpha$ activation	[4.7–6.9 $\times 10^{-1}$ ]	Vivacqua et al. (71)
ER $\alpha$ activation	[5.5–6.7 $\times 10^{-1}$ ]	Recchia et al. (211)
pS2 induction	[1.8 $\times 10^{-6}$ ]	Leffers et al. (220)
ER production	[7 $\times 10^{-8}$ ]	Olsen et al. (218)
Progesterone receptor production	[6.8 $\times 10^{-8}$ ]	Olsen et al. (218)
pS2 production	[10 $^{-7}$ ]	Olsen et al. (218)
pS2 mRNA	[1.1]	Vivacqua et al. (71)
pS2 mRNA	[8.9 $\times 10^{-1}$ ]	Recchia et al. (211)
Cathepsin D mRNA	[8.2 $\times 10^{-1}$ ]	Recchia et al. (211)
Transcription of human telomerase reverse transcriptase	[ $\sim 10^{-2}$ ]	Takahashi et al. (209)
Proliferation	[3.8 $\times 10^{-4}$ ]	Krishnan et al. (179)
Proliferation	1 $\times 10^{-3}$	Brotons et al. (65)
Proliferation	1 $\times 10^{-4}$	Soto et al. (221)
Proliferation	[ $\sim 1 \times 10^{-3}$ ]	Dodge et al. (186)

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Endpoint	Molar potency relative to 17 $\beta$ -estradiol	Reference
Proliferation	[1 $\times$ 10 <sup>-4</sup> ]	Perez et al. (188)
Proliferation	[9.8 $\times$ 10 <sup>-4</sup> ]	Schafer et al. (222)
Proliferation (3 different laboratories)	5–100 $\times$ 10 <sup>-7</sup>	Andersen et al. (170)
Proliferation	6 $\times$ 10 <sup>-5</sup>	Körner et al. (223)
Proliferation	3 $\times$ 10 <sup>-5</sup>	Kim et al. (181)
Proliferation	[2.5 $\times$ 10 <sup>-6</sup> ]	Suzuki et al. (173)
Proliferation	2 $\times$ 10 <sup>-5</sup>	Samuelsen et al. (217)
Proliferation	[9.2 $\times$ 10 <sup>-4</sup> ]	Nakagawa and Suzuki (190)
Proliferation	[~1 $\times$ 10 <sup>-3</sup> ]	Shimizu et al. (174)
Proliferation	[7 $\times$ 10 <sup>-9</sup> ]	Diel et al. (216)
Proliferation	1.6 $\times$ 10 <sup>-5</sup>	Olsen et al. (218)
Proliferation	[4.5–5 $\times$ 10 <sup>-1</sup> ]	Vivacqua et al. (71)
Proliferation	[1.1 $\times$ 10 <sup>-4</sup> ]	Strohecker et al. (192)
Proliferation	[6 $\times$ 10 <sup>-1</sup> ]	Recchia et al. (211)
Proliferation	2 $\times$ 10 <sup>-5</sup>	Olsen et al. (177)
Proliferation, with denatured rat S9	[6.5 $\times$ 10 <sup>-5</sup> ]	Yoshihara et al (224)
Proliferation, with active rat S9	[3.4 $\times$ 10 <sup>-4</sup> ]	Yoshihara et al (224)
<i>Rat pituitary cells</i>		
Proliferation	1–10 $\times$ 10 <sup>-6</sup>	Chun and Gorski (183)
Proliferation	[~8.4 $\times$ 10 <sup>-3</sup> ]	Steinmetz et al. (225)
Prolactin release	1 $\times$ 10 <sup>-5</sup>	Chun and Gorski (183)
Prolactin release (GH <sub>3</sub> cell)	[6 $\times$ 10 <sup>-3</sup> ]	Steinmetz et al. (225)
Prolactin release (F344 pituitary)	2–10 $\times$ 10 <sup>-4</sup>	Steinmetz et al. (225)
Prolactin gene expression	[~1 $\times$ 10 <sup>-3</sup> ]	Steinmetz et al. (225)
<i>Rat uterine adenocarcinoma cells</i>		
Induction of complement C3 mRNA	[8 $\times$ 10 <sup>-3</sup> ]	Strunck et al. (182)
<i>Human uterine adenocarcinoma cells</i>		
Progesterone receptor mRNA/protein	[~1 $\times$ 10 <sup>-2</sup> ]	Bergeron et al. (189)
Proliferation	no effect at 10 <sup>-5</sup> M	Bergeron et al. (189)
<i>Vitellogenin production, fish hepatocytes</i>		
Carp	1 $\times$ 10 <sup>-4</sup>	Smeets et al. (226)
Carp	[3.1 $\times$ 10 <sup>-3</sup> ]	Segner et al. (176)
Carp	[1 $\times$ 10 <sup>-5</sup> ]	Letcher et al. (227)
Carp	[3 $\times$ 10 <sup>-4</sup> ]	Rankouhi et al. (228)
Trout	2 $\times$ 10 <sup>-5</sup>	Shilling et al. (229)
Trout	[8 $\times$ 10 <sup>-4</sup> ]	Segner et al. (176)
Trout	2.9 $\times$ 10 <sup>-5</sup>	Olsen et al. (177)
<i>Frog hepatocytes</i>		
Vitellogenin mRNA expression	[~1 $\times$ 10 <sup>-3</sup> ]	Kloas et al. (230)
Vitellogenin production	no effect at 100 $\mu$ M	Rankouhi et al. (231)
ER mRNA expression	~10 <sup>-2</sup>	Lutz et al. (232)

<sup>a</sup>Progesterone receptor was increased and androgen receptor was decreased by 17 $\beta$ -estradiol 10<sup>-10</sup> M.

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1 Table 53. In Vivo Estrogenicity Tests of Bisphenol A

Model and exposure	Husbandry <sup>a</sup>	Endpoint	Molar potency/comparator <sup>b</sup>	Reference
<i>Rat uterus</i>				
Adult ovariectomized Sprague Dawley, gavage × 4 days	TD89222 diet, metal cage	Uterine wet weight	[3.9 × 10 <sup>-3</sup> ]/ethinyl estradiol	Dodge et al. (186)
Immature Sprague Dawley, bisphenol A given “orally” × 3 days; 17β-estradiol ip × 3 days	not indicated	Uterine weight	Not affected by bisphenol A at up to 150 mg/kg bw/day; 17β-estradiol was positive at 0.005 mg/day [ <b>~0.089 mg/kg bw/day</b> ]	Gould et al. (212)
		Progesterone receptor Peroxidase activity	[5.9 × 10 <sup>-3</sup> ]/17β-estradiol [7.6 × 10 <sup>-3</sup> ]/17β-estradiol	
Adult ovariectomized Crl:CD BR, gavage × 4 days	Purina 5002 diet, steel cage	Uterine weight	[3.5 × 10 <sup>-5</sup> ]/17β-estradiol	Cook et al. (233)
Adult ovariectomized F344, ip × 1	Not indicated	Stromal cell proliferation	[4.1 × 10 <sup>-5</sup> ]/17β-estradiol	Steinmetz et al. (234)
Adult ovariectomized F344 or Sprague Dawley, silastic implant × 3 days	Not indicated	<i>cfos</i> expression	[2.1 × 10 <sup>-4</sup> ]/17β-estradiol	Steinmetz et al. (234)
		Uterine wet weight: F344	[8.2 × 10 <sup>-3</sup> ]/17β-estradiol	
		Sprague Dawley	[6.0 × 10 <sup>-3</sup> ]/17β-estradiol	
		Uterine cell height: F344	[1.1 × 10 <sup>-2</sup> ]/17β-estradiol	
		Sprague Dawley	[9.2 × 10 <sup>-3</sup> ]/17β-estradiol	
Juvenile ovariectomized DA/Han, Wistar, or Sprague Dawley, gavage × 3 days	Not indicated	Uterine wet weight: DA/Han	[1.8 × 10 <sup>-5</sup> ]/ethinyl estradiol	Diel et al. (235)
		Wistar	No response to 200 mg/kg/d	
		Sprague Dawley	[1.7 × 10 <sup>-5</sup> ]/ethinyl estradiol	
		Uterine epithelium	No response to 200 mg/kg/day	
		Vaginal epithelium	No response to 200 mg/kg/day	
		Clusterin mRNA	No response to 200 mg/kg/day	
Immature Alpk:AP, sc × 3 days	RM3 diet, wire cage	Uterine wet weight	[2.6–2.7 × 10 <sup>-5</sup> ]/diethylstilbestrol	Ashby and Tinwell (236)
		Uterine dry weight	[2.5–3.0 × 10 <sup>-5</sup> ]/diethylstilbestrol	
Immature Alpk:AP, gavage × 3 days	RM3 diet, wire cage	Uterine wet weight	[2.3–3.1 × 10 <sup>-5</sup> ]/diethylstilbestrol	
		Uterine dry weight	[2.7–3.6 × 10 <sup>-5</sup> ]/diethylstilbestrol	
Immature Long Evans, gavage × 3 days	Purina 5001 diet	Uterine wet weight 6 hours after dosing	[1.4 × 10 <sup>-5</sup> ]/17β-estradiol	Laws et al. (237)

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Model and exposure	Husbandry <sup>a</sup>	Endpoint	Molar potency/comparator <sup>b</sup>	Reference
Adult ovariectomized Long Evans	Purina 5001 diet	Uterine wet weight 24 hours after dosing	No effect at bisphenol A at $\leq 400$ mg/kg bw/day	Laws et al. (237)
Juvenile ovariectomized DA/Han, gavage $\times 3$ days	Ssniff R-10 diet	Uterine wet weight relative to bw <i>Expression of:</i> Androgen receptor <i>ER</i> Progesterone receptor	No effect of bisphenol A at $\leq 100$ mg/kg bw/day [ $1.2 \times 10^{-5}$ ]/ethinyl estradiol [ $3.9 \times 10^{-4}$ ]/ethinyl estradiol [ $1.9 \times 10^{-4}$ ]/ethinyl estradiol bisphenol A and ethinyl estradiol produced opposite effects	Diel et al. (238)
Adult ovariectomized Alpk:ApfSD, sc $\times 3$ days	Not indicated	Complement C3 Clusterine Glyceraldehyde phosphate dehydrogenase Uterine wet weight	[ $2.2 \times 10^{-5}$ ]/ethinyl estradiol No bisphenol A effect at 200 mg/kg bw/day; ethinyl estradiol showed an effect at 0.1 mg/kg bw/day.	Ashby et al. (239)
Immature Crj:CD (SD), sc $\times 3$ days	MF diet, steel cage	Uterine dry weight	[ $1.7 \times 10^{-4}$ ]/17 $\beta$ -estradiol [ $1.8 \times 10^{-4}$ ]/17 $\beta$ -estradiol	Yamasaki et al. (125)
Immature Crj:CD (SD), gavage $\times 3$ days	MF diet, steel cage	Wet and blotted uterine weight	Effect noted at $\geq 8$ mg/kg bw/day bisphenol A/no comparator	
Adult ovariectomized Wistar, sc $\times 7$ days	Not indicated	Wet and blotted uterine weight	Effect noted at $\geq 160$ mg/kg bw/day bisphenol A/no comparator	
Adult ovariectomized Wistar, sc $\times 7$ days	Not indicated	Blotted uterine weight	Increased relative weight compared to placebo at $\geq 11$ mg/kg bw/day; uterus reached 83% of weight of sham-ovariectomized control at bisphenol A dose of 250 mg/kg bw/day.	Goloubkova et al. (240)
Adult ovariectomized Sprague Dawley, exposed in drinking water $\times 3$ days	Glass water bottles, plastic cage (negative E-Screen of ethanol cage washes)	Uterine wet weight	No effect of bisphenol A at up to 16.9 mg/kg bw/day; estrone positive at 0.12 mg/kg bw/day	Rubin et al. (241)

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<b>Model and exposure</b>	<b>Husbandry<sup>a</sup></b>	<b>Endpoint</b>	<b>Molar potency/comparator<sup>b</sup></b>	<b>Reference</b>
Adult ovariectomized Sprague Dawley, sc × 3 days	PMI Certified Rodent Diet, polycarbonate cage, elm bedding	Uterine wet weight Uterine dry weight	[1.7 × 10 <sup>-6</sup> ]/17β-estradiol [2.3 × 10 <sup>-6</sup> ]/17β-estradiol	Kim et al. (181)
Immature Alpk:ApfSD, sc × 3 days	RM1 diet	Uterine wet weight Uterine dry weight	[2.9 × 10 <sup>-4</sup> ]/17β-estradiol No effect of bisphenol A at 800 mg/kg bw/day; 17β-estradiol positive at 0.4 mg/kg bw/day	Matthews et al. (169)
Immature Alpk:ApfSD, gavage × 3 days	RM1 diet	Uterine wet weight Uterine dry weight	[2.3–5.5 × 10 <sup>-4</sup> ]/17β-estradiol [2.4–7.1 × 10 <sup>-4</sup> ]/17β-estradiol	An et al. (242)
Immature Sprague Dawley, sc × 3 days	Soy-free diet, polycarbonate cage	Uterine wet weight	No effect of bisphenol A at ≤ 1000 mg/kg bw/day; 17β-estradiol was positive at 0.04 mg/kg bw/day	
Immature Crj:CD (SD), sc × 3 days	MF diet, steel cage	Calbindin D <sub>9k</sub> expression <i>ERα</i> expression Uterine wet weight	[8.4 × 10 <sup>-6</sup> ]/17β-estradiol [3.4 × 10 <sup>-5</sup> ]/17β-estradiol [5.1 × 10 <sup>-5</sup> ]/ethinyl estradiol	Yamasaki et al. (208)
Immature Sprague Dawley, sc × 3 days	Soy-free diet, polycarbonate cage, corncob bedding	Blotted uterine weight Epithelial cell height	[8 × 10 <sup>-7</sup> ]/ethinyl estradiol [1.2 × 10 <sup>-6</sup> ]/ethinyl estradiol	Wade et al. (243)
Pubertal Sprague Dawley, sc gavage PND 22–42/43	Purina 5002 diet, polycarbonate cage, chip bedding	Blotted uterine weight  Vaginal opening	Absolute organ weight decreased with increase dose (400 and 600 mg/kg bw/day); no effect on relative organ weight No effect at 400 and 600 mg/kg bw/day	George et al. (244)
Pregnant Sprague Dawley, sc bisphenol A on GD 17–19 (17β-estradiol sc × 1)	Soy-free diet, polycarbonate cage	Maternal uterine weight	[1.8 × 10 <sup>-5</sup> ]/17β-estradiol	Hong et al. (245)
Pregnant Sprague Dawley, sc bisphenol A on GD 17–19 (17β-estradiol sc × 1)	Soy-free diet, polycarbonate cage	Maternal uterine calbindin D <sub>9k</sub> protein	[1.7 × 10 <sup>-5</sup> ]/17β-estradiol	Hong et al. (245)

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<b>Model and exposure</b>	<b>Husbandry<sup>a</sup></b>	<b>Endpoint</b>	<b>Molar potency/comparator<sup>b</sup></b>	<b>Reference</b>
Lactating Sprague Dawley, sc bisphenol A × 5 days (17β-estradiol sc × 1)	Soy-free diet	Maternal uterine calbindin D <sub>9k</sub> mRNA calbindin D <sub>9k</sub> protein	[2.2 × 10 <sup>-5</sup> ]/17β-estradiol [6.9 × 10 <sup>-5</sup> ]/17β-estradiol	Hong et al. (246)
Immature and adult ovariectomized Wistar, gavage × 4 days	AO4C diet, wire cage	Uterine wet and dry weight	No effect in either model of bisphenol A at ≤ 200 mg/kg bw/day/17β-estradiol positive at 0.025–0.035 mg/kg bw/day	Strohecker et al. (247)
Immature Sprague Dawley, sc × 3 days	Soy-free feed, polycarbonate cage	Calbindin D <sub>9k</sub> protein	[5.1 × 10 <sup>-5</sup> ]/17β-estradiol	An et al. (248)
Immature Sprague Dawley, sc × 3 days	Shinchon diet	Uterine wet weight Uterine wet weight relative to bw Glutathione peroxidase activity	[1.5 × 10 <sup>-6</sup> ]/17β-estradiol [1.3 × 10 <sup>-6</sup> ]/17β-estradiol [4.2 × 10 <sup>-3</sup> ]/17β-estradiol	Kim et al. (215)
Immature Alpk:ApfSD, gavage × 3 days	RM1 diet, polycarbonate cage	Blotted uterine weight <i>Expression of:</i> Progesterone receptor A Progesterone receptor B Complement C3 Lipocalcin	[2.5 × 10 <sup>-4</sup> ]/17β-estradiol [3.8 × 10 <sup>-4</sup> ]/17β-estradiol [4.2 × 10 <sup>-4</sup> ]/17β-estradiol [1.8 × 10 <sup>-4</sup> ]/17β-estradiol [2.3 × 10 <sup>-4</sup> ]/17β-estradiol	Ashby and Odum (249)
Immature AP, sc × 3 days	RM1 diet, polypropylene cages, sawdust and shredded paper bedding	Uterine wet weight Uterine dry weight	[1.0 × 10 <sup>-6</sup> ]/ethinyl estradiol [1.2 × 10 <sup>-6</sup> ]/ethinyl estradiol	Tinwell and Ashby (250)
Adult ovariectomized Sprague Dawley, diet × 3 months	Phytoestrogen-free diet	Uterine weight, endometrial thickness, ERα, ERβ expression Complement C3 expression	No bisphenol A effect at 0.37 mg/kg bw/day; estradiol benzoate positive control Bisphenol A and estradiol benzoate produced opposite effects	Seidlová-Wuttke et al. (167)
Immature Sprague Dawley, sc × 3 days	PMI Certified Rodent Diet	Uterine wet weight Uterine dry weight	[4.5 × 10 <sup>-7</sup> ]/ethinyl estradiol [4.9 × 10 <sup>-7</sup> ]/ethinyl estradiol	Kim et al. (251)

## 2.0 General Toxicology and Biological Effects

<b>Model and exposure</b>	<b>Husbandry<sup>a</sup></b>	<b>Endpoint</b>	<b>Molar potency/comparator<sup>b</sup></b>	<b>Reference</b>
Adult ovariectomized Crj:CD (SD), sc × 3 days	Estrogen-free NIH-07PLD diet, aluminum cage, paper bedding	Uterine wet weight, relative to bw Blotted uterine weight, relative to bw	[2.1 × 10 <sup>-5</sup> ]/17β-estradiol [1.7 × 10 <sup>-6</sup> ]/17β-estradiol	Koda et al. (252)
Adult Holzman, progesterone-treated to delay implantation, given test agent sc on GD 7	Unspecified Purina rodent chow, plastic cage, pine shavings	Implantation	[4–34 × 10 <sup>-6</sup> ]/estrone	Cummings et al. (253)
<i>Rat vagina</i>				
Adult ovariectomized F344, ip × 1	Not indicated	BrdU labeling	Increased at bisphenol A dose of 37.5 but not 18.5 mg/kg bw/no comparator [1.3 × 10 <sup>-4</sup> ]/17β-estradiol	Steinmetz et al. (234)
Adult ovariectomized Long Evans, bisphenol A by gavage × 11 days; 17β-estradiol by sc	Purina 5001 diet	<i>cfos</i> expression Vaginal cytology	No effect at bisphenol A dose of 100 mg/kg bw/day; 17β-estradiol 0.005 mg/kg bw/day resulted in persistent estrus.	Laws et al. (237)
Long Evans treated PND 21–35 by gavage	Purina 5001 diet	Vaginal opening	No effect at bisphenol A dose ≤ 400 mg/kg bw/day; ethinyl estradiol was active at 0.01 mg/kg bw/day.	Laws et al. (237)
Adult ovariectomized F344 and Sprague Dawley, ip × 1	Not indicated	BrdU labeling	F344: [4.5 × 10 <sup>-6</sup> ]/17β-estradiol Sprague Dawley: [1.4 × 10 <sup>-6</sup> ]/17β-estradiol [3.8 × 10 <sup>-4</sup> ]/17β-estradiol	Long et al. (254)
Immature Wistar, gavage × 4 days	AO4C diet, wire cage	Vaginal cornification		
Adult ovariectomized Wistar, gavage × 4 days	AO4C diet, wire cage	Vaginal cornification	No effect at bisphenol A dose of 100 mg/kg bw/day; 17β-estradiol was positive at 0.1 mg/kg bw/day	Strohecker et al. (247)
Immature Sprague Dawley, sc × 3 days	PMI Certified Rodent Diet	Vaginal weight	[5.3 × 10 <sup>-7</sup> ]/ethinyl estradiol	Kim et al. (251)
<i>Other rat organs</i>				
Ovariectomized Sprague Dawley, daily gavage for 5 weeks	TD89222 diet, metal cage	Prevention of bone mineral density decline	No effect at bisphenol A dose up to 10 mg/kg bw/day; no standard estrogen comparator.	Dodge et al. (186)

## 2.0 General Toxicology and Biological Effects

Model and exposure	Husbandry <sup>a</sup>	Endpoint	Molar potency/comparator <sup>b</sup>	Reference
Adult ovariectomized Sprague Dawley, treated in feed	Phytoestrogen-free diet	Prevention of bone mineral density decline	No effect at bisphenol A dose $\leq$ 370 $\mu\text{g}/\text{kg}$ bw/day; estradiol benzoate was effective at 1.18 mg/kg bw/day.	Seidlová-Wuttke et al. (167)
Adult ovariectomized Sprague Dawley and F344, by sc implant $\times$ 3 days	Not indicated	Serum prolactin	F344: $[1.7 \times 10^{-2}]$ /17 $\beta$ -estradiol Sprague Dawley: no effect of bisphenol A at 40–45 $\mu\text{g}/\text{day}$ or 17 $\beta$ -estradiol at 1.2–1.5 $\mu\text{g}/\text{day}$ .	Steinmetz et al. (225)
Adult ovariectomized Wistar, sc $\times$ 7 days	Not indicated	Pituitary weight	Increased compared to vehicle control at 128 but not 78 mg/kg bw/day	Goloubkova et al. (240)
		Serum prolactin	Increased compared to vehicle control at 128 mg/kg bw/day	
<i>Mouse uterus</i>				
Immature CFLP, sc $\times$ 3 days	Not indicated	Relative uterine weight	No response at up to 0.5 mg [ $\sim$ 50 mg/kg bw/day]	Coldham et al. (193)
Adult ovariectomized CD-1, sc $\times$ 1	Not indicated	<i>IGF1</i> expression	$[8.4 \times 10^{-4}]$ /17 $\beta$ -estradiol	Klotz et al. (255)
Juvenile-adult ovariectomized B6C3F <sub>1</sub> , sc $\times$ 4 days	Purina 5001, polypropylene cage, chip bedding	Uterine wet weight Endothelial proliferation	$[2.3 \times 10^{-5}]$ /17 $\beta$ -estradiol $[6.9 \times 10^{-6}]$ /17 $\beta$ -estradiol	Papconstantinou et al. (256)
Juvenile-adult ovariectomized B6C3F <sub>1</sub> , sc $\times$ 4 days	Purina 5001, polypropylene cage, cellulose fiber bedding	Induction of grp94 Induction of hsp72 Induction of hsp90	$[2.4 \times 10^{-5}]$ /17 $\beta$ -estradiol $[3.5 \times 10^{-6}]$ /17 $\beta$ -estradiol $[5.3 \times 10^{-6}]$ /17 $\beta$ -estradiol	Papconstantinou et al. (257)
Juvenile-adult ovariectomized B6C3F <sub>1</sub> , sc $\times$ 4 days	Purina 5001, polypropylene cage, cellulose fiber bedding	Uterine weight Induction of hsp90 $\alpha$ Induction of grp24	$[5.3 \times 10^{-6}]$ /17 $\beta$ -estradiol $[1.2 \times 10^{-5}]$ /17 $\beta$ -estradiol $[8.4 \times 10^{-6}]$ /17 $\beta$ -estradiol	Papconstantinou et al. (258)
Juvenile-adult ovariectomized B6C3F <sub>1</sub> , sc $\times$ 1	Purina 5001, polypropylene cage, cellulose fiber bedding	Blotted uterine weight, 6 hours after dose Blotted uterine weight, 12 hours after dose	$[8.4 \times 10^{-6}]$ /17 $\beta$ -estradiol $[4.2 \times 10^{-6}]$ /17 $\beta$ -estradiol	Papconstantinou et al. (259)
Adult ovariectomized transgenic ER-reporter, sc $\times$ 1	Purina 5001, polystyrene cage	Uterine wet weight ER activation	$[2.9 \times 10^{-5}]$ /diethylstilbestrol $[1.0 \times 10^{-4}]$ /diethylstilbestrol	Nagel et al. (260) Nagel et al. (260)

## 2.0 General Toxicology and Biological Effects

Model and exposure	Husbandry <sup>a</sup>	Endpoint	Molar potency/comparator <sup>b</sup>	Reference
Immature AP, sc × 3 days	RM1 diet, plastic cage, sawdust and shredded paper bedding	Blotted uterine weight	[ $2.3 \times 10^{-5}$ ]/diethylstilbestrol in 4 of 8 trials; other trials showed no effect at bisphenol doses up to 300 mg/kg bw/day.	Tinwell and Joiner (261)
Immature AP, gavage × 3 days	RM1 diet, plastic cage, sawdust and shredded paper bedding	Blotted uterine weight	No effect at bisphenol A doses up to 300 mg/kg bw/day; diethylstilbestrol produced response at 10 µg/kg bw/day.	Tinwell and Joiner (261)
Immature CD-1, sc × 3 days	RM1 diet	Lactoferrin expression	No effect at bisphenol A doses up to 1000 mg/kg bw/day; diethylstilbestrol showed effect at 0.1 µg/kg bw/day.	Mehmood et al. (262)
		Uterine weight, BrdU incorporation, peroxidase production	No effect at bisphenol A doses up to 100 mg/kg bw/day; diethylstilbestrol showed effect at 1–5 µg/kg bw/day.	
Immature CD-1, sc minipump × 3 days	RMH 3000 diet, cage, and bedding estrogen-negative by E-Screen	Uterine wet weight Epithelial cell height Lactoferrin expression	[ $1.6 \times 10^{-5}$ ]/17β-estradiol [ $3.8 \times 10^{-5}$ ]/17β-estradiol [ $3.9 \times 10^{-5}$ ]/17β-estradiol	Markey et al. (263)
Ovariectomized adult B6C3F <sub>1</sub> , ip × 3 days	Not indicated	Relative uterine to body weight	[ $3.6\text{--}74 \times 10^{-5}$ ]/17β-estradiol	Kitamura et al. (219)
Ovariectomized adult Swiss, sc × 1	Economy Rodent Maintenance diet	Increased uterine vascular permeability	$\sim 1 \times 10^{-4}$ /17β-estradiol	Milligan et al. (264)
<i>Other mouse organs</i>				
Juvenile-adult aromatase knock-out, diet × 4 months	NMF diet	Uterine and ovarian histology, bone mineral density	Dietary bisphenol A (0.1%) exerted estrogenic effects. Mean ± SD serum bisphenol A 84.3 ± 8.7 µg/L. No comparator estrogen was used for these endpoints.	Toda et al. (265)
<i>Fish</i>				
Immature rainbow trout, injected		Plasma vitellogenin	[ $3 \times 10^{-4}$ ]/17β-estradiol	Christiansen et al. (266)
Juvenile rainbow trout, injected		Plasma vitellogenin	[ $5.6 \times 10^{-3}$ ]/17β-estradiol	Andersen et al. (170)

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<b>Model and exposure</b>	<b>Husbandry<sup>a</sup></b>	<b>Endpoint</b>	<b>Molar potency/comparator<sup>b</sup></b>	<b>Reference</b>
Juvenile rainbow trout, exposed in water		Plasma vitellogenin	[~ <b>8.4 × 10<sup>-5</sup></b> ]/17β-estradiol	Lindholst et al. (267)
Male medaka, exposed in feed		Plasma vitellogenin	[ <b>1.4 × 10<sup>-4</sup></b> ]/ethinyl estradiol	Chikae et al. (268)
Male medaka, exposed in water		Hepatic vitellogenin and ERα mRNA	[ <b>8.4 × 10<sup>-6</sup></b> ]/17β-estradiol	Yamaguchi et al. (269)
Male killfish, injected		Plasma vitellogenin	[ <b>2.7 × 10<sup>-4</sup></b> ]/17β-estradiol	Pait et al. (270)
Male zebrafish, juvenile rainbow trout, exposed in water		Plasma vitellogenin	[~ <b>0.2</b> ]/ethinyl estradiol	Van den Belt et al.(271)
<i>Invertebrates</i>				
Mudsnail, exposed in water		New embryo production	[ <b>1.5 × 10<sup>-4</sup></b> ]/ethinyl estradiol	Jobling et al. (272)
Ramshorn snail, exposed in water		Egg production	Increased (EC <sub>10</sub> 13.9 ng/L); blocked by faslodex and tamoxifen. No comparison to reference estrogen	Oehlmann et al. (273)

<sup>a</sup>Husbandry information for rodent studies includes caging and bedding materials and diet when indicated by the authors.

<sup>b</sup>Estimates include comparison of administered dose, magnitude of effect, and molecular weight.

## 2.0 General Toxicology and Biological Effects

1 In vivo tests (Table 53) have been conducted principally in rats and mice. Most endpoints in these studies  
2 involved the uterus, and effects on uterine weight in immature or ovariectomized animals are the most  
3 commonly reported uterine endpoints. The potency of bisphenol A in increasing uterine weight varies  
4 over ~4 orders of magnitude. Some of this variation may be related to the short half-life of bisphenol A.  
5 Uterotrophic evaluations are typically performed 24 hours after the last dose of the test agent is  
6 administered. Laws et al. (237) showed no significant effect of bisphenol A at doses  $\leq 400$  mg/kg bw/day  
7 given orally on uterine wet weight assessed 24 hours after administering the last dose. When assessed 6  
8 hours after the last oral dose, bisphenol A 200 mg/kg bw/day increased uterine wet weight to ~2.5 times  
9 the control [estimated from a graph], which was about the same as the increase produced by  
10 administering  $17\beta$ -estradiol 0.005 mg/kg bw/day sc. Increase in uterine weight in the first 6 hours after  
11 treatment represents fluid inhibition and not true tissue growth. A dose-related decrease in blotted uterine  
12 weight and body weight, with no effect on weight-adjusted uterine weight, was shown in pubertal rats  
13 treated on PND 22–42/43 with bisphenol A by gavage at 400 or 600 mg/kg bw/day (244).

14  
15 For studies showing an increase in uterine weight after bisphenol A treatment, dose route affects  
16 response; bisphenol A given by gavage increased uterine weight by approximately 25% while the same  
17 dose given sc increased uterine weight by approximately 170%(237). A greater response by the sc than  
18 oral route was also shown by Yamasaki et al. (125) and Kanno et al. in the OECD multilaboratory study  
19 (274) who showed a lowest effective bisphenol A dose of 8 mg/kg bw/day by the sc route and 160 mg/kg  
20 bw/day by the oral route. The greater activity per unit dose of sc than oral bisphenol A is presumably due  
21 to glucuronidation of the orally administered compound with consequent loss of estrogenicity (169). A  
22 few studies could not confirm the greater effect of sc compared to oral bisphenol A on uterine weight.  
23 Ashby and Tinwell (236) concluded that the magnitude of uterine weight response was similar for sc and  
24 oral routes. [The Expert Panel notes a greater numerical magnitude of response after sc than oral  
25 exposure in most of the experiments reviewed in this report, and that statistical comparison of the  
26 dose routes was not reported.] Matthews et al. (169) found a similar increase in uterine weight in rats  
27 given sc or oral bisphenol A at 800 mg/kg bw/day.

28  
29 Nagel et al. (275, 276) noted that  $17\beta$ -estradiol is extensively protein-bound in vivo and bisphenol A is  
30 minimally protein-bound. A recent study indicated more extensive binding of bisphenol A to plasma  
31 binding proteins(139). Nagel suggested that estrogenicity of BPA (as well as other steroid hormones)can  
32 be more accurately predicted in rats by considering the free fraction of a chemical in human serum. [The  
33 Expert Panel notes that Figure 2 does not suggest that bisphenol A is more potent than  $17\beta$ -  
34 estradiol in vivo than in vitro. The developmental effects of bisphenol A in the prostate are  
35 discussed in Section 3.2.]

36  
37 Inter-strain variability in rats has been evaluated as a source of variability in estrogenicity assays.  
38 Inspection of Table 53 does not suggest large sensitivity differences between Sprague Dawley, Wistar,  
39 and Long Evans rats. Greater sensitivity of F344 than Sprague Dawley rats has been shown with respect  
40 to uterine weight and epithelial cell height (234), where  $17\beta$ -estradiol-adjusted potencies differed by 20–  
41 37% between the strains. BrdU labeling of vaginal epithelium was 3 times greater in F344 than Sprague  
42 Dawley rats in another study (254), and a third study (225) showed that both bisphenol A and  $17\beta$ -  
43 estradiol increase serum prolactin in ovariectomized F344 but not ovariectomized Sprague Dawley rats.  
44 Diel et al. (235) evaluated estrogenic response to bisphenol A in juvenile ovariectomized DA/Han,  
45 Sprague Dawley, and Wistar rats. After 3 days of treatment with bisphenol A 200 mg/kg bw/day, there  
46 were small statistically significant increases in uterine weight in DA/Han and Sprague Dawley rats but  
47 not in Wistar rats. There were no alterations in uterine or vaginal epithelium or in uterine clusterin mRNA  
48 expression in any of the strains after bisphenol A treatment.

## 2.0 General Toxicology and Biological Effects

1 Inter-laboratory variation in the uterotrophic assay was evaluated by the Organisation for Economic  
 2 Cooperation and Development (OECD) (274). Coded chemicals, including bisphenol A, were sent to up  
 3 to 212 different laboratories. Four assay protocols were evaluated including oral treatment of intact  
 4 immature rats for 3 days, sc treatment of intact immature rats for 3 days, sc treatment of ovariectomized  
 5 6–8-week-old rats for 3 days, and sc treatment of ovariectomized 6–8-week-old rats for 7 days. Not all  
 6 laboratories used all protocols or tested all compounds. Rat strains and suppliers were not standardized  
 7 across laboratories. Comparisons were made between labs based on the lowest dose level at which body  
 8 weight-adjusted blotted uterine weight was significantly different from the control. Results are  
 9 summarized in Table 54. The lowest effective dose of bisphenol A was uniformly identified for the assays  
 10 performed in ovariectomized adults. Assays performed in immature animals varied in identification of the  
 11 lowest effective bisphenol A dose level. There was no apparent effect of strain on sensitivity of the  
 12 uterotrophic response in immature (intact or castrate) or adult female rats.

13  
 14 **Table 54. Differences Between Laboratories in Rat Uterotrophic Assay with Bisphenol A**

Laboratory	Rat strain	Lowest effective dose level (mg/kg bw/day)				
		200	375	600	1000	
Immature, gavage × 3 days		200	375	600	1000	
2	CD(SD)IGS		×			
7	CD(SD)IGS			×		
12	CD(SD)IGS BR		×			
13	Wistar				×	
Immature, sc × 3 days		10	100	300	600	1000
2	CD(SD)IGS		×			
6	CD(SD)IGS BR			×		
7	CD(SD)IGS		×			
8	Alpk:ApfSD		×			
12	CD(SD)IGS BR				×	
13	Wistar			×		
15	Wistar			×		
18	Sprague Dawley	×				
20	Sprague Dawley	×				
21	CD(SD) BR	×				
Adult, sc × 3 days		10	100	300	600	1000
2	CD(SD)IGS		×			
6	CD(SD)IGS BR		×			
7	CD(SD)IGS		×			
8	Alpk:ApfSD		×			
12	CD(SD)IGS BR		×			
Adult, sc × 7 days		10	100	300	600	1000
2	CD(SD)IGS		×			
7	CD(SD)IGS		×			

From Kanno et al. (274)

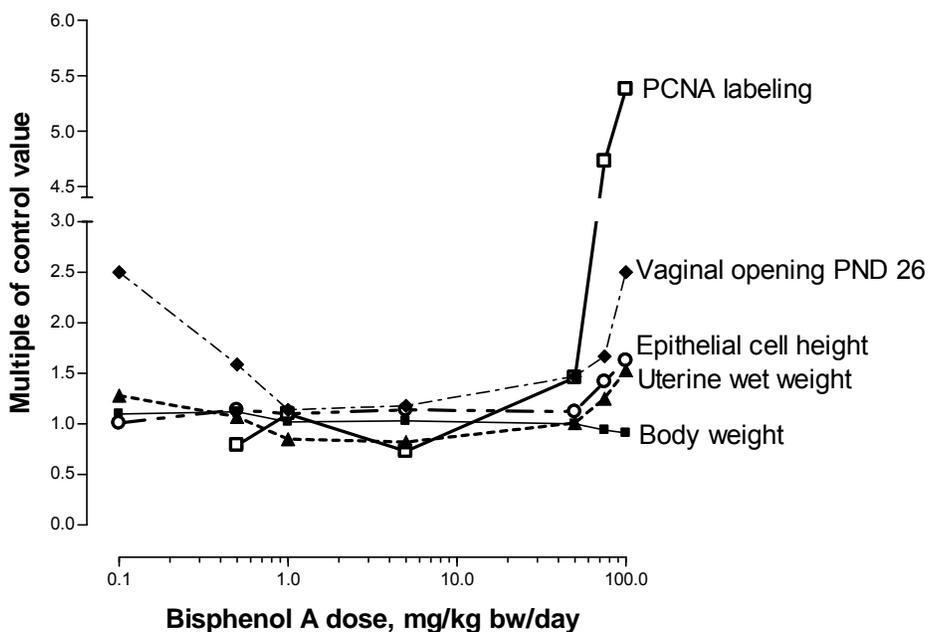
15  
 16 Intra-laboratory variability has been noted for the bisphenol A uterotrophic assay in immature mice (261).  
 17 Of 8 studies performed over a 2-year period at sc bisphenol A dose levels up to 200 or 300 mg/kg bw/day,  
 18 4 showed a significant increase in uterine weight at 200 mg/kg bw/day. The other 4 studies, including the  
 19 2 studies that went to 300 mg/kg bw/day, showed no effect of bisphenol A treatment on uterine weight  
 20 despite the expected response to diethylstilbestrol. Study authors noted that reducing the permissible body  
 21 weight of the mice selected for study resulted in lower and less variable control uterine weights and

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1 greater likelihood of bisphenol A effect (261, 277). [The Expert Panel notes that these studies all used  
2 high sc doses of bisphenol A.]

3  
4 Markey et al. proposed that the rodent uterotrophic assay is relatively insensitive to the estrogenic effects  
5 of bisphenol A (263). These authors treated immature CD-1 mice with bisphenol A in subcutaneous  
6 minipumps and evaluated uterine weight, relative area of uterine compartments, epithelial height,  
7 expression of lactoferrin and proliferating cell nuclear antigen (PCNA), and induction of vaginal opening.  
8 Dose-response curves for the endpoints that showed significant changes from control are illustrated in  
9 Figure 4. The study authors also noted that significant alterations in some endpoints were observed at  
10 much lower doses (0.1 mg/kg bw/day for vaginal opening and 5 mg/kg bw/day for epithelial cell height),  
11 giving rise to a U-shaped dose-response curve. [The assertions of some investigators notwithstanding,  
12 the Expert Panel notes that oral bisphenol A does not consistently produce robust estrogenic  
13 responses and, when seen, estrogenic effects after oral treatment occur at high dose levels.]

14  
15 Transgenic reporter mice have permitted in vivo identification of activation of the estrogen response  
16 element. Eight hours after ip injection on GD 13.5 of wild type dams carrying transgenic fetuses,  
17 luciferase reporter activity was increased for bisphenol A 1 and 10 mg/kg bw (214). The luciferase  
18 response after bisphenol A was about half that after a similar dose of estradiol dipropionate and ~25% of  
19 that after a 10-fold higher dose of diethylstilbestrol [estimated from a graph]. Use of an in vitro reporter  
20 system showed bisphenol A potency to be 3–4 orders of magnitude less than that of diethylstilbestrol  
21 (Table 52). The authors concluded that the in vivo estrogenic potency of bisphenol A may be greater than  
22 predicted by in vitro assays.



24  
25  
26 **Figure 4. Dose-Response Curves for Endpoints of Estrogenic Activity in sc-Dosed Mice**

27 On pair-wise testing, body weight was increased at 0.5 mg/kg bw/day and decreased at 100 mg/kg bw/day;  
28 vaginal opening was advanced at 0.1 and 100 mg/kg bw/day; epithelial cell height was increased at 5, 75,  
29 and 100 mg/kg bw/day; PCNA labeling was increased at 75 and 100 mg/kg bw/day; and uterine wet weight  
30 was increased at 100 mg/kg bw/day. Data from Markey et al. (263).

31  
32 Nagel et al. (260) developed a transgenic mouse with a thymidine kinase-*lacZ* reporter linked to 3 copies  
33 of the vitellogenin estrogen response element. This model showed an increase in ER activity after a single

## 2.0 General Toxicology and Biological Effects

1 sc bisphenol A dose of 25 µg/kg bw ( $P = 0.052$ ), with further increases in activity after 0.8 and 25 mg/kg  
 2 bw. Uterine weight was only increased at the 25 mg/kg bw dose level. Normalized to the diethylstilbestrol  
 3 response, uterine weight response to bisphenol A 25 mg/kg bw was less than one-third the response in  
 4 ER activity [estimated from a graph].

5  
 6 Gene expression profiles have been performed to compare the presumably ER-mediated response to  
 7 bisphenol A with the response to reference ER agonists. Naciff et al. (278) evaluated expression in the  
 8 uteri and ovaries of Sprague Dawley fetuses after sc dosing of dams on GD 11–20 with ethinyl estradiol  
 9 0, 0.5, 1 or 10 µg/kg bw/day or bisphenol A 0, 5, 50, or 400 mg/kg bw/day. The high dose of both  
 10 compounds induced nipples and areolae in male and female fetuses. There were 366 genes in which  
 11 expression was altered by ethinyl estradiol and 397 genes in which expression was altered by bisphenol  
 12 A. Expression of 66 genes was changed in the same direction with high doses of ethinyl estradiol,  
 13 bisphenol A, and genistein (which was also tested in this model). Of the 40 genes with at least a 1.8-fold  
 14 change in expression, 17 responded similarly to ethinyl estradiol and bisphenol A. The authors identified  
 15 50 mg/kg bw/day as the lowest dose level at which estrogen-like gene expression activity could be  
 16 identified, which is lower than the 400–800 mg/kg bw/day dose range at which uterotrophic activity is  
 17 typically reported in rats (236).

18  
 19 Terasaka et al. (279) used expression of 120 estrogen-responsive genes (based on previous work) in  
 20 MCF-7 cells to compare the profiles of bisphenol A and 17β-estradiol. Response was highly correlated ( $R$   
 21 = 0.92) between the 2 compounds. Another gene array study (280) used MCF-7 cells that had lost ER and  
 22 were re-engineered to express ERα. Among 40 estrogen-responsive genes, 12 responded to both  
 23 bisphenol A and 17β-estradiol, 9 responded only to bisphenol A, and 19 responded only to 17β-estradiol.  
 24 In the ER-deficient MCF-7 cell line from which these cells had been engineered, 1 gene responded to  
 25 both bisphenol A and 17β-estradiol and 14 responded to bisphenol A alone, suggesting ER-independent  
 26 activity. The same group reported the response of an additional 31 genes, associated with growth and  
 27 development, from the same chip (203). In the ERα-containing cells, 5 of these genes showed regulation  
 28 with both 17β-estradiol and bisphenol A, 13 were regulated only by bisphenol A, and 13 were regulated  
 29 only by 17β-estradiol.

30  
 31 Differences in the estrogenic activity of bisphenol A and reference estrogens may be due to differences in  
 32 recruiting by the liganded receptor of co-regulatory proteins. Singleton et al. (203) used a co-regulator-  
 33 independent yeast reporter system to evaluate the estrogenicity of bisphenol A and 17β-estradiol.  
 34 Bisphenol A activity was more than 3 orders of magnitude less than 17β-estradiol in the yeast system,  
 35 compared to about a 2-order-of-magnitude difference in an MCF-7 cell assay, leading the authors to  
 36 postulate that mammalian co-activators may be involved in enhancing bisphenol A activity. In a  
 37 comparison of ER binding and co-activator recruitment, Routledge et al. (166) showed bisphenol A to  
 38 bind the receptor more avidly than the liganded receptor recruited 2 co-activator proteins, normalized to  
 39 17β-estradiol (Table 55).

40  
 41 **Table 55. Bisphenol A Receptor Binding and Recruitment of Co-Activator Proteins**

Assay	Activity relative to 17β-estradiol	
	ERα	ERβ
Receptor binding	$7.3 \times 10^{-4}$	$7.5 \times 10^{-3}$
TIF2 recruitment	$< 1 \times 10^{-6}$	$5 \times 10^{-4}$
SRC-1a recruitment	$3 \times 10^{-4}$	$2 \times 10^{-4}$

From Routledge et al. (166).

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1 The classical ERs are receptors that, when bound, produce their activity through alterations in genomic  
2 transcription. In contrast, a membrane-bound ER has been described in murine pancreatic islet cells (281-  
3 284). This membrane-bound receptor regulates calcium channels and modulates insulin and glucagon  
4 release. Bisphenol A has been shown to activate this receptor in vitro at a concentration of 1 nM, which is  
5 similar to the active concentration of diethylstilbestrol (281, 283). Treatment of mice with bisphenol A or  
6 17 $\beta$ -estradiol sc at 10  $\mu$ g/kg bw acutely or daily for 4 days resulted in decreased plasma glucose and  
7 increased insulin (285). By contrast, Adachi et al. (286) reported that exposure of rat pancreatic islets to  
8 0.1–1  $\mu$ g/L [**0.4–4.4 nM**] bisphenol A did not alter insulin secretion over a 1-hour period. Exposure of  
9 islets to bisphenol A 10  $\mu$ g/L [**44 nM**] for 24 hours increased insulin release. This response was prevented  
10 by actinomycin D and by ICI 182,780, supporting the conclusion that bisphenol A insulin release occurs  
11 through interaction with the cytoplasmic ER rather than the membrane-bound receptor.  
12

13 A membrane-bound ER $\alpha$  in the pituitary could be related to regulation of the release of stored prolactin in  
14 response to estrogens, a non-genomic response mediated by calcium influx. Using a rat prolactinoma cell  
15 line, bisphenol A was shown to promote calcium influx and release prolactin over a concentration range  
16 similar to that for 17 $\beta$ -estradiol (287, 288). The response to bisphenol was bimodal, with maximal  
17 responses at concentrations of 10<sup>-12</sup> and 10<sup>-8</sup> M and little-to-no response at intermediate concentrations.  
18 Calcium influx in MCF-7 cells has been shown to occur rapidly after exposure to bisphenol and 17 $\beta$ -  
19 estradiol concentrations of 10<sup>-10</sup> M through a non-ER-mediated mechanism (289).  
20

21 Recently, bisphenol A was identified as competitor to 17 $\beta$ -estradiol for binding to the GPR30 receptor; a  
22 novel seven-transmembrane receptor that mediates nongenomic estrogen actions to up-regulate adenylyl  
23 cyclase and MAPK activities (290). Similar to previously reported findings with nuclear estrogen  
24 receptors and membrane estrogen receptors, bisphenol A was identified as a relatively effective  
25 competitor of 17 $\beta$ -estradiol binding, with relative binding affinities of 2.8 % that of the natural estradiol  
26 ligand and an IC<sub>50</sub> of 630 $\times$ 10<sup>-9</sup>M. Bisphenol A, at a concentration of 200nM significantly increased  
27 cAMP levels in transfected cells 30 minutes after compound addition.  
28

29 Bisphenol A has been found to bind estrogen-related receptor  $\gamma$ , a nuclear receptor with no known natural  
30 ligand that shows little affinity for 17 $\beta$ -estradiol (164). Estrogen-receptor  $\gamma$  demonstrates high constitutive  
31 activity that is maintained by bisphenol A in the presence of 4-hydroxytamoxifen, which otherwise blocks  
32 nuclear ER activity. This observation led to the suggestion that bisphenol A may maintain estrogen-  
33 related receptor  $\gamma$  activity in the presence of a yet-to-be-identified natural antagonist and that cross talk  
34 between the estrogen-related receptor and ER systems could be responsible for the estrogenic activity of  
35 bisphenol A in spite of low binding affinity for ER $\alpha$  and  $\beta$  (164).  
36

37 In addition to the studies reviewed for this section, there are studies in which the putative estrogenicity of  
38 environmental samples or synthetic products were evaluated using one or another assay. For example,  
39 Olea et al. (75) evaluated resin-based dental composites in an MCF-7 culture system. The response of the  
40 system was attributed to the bisphenol and its methacrylate detected in the composites, but bisphenol A  
41 was not specifically tested. These papers were not reviewed for this section.  
42

### 43 2.2.3 Androgen activity

44 Transfected cell-based assays have not identified bisphenol A as having androgenic activity (196, 213,  
45 219, 291). However, bisphenol A is mitogenic in cultured human prostate carcinoma cells at a  
46 concentration of 1 nM (292). Based on stimulated cell growth in this system, the potency of bisphenol A  
47 is about 5% that of dihydrotestosterone [**estimated from a graph**]. This bisphenol A activity was shown  
48 to be mediated by interaction with a mutant tumor-derived androgen receptor called AR-T877A. Anti-  
49 androgenic activity has been demonstrated using cells transfected with androgen receptor reporting  
50 systems (Table 56). The anti-androgenic activity of bisphenol A is expressed as the concentration needed

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1 to halve the androgen reporter response to a reference androgen. Studies in transfected cells have shown  
 2 that bisphenol A interferes with the binding of dihydrotestosterone to the androgen receptor, interferes  
 3 with translocation of the liganded receptor to the nucleus, and prevents transactivation at the androgen-  
 4 response element (293).

5  
 6 **Table 56. Anti-androgenicity Studies of Bisphenol A in Cells Transfected with Androgen Receptor**  
 7 **Reporter**

Cell type	Reference androgen concentration (nM)	Bisphenol A median inhibitory concentration (IC <sub>50</sub> ), $\mu$ M [mg/L]	Reference
Human prostate adenocarcinoma	R1881 0.1	7 [1.6]	Paris et al. (163)
Chinese hamster ovary	R1881 0.1	19.6 [4.5]	Roy et al. (294)
Yeast	Testosterone 10	1.8 [0.4]	Lee et al. (293)
Yeast	Dihydrotestosterone 1.25	2 <sup>a</sup> [0.5]	Sohoni and Sumpter (196)
Monkey kidney	Dihydrotestosterone 1	0.746 [0.2]	Xu et al. (291)
Monkey kidney	Dihydrotestosterone 1	2.14 [0.5]	Sun et al. (295)
Mouse fibroblast	Dihydrotestosterone 0.01	4.3 [1.0]	Kitamura et al. (219)
Human hepatoma	Dihydrotestosterone 100	No anti-androgenic activity	Gaido et al. (213)

<sup>a</sup>Estimated from a graph.

8  
 9 Kim et al. (296) conducted a Hershberger assay to determine the effects of bisphenol A exposure on  
 10 reproductive organs of rats. Sprague Dawley rats were fed PMI Certified Rodent LabDiet and housed in  
 11 polycarbonate cages. No information was provided about bedding materials. One experiment was  
 12 conducted to determine the optimum dose and age for observing testosterone exposure effects. In a  
 13 second experiment, 10 rats/group rats were castrated at 5 weeks of age and 7 days later gavaged with  
 14 bisphenol A (99% purity) at doses of 0 (ethanol/corn oil vehicle) 10, 100, or 1000 mg/kg bw/day for 7  
 15 days. A second group of castrated 6-week-old males rats was gavaged with bisphenol A at 0, 50, 100,  
 16 250, or 500 mg/kg bw/day for 7 days. In a third experiment, 10 castrated 6-week-old rats/group were  
 17 treated with 0.4 mg/kg bw/day testosterone by sc injection in addition to gavaged bisphenol A at 50, 100,  
 18 250, or 500 mg/kg bw/day or flutamide at 1, 5, 10, or 25 mg/kg bw/day for 7 days. A positive control  
 19 group was given 0.4 mg/kg bw/day testosterone for 7 days. **[There is some confusion in the paper**  
 20 **regarding ages at castration and start of treatment. For the first group of bisphenol A-treated rats,**  
 21 **it is reported that rats were castrated at 5 weeks of age and treated at 6 weeks of age. For the other**  
 22 **groups of bisphenol A-treated rats, the Methods section reported that treatment began at 6 weeks**  
 23 **of age, but tables in the Results section indicated that rats were castrated at 6 weeks of age.]** During  
 24 the study, clinical signs were observed and body weights were measured. Blood was collected and rats  
 25 were killed ~24 hours after administration of the last dose. Accessory reproductive organs were removed  
 26 and weighed. Serum luteinizing hormone (LH) and testosterone concentrations were measured by  
 27 radioimmunoassay (RIA). Statistical analyses included Bartlett test, analysis of covariance (ANCOVA),  
 28 Dunnet test, and Bonferroni test. Exposure to bisphenol A did not affect weights of the ventral prostate,  
 29 seminal vesicles, glans penis, or levator ani plus bulbocavernosus muscle; or serum concentrations of LH  
 30 or testosterone. Testosterone increased the weights of accessory reproductive organs. Flutamide increased  
 31 serum LH concentrations and inhibited testosterone-induced increases in accessory reproductive organ  
 32 weights. Study authors concluded that bisphenol A did not exhibit androgenic or antiandrogenic effects in  
 33 rats.

34  
 35 Yamasaki et al. (297) conducted a Hershberger assay in rats exposed to bisphenol A or 1 of 29 other  
 36 chemicals. In this study, which was conducted according to GLP, animals were housed in stainless steel  
 37 wire-mesh cages. Assuming these males were fed the same diets as rats used in an uterotrophic assay also

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1 described in this study, they received MF Oriental Yeast feed. Rats were randomly assigned to treatment  
2 groups. Beginning at 56 days of age and continuing for 10 days, 6 castrated male Brl Han: WIST Jcl  
3 (GALAS) rats/group were administered bisphenol A by stomach tube at doses of 0 (olive oil vehicle), 50,  
4 200, or 600 mg/kg bw/day. An additional group of rats was administered the same vehicle and doses of  
5 bisphenol A in addition to 0.2 mg/kg bw/day testosterone propionate by sc injection. Dose selection was  
6 based on results of preliminary studies. A positive control group was given 10 mg/kg bw/day flutamide in  
7 addition to 0.2 mg/kg bw/day testosterone propionate. Rats were killed 24 hours after receiving the final  
8 dose. Ventral prostate with fluid, seminal vesicles with fluid, bulbocavernosus/levator ani muscle, glans  
9 penis, and Cowper gland were collected and weighed. Data were analyzed by Student *t*-test. Bisphenol A  
10 did not affect body weight and there were no clinical signs of toxicity. The only statistically significant  
11 effect on relative organ weight was a [24%] increase in glans penis weight in rats given 600 mg/kg  
12 bw/day bisphenol A without co-administration of testosterone. In contrast, rats treated with flutamide plus  
13 testosterone propionate experienced increases in weights of ventral prostate, seminal vesicle,  
14 bulbocavernosus/levator ani muscle, glans penis, and Cowper gland. **[Absolute organ weights were not  
15 reported. It is assumed but was not stated that relative weights were based on body weight.]** Study  
16 authors noted that because glans penis weights were variable in control rats and weights of other  
17 accessory reproductive organs were not affected, bisphenol A could not be clearly determined to have  
18 androgen agonistic properties.

19  
20 Nishino et al. (298) performed a Hershberger assay in Wistar rats. At 2 weeks of age, rats were given  
21 ssniffR 10 diet and housed in Makrolon cages with ssniff bedding. Seven days after orchietomy, rats  
22 were placed in groups of 13 **[randomization not discussed]** and treated orally **[gavage assumed]** with  
23 bisphenol A **[purity not indicated]** in propylene glycol at 0, 3, 50, 200, or 500 mg/kg bw/day for 7 days  
24 or sc with testosterone propionate 1 mg/kg bw. Another group was given oral bisphenol A 500 mg/kg  
25 bw/day and flutamide 3 mg/kg bw/day. Rats were killed by decapitation after treatment. Seminal vesicles  
26 and prostates were weighed and fixed in 4% neutral buffered paraformaldehyde. Immunohistochemical  
27 evaluation of androgen receptor, PCNA, and MIB-5 was performed. Epithelial cell height and duct  
28 luminal area were determined morphometrically. Review by the Expert Panel indicated that this paper  
29 was inadequate due to methodological issues.

### 31 **2.3 Genetic Toxicity**

32 Assessment of mutagenicity associated with bisphenol A was based primarily on reviews by the European  
33 Union (2) and Haighton et al. (47). CERHR summarized a limited number of studies that were not  
34 included in reviews. Results of *in vitro* genetic toxicity testing are summarized in [Table 57](#), and results of  
35 *in vivo* genetic toxicity tests are summarized in [Table 58](#).

36  
37 The European Union (2) noted that bisphenol A demonstrated aneugenic potential and micronuclei  
38 formation in *in vitro* tests without metabolic activation. However, there was no evidence of micronuclei  
39 formation in an *in vivo* mouse study. Other studies demonstrated disruption of microtubule formation and  
40 the presence of DNA adducts. In the studies reviewed by the European Union, there was no evidence of  
41 gene mutations or structural chromosomal aberrations in *in vitro* tests and negative results were obtained  
42 in a dominant lethal test in rats; however, the European Union noted several limitations for those studies.  
43 Based on their review of genotoxicity data and the lack of significant tumors reported in animal studies,  
44 the European Union (2) concluded that bisphenol A does not appear to have significant mutagenicity  
45 potential *in vivo*. Because aneugenic potential was apparently observed only in *in vitro* tests, it was  
46 judged to be of no concern. The relevance of DNA adduct formation was unclear, but based on weight of  
47 evidence, i.e. negative findings for gene mutation and clastogenicity in cultured mammalian cells, DNA  
48 adduct formation was thought unlikely to be of concern for humans.

49  
50 Haighton et al. (47) concluded that results of standardized and validated genetic toxicity tests  
51 demonstrated the lack of mutagenic and genotoxic activity of bisphenol A *in vivo*. Studies demonstrating

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1 disrupted microtubule formation or DNA adduct formation were noted, but because the studies used high  
2 doses, they were judged to be of limited relevance. The lack of activity in an in vivo micronucleus assay  
3 in mice was said to confirm negative results observed in in vivo tests. Lastly, it was concluded that  
4 bisphenol A (parent) had no structural features that suggested mutagenic activity.

5  
6 Subsequent to the release of the European Union (2) and Haighton et al. (47) reviews, Hunt et al. (299),  
7 published a study examining meiotic aneuploidy potential of bisphenol A in female mice. In 1998, a large  
8 increase in background rate of congression failure (from 1–2 to 40%) and in aneuploidy (from 0.7 to  
9 5.8%) was observed in the study authors' laboratory. The increase was found to coincide with damage to  
10 polycarbonate caging material. Removal of the most damaged cages and change to polysulfone cages  
11 resulted in decreased background rates of congression failure. Intentionally damaging polycarbonate  
12 cages and water bottles resulted in increased rates of congression failure. As noted in Table 58,  
13 congression failure rates were increased in juvenile female mice orally exposed to  $\geq 20$   $\mu\text{g}/\text{kg}$  bw/day  
14 bisphenol A for 6–8 days or 20  $\mu\text{g}/\text{kg}$  bw/day for 7 days. The study authors concluded that bisphenol A  
15 was a potential meiotic aneugen.

16  
17 In a follow-up study (300), pregnant C57Bl/6 mice on GD 11.5 were implanted with sc pellets designed  
18 to release bisphenol A 0 or 0.4  $\mu\text{g}/\text{day}$ . [**The authors assume a 20 g bw, giving an estimated dose level**  
19 **of 20  $\mu\text{g}/\text{kg}$  bw/day.**] Oocytes from GD 18.5 female fetuses showed an increase in pachytene synaptic  
20 abnormalities including incomplete synapsis and end-to-end associations of sister chromatids. There was  
21 also paradoxically an increase in recombinant foci in pachytene oocytes of bisphenol A-exposed females.  
22 Some female offspring of bisphenol A-treated dams were fostered to untreated dams. Eggs or 2-cell  
23 embryos from these female offspring at 4–5 weeks of age showed an increase in hyperploidy. Pachytene  
24 oocyte abnormalities similar to those identified in fetuses exposed to bisphenol A were seen in oocytes  
25 obtained from ER $\beta$  knock-out mice, suggesting to the authors that bisphenol A may exert adverse effects  
26 on meiosis by blocking ER $\beta$ .

27  
28 In response to the study of Hunt et al. (299), Pacchierotti et al. (301) investigated the aneugenic effects of  
29 bisphenol A in mouse somatic and germ cells. C57Bl/6 female mice were superovulated using pregnant  
30 mare serum and hCG following which they were gavaged with bisphenol A 0.2 or 20 mg/kg bw.  
31 Metaphase II oocytes were collected after 17 hours and evaluated using C-banding. Additional female  
32 mice were gavaged with bisphenol A 0.04 mg/kg bw/day for 7 days or were given bisphenol A in  
33 drinking water at a concentration of 0.4 mg/L for 7 weeks. These mice were superovulated at the end of  
34 the 7-day or 7-week treatment period and housed overnight with untreated males. Females without  
35 vaginal plugs were killed for evaluation of oocytes by C-banding. Females with vaginal plugs were  
36 treated with colchicine to prevent the first embryonic cleavage, and zygotes were collected the next  
37 morning for evaluation by C-banding. There were no bisphenol A effects on induction of aneuploidy.  
38 There was a statistically significant increase in premature centromere separation in the group treated for 7  
39 weeks, but there was no effect of bisphenol A treatment on the proportion of zygotes with structural or  
40 numeric chromosome changes. Male mice were treated with bromodeoxyuridine 8 days before being  
41 treated with bisphenol A 0.2 mg/kg bw/day for 6 days. Evaluation of sperm after 21–25 days did not  
42 show a significant mitotic delay in spermatocytes. Additional male mice were given bisphenol A orally at  
43 doses of 0, 0.002, 0.02, and 2 mg/kg bw/day for 6 days. Epididymal sperm were collected 22 days after  
44 the end of bisphenol A treatment and multicolor fluorescent in situ hybridization was used to evaluate  
45 decondensed sperm for aneuploidy. Sperm count was decreased by bisphenol A 0.002 mg/kg bw/day, but  
46 there was no increase in the frequency of hyperhaploidy or diploidy. Bisphenol A was negative in a bone  
47 marrow micronucleus test at dose levels up to 2 mg/kg/day for 2 days.

1 Table 57. In Vitro Genetic Toxicity Studies of Bisphenol A

Concentration	Cell	Endpoint	Results	Reference
3.3–333.3 µg/plate, with and without metabolic activation	<i>Salmonella typhimurium</i> strains TA98, TA100, TA1535, TA1537	Mutagenicity	Negative	Haworth et al. (1983) <sup>a,b</sup>
50–500 µg/plate, with and without metabolic activation	<i>Salmonella typhimurium</i> strains TA97a, TA98, TA100, TA102	Mutagenicity	Negative	Schweikl et al. (1998) <sup>a,b</sup>
≤5000 µg/plate with and without metabolic activation	<i>Salmonella typhimurium</i> strains TA97, TA98, TA100, TA102	Mutagenicity	Negative	Takahata et al. (1990) <sup>a,b</sup>
≤1000 µg/mL, with and without metabolic activation	<i>Salmonella typhimurium</i> strain TA1538 and <i>Escherichia coli</i> strains WP2 and WP2uvrA	Mutagenicity	Negative	Dean and Brooks (1978) <sup>a,b</sup>
5–1250 µg/plate, with and without metabolic activation	<i>Salmonella typhimurium</i> strains TA98, TA100, TA1535, TA1537 and <i>Escherichia coli</i> strain WP2uvrA	Mutagenicity	Negative	JETOC (1996) <sup>a,b</sup>
1 mM [228 mg/L], with and without metabolic activation	<i>Salmonella typhimurium</i> strains TA98 and TA100	Mutagenicity	Negative	Masuda et al. (302)
0.1–0.2 mM [23–46 mg/L], without metabolic activation	Chinese hamster V79 cells, hprt locus	Mutagenicity	Negative	Schweikl et al. (1998) <sup>a,b</sup>
5–60 mg/L without metabolic activation, 25–200 mg/L with metabolic activation, or 5–60 mg/L with and without metabolic activation <sup>d</sup>	Mouse lymphoma L5178Y cells, tk <sup>+/−</sup> locus	Mutagenicity	Negative (results questioned due to possible inability to count small colonies)	Myhr and Caspary (1991) <sup>a,b</sup>
Concentrations not specified, with and without metabolic activation	Mouse lymphoma L5178Y cells, tk <sup>+/−</sup> locus	Mutagenicity	Inconclusive without and negative with metabolic activation	Honma et al. (1999) <sup>a,b</sup> and Moore et al. (1999) <sup>a,b</sup>
25–200 µM [5.7–46 mg/L], without metabolic activation	Syrian hamster embryo cells (Na <sup>+</sup> /K <sup>+</sup> ATPase and hprt loci)	Mutagenicity	Negative	Tsutsui et al. (1998) <sup>a,b</sup>
10 <sup>−8</sup> –10 <sup>−5</sup> M [0.002–2 mg/L], without metabolic activation	Human R5a cells	Mutagenicity	↑ at all doses	Takahashi et al. (303)
≤500 mg/L, with and without metabolic activation	<i>Saccharomyces cerevisiae</i> strain JDI	Mutagenicity	Negative	Dean and Brooks (1978) <sup>a,b</sup>
10 <sup>−8</sup> –10 <sup>−4</sup> M [0.002–23 mg/L], without metabolic activation	MCF-7 cells	DNA damage (assessed by comet assay)	↑ at ≥10 <sup>−6</sup> M [0.2 mg/L]	Iso et al. (304)
10 <sup>−4</sup> M [23 mg/L], without metabolic activation	MDA-MB-231 cells	DNA damage (assessed by comet assay)	↑	

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Concentration	Cell	Endpoint	Results	Reference
20–40 mg/L, without metabolic activation and 30–50 mg/L with metabolic activation	Chinese hamster ovary (CHO) cells	Chromosomal aberration	Negative (inconsistent ↑ at high dose with metabolic activation)	Ivett et al. (1989) <sup>a,b</sup> and Tennant et al. (1986, 1987) <sup>b</sup>
350–450 μM [ <b>80–103 mg/L</b> ], without metabolic activation and ≤250 μM [ <b>57 mg/L</b> ] with metabolic activation	CHO cells, clone WBL	Chromosomal aberration	Positive at ≥400 μM [ <b>91.3 mg/L</b> ] without metabolic activation <sup>c</sup> ; negative with metabolic activation	Hilliard et al. (1998) <sup>a</sup>
400 and 450 μM [ <b>91 and 103 mg/L</b> ], without metabolic activation	CHO cells, clone WBL	Chromosomal aberration	Positive <sup>c</sup>	Galloway et al. (1998) <sup>a</sup>
25–200 μM [ <b>5.7–46 mg/L</b> ], without metabolic activation	Syrian hamster embryo cells	Chromosomal aberration	Negative	Tsutsui et al. (1998) <sup>a,b</sup>
10–30 mg/L	Epithelial-type rat liver cell line (RL1)	Chromosomal aberration	Negative	Dean and Brooks (1978) <sup>b</sup>
25–200 μM [ <b>5.7–46 mg/L</b> ], without metabolic activation	Syrian hamster embryo cells	Aneuploidy/polyploidy	Inconclusive (non-dose-related ↑ in aneuploidy at ≥50 μM [ <b>11 mg/L</b> ]); apparently positive <sup>f</sup>	Tsutsui et al. (1998) <sup>a,b</sup>
0.8–25 mg/L, without metabolic activation and 30–50 μg/mL, with metabolic activation	CHO cells	Sister chromatid exchange	Negative (one small ↑ was not reproducible)	Ivett et al. (1989) <sup>a,b</sup> and Tennant et al. (1986) <sup>b</sup>
0.2–0.5 mM or nM <sup>d</sup> [ <b>46–114 mg/L or μg/L</b> ]	Rat hepatocytes	DNA strand breaks	Negative (↑ noted but scored as negative by study authors due to excessive cytotoxicity)	Storer et al. (1996) <sup>a,b</sup>
10 <sup>-9</sup> –10 <sup>-5</sup> M [ <b>0.0002–2 mg/L</b> ], without metabolic activation	Human RSa cells	Unscheduled DNA synthesis	↑ at 10 <sup>-6</sup> M [ <b>0.2 mg/L</b> ] and ↓ at 10 <sup>-7</sup> [ <b>0.02 mg/L</b> ] and 10 <sup>-5</sup> M [ <b>2 mg/L</b> ]	Takahashi et al. (303)
Not specified, but stated to cover range of cytotoxicity	A31-1-13 clone of BALB/c-3T3 cells	Transformation	Negative	Matthews et al. (1993) <sup>a</sup>
25–200 μM [ <b>5.7–46 mg/L</b> ], without metabolic activation	Syrian hamster embryo cells	Transformation	Positive at ≥50 μM [ <b>11.4 mg/L</b> ] (non-dose-related ↑) <sup>e</sup> ; equivocal <sup>f</sup>	Tsutsui et al. (1998, 2000) <sup>a,b</sup>
≤50 mg/L for 24 hours; ≤30 mg/L for 7 days, without metabolic activation	Syrian hamster embryo cells	Transformation	Negative	LeBoeuf et al. (1996) <sup>a</sup>
2–60 mg/L	Syrian hamster embryo cells	Transformation	Negative	Jones et al. (1988) <sup>b</sup>
50–200 μM [ <b>11.5–46 mg/L</b> ], without metabolic activation	Syrian hamster embryo cells	DNA adduct formation	Positive at ≥50 μM [ <b>11 mg/L</b> ] (dose-related ↑)	Tsutsui et al. (1998) <sup>a,b</sup>

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Concentration	Cell	Endpoint	Results	Reference
1000 µg presence of peroxidase and hydrogen peroxide	Purified rat DNA	DNA adduct formation	Positive	Atkinson and Roy (144)
10–100 µM [2.3–23 mg/L], metabolic activation unknown	Bovine brain microtubule protein	Inhibited microtubule polymerization	Positive	Metzler and Pfeiffer (1995) <sup>a</sup>
50–200 µM [11.5–46 mg/L], no metabolic activation	Bovine brain microtubule protein	Inhibited microtubule polymerization	Positive (dose-related)	Pfeiffer et al. (1996) <sup>b</sup>
20–200 µM [4.6–46 mg/L], metabolic activation unknown	Bovine brain microtubule protein	Inhibited microtubule polymerization	Positive (EC <sub>50</sub> = 150 µM [34 mg/L])	Pfeiffer et al. (1997) <sup>a,b</sup>
200 µM [46 mg/L], without metabolic activation; 100 µM [23 mg/L] for metaphase arrest assay	Chinese hamster V79 cells	Aneuploidogenic potential as assessed by micronuclei formation, microtubule assay, and metaphase arrest.	Positive	Pfeiffer et al. (1997) <sup>a,b</sup>
100–200 µM [23–46 mg/L], without metabolic activation	Chinese hamster V79 cells	Aneuploidogenic potential as assessed by micronuclei formation	Positive	Ochi (1999) <sup>a,b</sup>
10 or 30 µM [2.3 or 6.9 mg/L]	Oocytes from Balb/c mice	Meiotic spindle formation	Centrosomes and spindles disorganized	Can et al. (305)
0.05–0.4 mg/L	Oocytes from MF <sub>1</sub> mice	Aneuploidy	No hyperhaploidy but ↑ diploid metaphase II oocytes at 0.2 mg/L	Pacchierotti et al. (301)

↑, ↓ increase, decrease.

<sup>a</sup>Reviewed by Haighton et al. (47).

<sup>b</sup>Reviewed by the European Union (2).

<sup>c</sup>According to the Haighton et al. (47) review, positive results occurred at cytotoxic concentrations.

<sup>d</sup>Discrepancies noted between information presented by Haighton et al. (47) and European Union (2).

<sup>e</sup>Conclusion by Haighton et al. (47).

<sup>f</sup>Conclusion by European Union (2).

1 Table 58. In Vivo Genetic Toxicity Studies of Bisphenol A

Species and sex	Dose (route)	Cells	Endpoint	Results	Reference
Male rat	85 mg/kg bw/day for 5 days (ip)	Germ	Dominant lethality	Negative	Bond et al. (1980) <sup>a,b</sup> (abstract only)
Male rat	200 mg/kg bw (ip) and 200 mg/kg bw for 4, 8, 12, or 16 days (oral)	DNA	Adduct formation	Positive	Atkinson and Roy (145)
Male and female mouse	500–2000 mg/kg bw (oral)	Bone marrow	Micronuclei	Negative	Gudi and Krsmanovic (1999) <sup>a</sup> and Shell Oil Co. (1999) <sup>b</sup>
Male mouse	1 mmol/kg bw [228 mg/kg bw] (oral)	Peripheral blood reticulocyte	Micronuclei	Negative	Masuda et al. (302)
20–22-day-old female mouse	0.02–0.100 mg/kg bw/day (oral) for 6–8 days or 0.02 mg/kg bw for 3, 5, or 7 days	Oocyte	Congression failure	Positive at all doses; statistically significant with 7-day exposure	Hunt et al. (299)
Pregnant mouse GD 11.5–18.5	0.4 µg/day sc pellet [~20 µg/kg bw/day]	Oocyte	Evaluation of pachytene fetal oocyte and of ploidy in oocytes and 2-cell embryos from adults that were exposed in utero	Incomplete synapsis, end-to-end association of sister chromatids, ↑hyperploidy	Susiarjo et al. (300)
Female mouse	0.2 or 20 mg/kg bw acutely or daily for 7 days or 0.4 mg/L in drinking water for 7 weeks	Oocyte	Aneuploidy	Negative	Pacchierotti et al. (301)
Male (102/ElxC3H/El) F <sub>1</sub> mouse	0.002–0.2 mg/kg bw for 6 days (oral)	Spermatocyte	Meiotic delay and aneuploidy	Negative	Pacchierotti et al. (301)
<i>Drosophila melanogaster</i>	10,000 ppm (oral)	Offspring	Sex-linked recessive lethal test	Negative	Foureman et al. (1994) <sup>a,b</sup>
Turbot	50 ppb in aquarium water for 2 weeks	Erythrocyte	Micronuclei	Positive	Bolognesi et al. (306)

<sup>a</sup>Reviewed by Haighton et al. (47).<sup>b</sup>Reviewed by the European Union (2).

### 2.4. Carcinogenicity

No human data examining the carcinogenicity of bisphenol A were identified.

NTP (157, 307) examined carcinogenicity of bisphenol A in F344 rats and B6C3F<sub>1</sub> mice. Animals were randomly assigned to treatment groups. Bisphenol A (<98.2% purity) was administered through feed for 103 weeks to 50 rats/sex/dose at 0, 1000, or 2000 ppm, 50 male mice/group at 0, 1000, or 5000 ppm, and 50 female mice/group at 0, 5000, or 10,000 ppm. NTP estimated mean bisphenol A intakes of 74 and 148 mg/kg bw/day for male rats and 74 and 135 mg/kg bw/day for female rats. **[Data on bisphenol A intake, food intake, and body weights were not provided for mice.]** Using default values, the European Union (2) estimated bisphenol A intakes of 120 and 600 mg/kg bw/day in male mice and 650 and 1300 mg/kg bw/day in female mice. Concentration and stability of bisphenol A in feed were verified. Body weights and clinical signs were observed during the study. Following the exposure period, animals were killed and necropsied. Organs, including seminal vesicle, prostate, testis, ovary, and uterus, were preserved in 10% neutral buffered formalin and examined histologically. Statistical analyses included Cox and Tarone methods, 1-tailed Fisher exact test, Bonferroni inequality criterion, Cochran-Armitage test, and life table methods for linear trend.

In rats, body weights of males and females from both dose groups were lower than controls throughout the study. Feed intake was decreased in females of both dose groups beginning at week 12. No adverse effects on survival were observed. There were no non-neoplastic lesions **[including in male and female reproductive organs]** that appeared to be treatment-related. The incidence of leukemia was increased in males (13 of 50, 12 of 50, and 23 of 50 in control and each respective dose group) and females (7 of 50, 13 of 50, and 12 of 50). In males the trend for leukemia was significant by Cochran-Armitage test, but statistical significance was not shown by life table analysis for trend or incidence in the high-dose group, according to the unpublished version of the study. The published version of the study indicated statistical significance at the high dose. Statistical significance was not attained for leukemia incidence in female rats. An increased incidence of testicular interstitial cell tumors (35 of 49, 48 of 50, 46 of 49) was statistically significant in both dose groups. An increased incidence of mammary fibroadenomas in males of the high-dose group (0 of 50, 0 of 50, and 4 of 50) achieved statistical significance for trend by Cochran-Armitage test but not by Fisher exact test. In bisphenol A groups, there were decreased incidences of adrenal pheochromocytomas in males, adrenal cortical adenomas in females, and uterine endometrial stromal polyps. The NTP concluded that none of the increases in tumor incidence in rats was clearly associated with bisphenol A exposure.

In mice, body weights were lower in high-dose males and in females of both dose groups. Feed intake could not be accurately determined because of spillage. Bisphenol A did not affect the survival of mice. Incidence of multinucleated hepatocellular giant cells was increased in treated males (1 of 49, 41 of 49, and 41 of 50). **[A review of the data indicated no increases in incidence of non-neoplastic lesions in the reproductive organs of male or female mice.]** The incidence of leukemia or lymphoma in male mice by dose group (2 of 49, 9 of 50, and 5 of 50) was not statistically significant. The published version of the report indicated an increasing trend for lymphoma. The linear trend for increased pituitary chromophobe carcinomas in male mice (0 of 37, 0 of 36, 3 of 42) was reported to be statistically significant by Cochran-Armitage test but statistical significance was not shown by Fisher exact test. The study authors concluded that none of the increases in tumor incidence in mice could be unequivocally associated with bisphenol A exposure.

NTP concluded that under the conditions of this study, there was no convincing evidence the bisphenol A was carcinogenic in F344 rats or B6C3F<sub>1</sub> mice. However, study authors stated that there was suggestive evidence of increased cancer in the hematopoietic system based on marginally significant increases in leukemia in male rats, non-statistically significant increases in leukemia in female rats, and a marginally significant increase in combined incidence of lymphoma and leukemia in male mice. A statistically significant increase in testicular interstitial cell tumors in aging F344 rats was also considered suggestive

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evidence of carcinogenesis. The effect was not considered conclusive evidence because of the high incidence of the testicular neoplasm in aging F344 rats (88% incidence in historical controls).

The NTP study was reviewed by the European Union (2) and Haighton et al. (47). For increases in leukemia, mammary gland fibroadenoma, and Leydig cell tumors in male rats, both groups noted the lack of statistical significance using the appropriate analyses and the common occurrence of these tumor types in F344 rats. The European Union (2) concluded, “Overall, all of these [tumor] findings in rats and mice are not considered toxicologically significant. Consequently, it is concluded that bisphenol A was not carcinogenic in this study in both species.” Haighton et al. (47) concluded, “Overall, the results of this bioassay did not provide any compelling evidence to indicate that [bisphenol A] was carcinogenic in F344 rats or in B6C3F<sub>1</sub> mice.” Based on the experimental animal data, the European Union concluded that “. . . the evidence suggests that bisphenol A does not have carcinogenic potential.” Using a weight of evidence approach, Haighton et al. (47) concluded that bisphenol A was not likely to be carcinogenic to humans. This conclusion was based upon NTP study results; lack of activity at noncytotoxic concentrations in both in vitro genetic toxicity tests and in an in vivo mouse micronucleus test; and data from metabolism studies that show rapid glucuronidation and no formation of possibly reactive intermediates, with the possible exception of reactive intermediates potentially generated as a result of saturated detoxification pathways at high doses.

### 2.5 Potentially Susceptible Subpopulations

As noted in Section 2.1.1.3, one pathway of bisphenol A metabolism in humans and experimental animals is glucuronidation. Studies in experimental animals demonstrated that both the intestine and liver can glucuronidate bisphenol A. UGT2B1 was identified as the isoform involved in bisphenol A glucuronidation in rat liver (146). The UDPGT isoform involved in human intestinal glucuronidation of bisphenol A is not known to have been identified. Despite uncertain isoform identification, studies in humans and experimental animals demonstrate developmental changes in expression of activities of several UDPGT isoforms that potentially affect bisphenol A metabolism.

Coughtrie et al. (308) examined the ontogeny of UDPGT activity in human liver microsome samples obtained postmortem from adults and premature or full-term infants. Results of this analysis are listed in Table 59. Activities for isoenzymes catalyzing glucuronidation of bilirubin, testosterone, and 1-naphthol were very low at birth in premature and full-term infants. Activities increased with age for the isoenzymes catalyzing glucuronidation of bilirubin (~80% of adult levels by 8–15 weeks of age) and 1-naphthol (~30% of adult levels at 8–15 weeks of age). During the first 55 weeks of life, no consistent increase in activity was noted for the isoenzyme catalyzing glucuronidation of testosterone. Using an immunoblot technique with antibodies developed toward liver testosterone/4-nitrophenol and kidney naphthol/bilirubin, 1 immunoreactive protein was observed in microsomes of 18- and 27-week-old fetuses and 3 immunoreactive proteins were observed in microsomes of full-term infants. Most isoenzymes present in adults were observed in infants within 3 months of age at levels ~25% those of adults.

**Table 59. Development of UDPGT Activity in Humans**

Age	UDPGT activity, nmol/min/mg protein		
	Bilirubin	Testosterone	1-Naphthol
30 weeks gestation	0.05	0	0.56
30 weeks gestation with 10 weeks survival	0.4; 1	0.14; 0.85	3.0; 1.8
Full-term infants surviving 1–10 days (n = 7)	0.07 ± 0.04	0.10 ± 0.06	0.75 ± 0.68
Full-term infants surviving 8–15 weeks (n = 6)	0.64 ± 0.32	0.12 ± 0.05	2.4 ± 1.1
Full-term infants surviving 22–55 weeks (n = 5)	0.99 ± 1.1	0.09 ± 0.06	3.6 ± 2.1
Adult males (n = 3)	0.76 ± 0.43	0.46 ± 0.61	7.2 ± 2.2

Data presented as individual values or mean ± SD.

From Coughtrie et al. (308).

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1 Strassburg et al. (309) used a reverse transcript (RT)-polymerized chain reaction (PCR) technique to  
2 examine developmental changes in expression for 13 *UDPGT* genes in liver samples obtained from 16  
3 pediatric patients undergoing liver transplant for extrahepatic biliary atresia (6–24 months old) and 12  
4 adults undergoing liver transplant for carcinoma (25–75 years). Changes in gene expression were also  
5 assessed in hepatic RNA samples for two 20-week-old fetuses. No transcripts for *UDPGT* were detected  
6 in samples from 20-week-old fetuses. In infant and adult livers, transcripts were detected for *UGT1A1*,  
7 *UGT1A3*, *UGT1A4*, *UGT1A6*, *UGT1A9*, *UGT2B4*, *UGT2B7*, *UGT2B10*, and *UGT2B15*; there were no  
8 age-related differences in expression. Expression of *UGT1A9* and *UGT2B4* mRNA was lower in the  
9 pediatric samples. Western blot analyses of protein expression for *UGT1A1*, *UGT1A6*, and *UGT2B7*  
10 were consistent with findings for mRNA expression. Activities towards 18 specific substrates were  
11 assessed in microsomes. In 13–24-month-old children compared to adults, glucuronidation activity was  
12 lower for ibuprofen (24-fold), amitriptyline (16-fold), 4-tert-butylphenol (40-fold), estrone (15-fold), and  
13 buprenorphine (12-fold).

14  
15 Cappiello et al. (310) compared uridine 5'-diphosphoglucuronic acid concentrations in livers and kidneys  
16 of human fetuses and adults and in placenta. In adults undergoing surgery, liver samples were obtained  
17 from 1 man and 4 women (23–72 years of age) and kidney samples were obtained from 1 woman and 4  
18 men (55–63 years of age). Fetal livers and kidneys were obtained from 5 fetuses legally aborted between  
19 16 and 25 weeks gestation. Five placenta samples were obtained upon delivery at 17–25 weeks gestation.  
20 Compared to adults, fetal uridine 5'-diphosphoglucuronic acid concentrations were 5-fold lower in liver  
21 and 1.5-fold lower in kidney. Concentrations of uridine 5'-diphosphoglucuronic acid in placenta were 3–  
22 4-fold lower than in fetal liver. Based on these findings, study authors concluded that glucuronidation is  
23 potentially limited in the human fetus.

24  
25 As noted in Sections 2.1.2.2 and 2.1.2.3, rat fetuses appear to have no or low ability to glucuronidate  
26 bisphenol A (126, 150, 151). Although rats glucuronidate bisphenol A at birth, glucuronidation capacity  
27 appears to increase with age (2, 118, 151).

28  
29 Some possible interindividual or sex-related differences in the ability to produce the bisphenol A sulfate  
30 conjugate were identified in a limited number of human studies. As discussed in more detail in Section  
31 2.1.1.3 and shown in Table 8, higher amounts of urinary bisphenol A sulfate were detected in 15 adult  
32 women than in 15 adult males (98). In a study examining bisphenol A metabolism by human hepatocytes,  
33 an ~10-fold higher concentration of a bisphenol A glucuronide/sulfate conjugate was observed in the  
34 sample from 1 female than in samples from 2 other females and 2 males (143).

35  
36 Yang et al. (88) examined the effects of polymorphisms in sulfotransferase enzymes on urinary excretion  
37 of total bisphenol A (conjugated and free) in Korean volunteers. Urinary bisphenol A concentrations were  
38 measured by HPLC and a PCR method was used to determine sulfotransferase genotype. The  
39 *SULT1A1\*1* allele was reported to have greater enzyme activity than the *SULT1A1\*2* enzyme and it was  
40 expected that individuals with the *SULT1A1\*1* allele would be able to rapidly eliminate bisphenol A.  
41 However, no significant differences in urinary bisphenol A concentrations were observed between 57  
42 individuals with the *SULT1A1\*1* allele (geometric mean  $\pm$  SD = 10.10  $\pm$  8.71  $\mu$ g/L) and 15 individuals  
43 with the *SULT1A1\*2* enzyme (6.31  $\pm$  8.91  $\mu$ g/L). Adjustment for possible bisphenol A exposure through  
44 vinyl wrap use also did not result in significant differences between the 2 groups. The study authors  
45 concluded that additional enzymes involved in bisphenol A metabolism should be studied to determine  
46 possible sensitivity differences.

47  
48 One animal study demonstrated sex-related differences in sulfation. Male versus female Sprague Dawley  
49 and F344 rats were found to produce higher amounts of a bisphenol A glucuronide/sulfate conjugate  
50 (143).

51

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1 As noted in [Table 7](#), one human study reported ~2-fold higher blood bisphenol A concentrations in  
2 Japanese men than women Takeuchi et al. (90). Based on positive correlation between serum bisphenol A  
3 and testosterone concentrations, authors speculated that sex-related differences in bisphenol A  
4 concentrations might be due to androgen-related metabolism (90). There are no known human studies  
5 demonstrating inter-individual or sex-related variations in metabolism that could lead to higher bisphenol  
6 A concentrations in blood. Experimental animal studies have not consistently demonstrated higher  
7 concentrations of bisphenol A or radioactive dose in one sex (119, 127). In Wistar rats orally administered  
8 1 mg bisphenol A every 2 days for 2 or 4 weeks, liver microsomal UDPGT activity towards 17 $\beta$ -estradiol  
9 and testosterone and expression of UGT2B1 protein and mRNA were reduced in males but not females  
10 (311). One study reported an ~3-fold higher concentration of blood bisphenol A in male than in female  
11 Wistar–Imamichi rats that were apparently not treated, but there was no sex-related difference in  
12 percent glucuronidated bisphenol A in serum (312). However, in an in vitro study conducted with hepatic  
13 microsomes, glucuronidation of bisphenol A and expression of *UGT2B1* mRNA were higher in  
14 microsomes from female than male rats. As described in more detail in Section 2.1.2.3, one study  
15 demonstrated reduced capacity to glucuronidate bisphenol A in livers from pregnant than in non-pregnant  
16 rats (149).

### 2.6 Summary of General Toxicology and Biologic Effects

#### *Analytical considerations*

21 Free concentrations of BPA measured in various matrices can be affected by analytic techniques and  
22 methodology. Free bisphenol A contamination from reagents and plastic ware may contribute to the  
23 measured free concentration of bisphenol A (6, 7). Analytical techniques employed may incorrectly over-  
24 estimate the free concentration of measured bisphenol A. HPLC with ultraviolet, fluorescence, or  
25 electrochemical detection is unable to make definitive identification of bisphenol A or bisphenol A  
26 glucuronides, because similar retention times may occur for the metabolites of other endogenous and  
27 exogenous compounds (7). Bisphenol A glucuronide can also be hydrolyzed and in some cases degraded  
28 to unknown components either in acidic or basic pH solutions of diluted urine, adding another potential  
29 source of error in the measurement of sample levels of bisphenol A and its conjugates (17 2485). These  
30 considerations taken together, suggest that it is possible that free bisphenol A concentrations reported in  
31 biological samples may be overestimated.

#### *2.6.1 Toxicokinetics and metabolism*

34 Human toxicokinetic data for bisphenol A are summarized in [Table 60](#). In humans ingested bisphenol A  
35 is rapidly glucuronidated and circulated as bisphenol A glucuronide (109). There is no evidence of  
36 enterohepatic circulation (109). Most of the bisphenol A dose is excreted by humans through urine;  
37 bisphenol A recoveries in urine were reported at  $\geq 84\%$  within 5 hours of dosing (7) and 100% within 42  
38 hours of dosing (109). Human urinary profiles were reported at ~33–70% bisphenol A glucuronide, ~10–  
39 33% parent compound, and ~5–34% bisphenol A sulfate conjugate (96, 98). The presence of bisphenol A  
40 in human fetal tissues or fluids demonstrates that bisphenol A is distributed to the human conceptus (11,  
41 14, 91, 92, 103, 104) ([Table 61](#)). Results from a limited number of studies indicated that fetal bisphenol A  
42 concentrations are within the same order of magnitude as maternal blood concentrations (11, 104) and  
43 amniotic fluid bisphenol A concentrations are ~1 order of magnitude lower than maternal blood  
44 concentrations (92). Significantly higher mean bisphenol A concentrations were reported in the blood of  
45 male than female fetuses ( $3.5 \pm 2.7$  versus  $1.7 \pm 1.5$  ng/mL,  $P = 0.016$ ). Bisphenol A concentrations were  
46 measured in placenta samples at 1.0–104.9, median 12.7  $\mu\text{g}/\text{kg}$  (104). There were no differences between  
47 pregnant and non-pregnant blood levels (median in  $\mu\text{g}/\text{L}$  0.44, range 0.22–0.87 mean +SD 0.46 +0.20)  
48 (11). Median bisphenol A concentrations in human milk were reported to be  $\leq 1.4$   $\mu\text{g}/\text{L}$  (36, 37). One of  
49 the studies reported a milk/serum ratio of 1.3 (12).

1 **Table 60. Human Toxicokinetic Values for Total Bisphenol A Dose**

Endpoint	Value	Reference
Oral absorption, %	≥84%	Völkel et al.(7, 109)
Dermal absorption, in vitro, %	~10%	European Union (2)
T <sub>max</sub> , minutes	80	Völkel et al.(109)
Elimination half-life, hours	4–5.4	Völkel et al.(7, 109)

2

3 **Table 61. Concentrations of Bisphenol A in Maternal and Fetal Samples\***

Study description; analytical method	Bisphenol A concentrations, µg/L, median (range) or mean ± SD			Reference
	Serum or plasma		Other fetal compartments	
	Maternal	Fetal		
21 samples collected in women in the US before 20 weeks gestation; LC with electrochemical detection			0.5 (Non-detectable <0.5 -1.96) 10% of Amniotic fluid samples detectable	Engel et al. (103)
37 German women, 32–41 weeks gestation; GC/MS	3.1 (0.3–18.9); 4.4 ± 3.9	2.3 (0.2–9.2); 2.9 ± 2.5	12.7 (ng/g) (1.0-104.9) 11.2±9.1) Placental tissue	Schönfelder et al. (104)
9 sets of maternal and umbilical cord blood samples obtained at birth in Japanese patients; HPLC	0.43 (0.21–0.79) 0.46±0.2	0.64 (0.45–0.76) 0.62±0.13		Kuroda et al. (11)
180 Malaysian newborns; GC/MS		Non-detectable (<0.05) to 4.05 88% of samples detectable		Tan and Mohd (14)

\*As discussed in Section 1.1.5, ELISA may over-estimate bisphenol A concentrations so only results from studies based on HPLC, GC/MS and LC/MS are presented.

4

5 Animal toxicokinetic data for bisphenol A are summarized in Table 62. Following oral intake of  
6 bisphenol A by rats, most of the dose (≥77%) is glucuronidated and circulated as bisphenol A  
7 glucuronide (126, 127, 150). Most bisphenol A (90–95%) circulates bound to plasma proteins (138)  
8 [reviewed in (139)]. In a single study in mice injected with a low dose (0.025 mg/kg bw), the most  
9 abundant compound in most tissues was bisphenol A glucuronide (135). Most of a bisphenol A dose is  
10 circulated as the glucuronide in monkeys (124). As noted in Table 64, free bisphenol A in blood  
11 represents ≤8% of the dose in rats and ≤1% of the dose in monkeys following oral dosing; higher  
12 concentrations of free bisphenol A in blood were observed following parenteral dosing. The presence of 2  
13 or more C<sub>max</sub> values for radioactivity or bisphenol A, an indication of enterohepatic circulation, was noted  
14 in some rat studies (123, 126, 127). In rats, glucuronidation of bisphenol A was demonstrated to occur in  
15 intestine (147, 148) and liver (149). UGT2B1 was identified as a liver enzyme capable of glucuronidating  
16 bisphenol A, and possible involvement of other liver isoforms was noted (146). There are some data  
17 indicating that glucuronidation capacity is very limited in fetuses and lower in immature than adult  
18 animals. Little-to-no UGT2B activity towards bisphenol A was detected in microsomes of rat fetuses;  
19 activity of the enzyme increased linearly following birth (151). In an in vitro study comparing clearance

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1 of bisphenol A by hepatic microsomes from rats of different ages, activity was lower in microsomes from  
2 fetuses than in those from immature animals and adults [reviewed in (2)]. As noted in [Table 64](#), immature  
3 rats are capable of glucuronidating bisphenol A, but activity appears to increase with age. One study  
4 demonstrated that neonatal rats were able to glucuronidate a larger fraction of a lower (1 mg/kg bw) than  
5 higher (10 mg/kg bw) bisphenol A dose (118).

6  
7 Kurbayashi et al. (127) evaluated fetal and maternal rat bisphenol A during different stages of pregnancy.  
8 Bisphenol A labeled with carbon-14 was administered p.o. to male and female Fischer (F344) rats at  
9 relatively low doses (20, 100, and 500 micrograms/kg), and i.v. injected at 100 and 500 micrograms/kg).  
10 <sup>14</sup>C-bisphenol A (500 micrograms/kg) was also administered orally to pregnant and lactating rats to  
11 examine the transfer of radioactivity to fetuses, neonatal rats, and milk ([Table 63](#)). Radioluminographic  
12 determination using phosphor imaging plates was employed to achieve highly sensitive determination of  
13 radioactivity. Absorption ratios of radioactivity after three oral doses were high (35–82%); parent <sup>14</sup>C-  
14 bisphenol A in the circulating blood was quite low, however, suggesting considerable first-pass effect.  
15 After an oral dose of 100 micrograms/kg <sup>14</sup>C- bisphenol A, the radioactivity was distributed and  
16 eliminated rapidly, but remained in the intestinal contents, liver, and kidney for 72 h. The major  
17 metabolite in the plasma and urine was bisphenol A glucuronide, whereas most of the bisphenol A was  
18 excreted with the feces as free bisphenol A. A second peak in the time-course of plasma radioactivity  
19 suggested enterohepatic recirculation of bisphenol A glucuronide. There was limited distribution of <sup>14</sup>C-  
20 bisphenol A to the fetus and neonate after oral administration to the dam. Significant radioactivity was not  
21 detected in fetuses on gestation days 12 and 15. On day 18, however, radioactivity was detected in the  
22 fetal intestine and urinary bladder 24 h after oral dosing of <sup>14</sup>C- bisphenol A to the dam. The distribution  
23 pattern of radioactivity in pregnant rats was essentially the same as that in non-pregnant female rats. The  
24 distribution levels were dose-dependent in most of the tissues. There was limited distribution of <sup>14</sup>C-  
25 bisphenol A to the fetus. Radioactivity in fetal tissues was undetectable except on gestation day 18 in the  
26 fetal urinary bladder and intestine. On gestation day 18, the amount of radioactivity in fetal tissues at 24 h  
27 was about 30% that in maternal blood, and the yolk sac contained a much higher level of radioactivity  
28 than the maternal blood. The Expert Panel thought these differences were a consequence of the routes of  
29 administration, i.v. or p.o., because only trace amounts of parent bisphenol A dosed orally appeared in the  
30 plasma.

31  
32 The major metabolite of bisphenol A is the glucuronide conjugate. Another metabolite that has been  
33 commonly detected in urine is bisphenol A sulfate. Excretion patterns for bisphenol A are summarized in  
34 [Table 65](#). As noted in [Table 65](#), the major elimination routes for bisphenol A in rodents are feces and bile;  
35 a lower percentage of the dose is eliminated through urine. The major compound detected in feces is  
36 bisphenol A and the major compound detected in bile and urine is bisphenol A glucuronide. Excretion  
37 patterns and metabolic profiles observed in rodents dosed orally or parenterally with low (< 1 mg/kg  
38 bw/day) or high doses (10–100 mg/kg bw/day) were similar. In contrast to rodents and similar to humans,  
39 most of the dose in orally or iv exposed monkeys was eliminated through urine.

1 Table 62. Toxicokinetic Values for Bisphenol A in Non-Pregnant Animals

Model	Endpoint	Value	Reference
Rats orally exposed to $\leq 100$ mg/kg bw	T <sub>max</sub> , hours	0.083–0.75	Domoradzki et al., Pottenger et al., Negishi et al., Takahashi et al., Yoo et al. (118-122)
Ovariectomized, adult rats gavaged with bisphenol A at 10 and 100 mg/kg bw	T <sub>max1</sub> / T <sub>max2</sub> , hours	0.5–1.5 / 3–6	Upmeier et al. (123)
Immature rats orally dosed with $\leq 10$ mg/kg bw	T <sub>max</sub> hours	0.25–3	Domoradzki et al. (118)
Monkeys orally dosed with $\leq 100$ mg/kg bw	T <sub>max</sub> , hours	0.7	Negishi et al. (120)
Chimpanzees orally dosed with 10 mg/kg bw	T <sub>max</sub> , hours	0.5	Negishi et al. (120)
Rats sc dosed with $\leq 100$ mg/kg bw	T <sub>max</sub> , hours	1	Negishi et al. (120)
Monkeys sc dosed with $\leq 100$ mg/kg bw	T <sub>max</sub> , hours	2	Negishi et al. (120)
Chimpanzees sc dosed with 10 mg/kg bw	T <sub>max</sub> , hours	2	Negishi et al. (120)
Ovariectomized, adult rats orally dosed with bisphenol A at 10 and 100 mg/kg bw	Bioavailability, %	16.4 and 5.6 <sup>a</sup>	Upmeier et al. (123)
Rats orally dosed with 10 mg/kg bw	Bioavailability, %	5.3	Yoo et al. (122)
Rat	Plasma protein binding, %	90–95%	Kurebayashi et al. (138); reviewed in (139)
Rats orally dosed with 10 mg/kg bw	C <sub>max</sub> , $\mu\text{g/L}$	14.7–63	Domoradzki et al., Yoo et al. (118, 122)
Rats orally dosed with 100 mg/kg bw	C <sub>max</sub> , $\mu\text{g/L}$	580	Negishi et al. (120).
Ovariectomized, adult rats orally dosed with (mg/kg bw):	C <sub>max1</sub> /C <sub>max2</sub> , $\mu\text{g/L}$		Upmeier et al. (123)
10		30/40	
100		150/134	
Oral doses (mg/kg bw) in immature rats at each age:	C <sub>max</sub> ( $\mu\text{g/L}$ )	Range of values for males and females:	Domoradzki et al. (118)
1 (PND 4)		30–60	
10 (PND 4)		10,200–48,300	
1 (PND 7)		40–80	
10 (PND 7)		1100–1400	
1 (PND 21)		5–6	
10 (PND 21)		200	
Monkeys orally dosed with 10 and 100 mg/kg bw	C <sub>max</sub> , $\mu\text{g/L}$	2793 and 5732 <sup>a</sup>	Negishi et al. (120)
Monkeys orally dosed with 10 mg/kg bw	C <sub>max</sub> , $\mu\text{g/L}$	96–325	Negishi et al. (120)
Rats sc dosed with 10 and 100 mg/kg bw	C <sub>max</sub> , $\mu\text{g/L}$	872 and 3439 <sup>a</sup>	Negishi et al. (120)
Monkeys sc dosed with 10 and 100 mg/kg bw	C <sub>max</sub> , $\mu\text{g/L}$	57,934 and 10,851 <sup>a</sup>	Negishi et al. (120)
Chimpanzees sc dosed with 10 mg/kg bw	C <sub>max</sub> , $\mu\text{g/L}$	1026–2058	Negishi et al. (120)

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Model	Endpoint	Value	Reference
Oral doses (mg/kg bw) in immature rats at each age:	AUC, µg-hour/L	Range of values for males and females:	Domoradzki et al.(118)
1 (PND 4)		100–200	
10 (PND 4)		7200–23,100	
1 (PND 7)		100	
10 (PND 7)		1700–1900	
10 (PND 21)		1000–1100	
Rats orally dosed with 10 mg/kg bw	AUC, µg-hour/L	85.6	Yoo et al.(122)
Rats orally dosed with 100 mg/kg bw	AUC <sub>0–24h</sub> , µg-hour/L	1353	Negeshi et al.(120).
Monkeys orally dosed with 10 and 100 mg/kg bw	AUC <sub>0–24h</sub> , µg-hour/L	3247 and 52,595 <sup>a</sup>	Negeshi et al.(120).
Chimpanzees orally dosed with 10 mg/kg bw	AUC <sub>0–24h</sub> , µg-hour/L	813–1167	Negeshi et al.(120).
Rats sc dosed with 10 and 100 mg/kg bw	AUC <sub>0–24h</sub> , µg-hour/L	3377 and 23,001 <sup>a</sup>	Negeshi et al.(120).
Monkeys sc dosed with 10 and 100 mg/kg bw	AUC <sub>0–24h</sub> , µg-hour/L	3247 and 39,040 <sup>a</sup>	Negeshi et al.(120).
Chimpanzees sc dosed with 10 mg/kg bw	AUC <sub>0–24h</sub> , µg-hour/L	12,492–21,141	Negeshi et al.(120).
Rats orally dosed with 10 mg/kg bw	Apparent volume of distribution, L/kg	4273	Yoo et al.(122)
Immature rats orally dosed with ≤10 mg/kg bw	Half-life, hours	4.3–21.8	Domoradzki et al.(118)
Rats orally dosed with 10 mg/kg bw	Terminal elimination half-life, hours	21.3	Yoo et al.(122)
Rats orally dosed with 10 mg/kg bw	Oral clearance, mL/minute/kg	2352.1	Yoo et al.(122)

<sup>a</sup>Results presented for low and high dose

1

2 **Table 63. Tissue Radioactivity in Pregnant and Fetal Rats after Oral Administration of**

3 **500 µg/kg <sup>14</sup>C-bisphenol A to Dams**

Dam and Fetal Tissues	Radioactivity concentration (ng bisphenol A eq. g <sup>-1</sup> or mL <sup>-1</sup> )					
	12 days of gestation		15 days of gestation		18 days of gestation	
	30 min <sup>a</sup>	24 h	30 min <sup>a</sup>	24 h	30 min <sup>a</sup>	24 h
<i>Dams</i>						
Amniotic fluid	ND	ND	NQ	NQ	NQ	NQ
Blood	43.32	4.33	37.51	3.83	30.99	10.79
Ovary	21.94	3.96	13.91	NQ	15.67	3.49
Placenta	15.43	NQ	18.12	NQ	9.91	3.86
Uterus	22.68	ND	NQ	NQ	15.31	NQ
Fetus	NQ	NQ	NQ	NQ	NQ	3.28
Fetal membrane	NQ	NQ	NQ	NQ	NQ	10.87
Yolk sac	NQ	ND	ND	ND	NQ	54.14

NQ - Nonquantifiable (below LOQ)

ND - Not determined (indistinguishable)

<sup>a</sup>Each time shows the sacrifice time after oral administration of <sup>14</sup>C-bisphenol A to each pregnant rat

1 **Table 64. Age and Route Factors Affecting Free Bisphenol A Concentrations in Blood**

Model and Regimen	Findings for free bisphenol A in blood	Reference
Age effects of rat oral dosing at 1 or 10 mg/kg:		Domoradzki et al.(118)
4 days of age	1.5–56.8 mg/L	
7 days of age	1.1–12.2 mg/L	
21 days of age	0.8–8 mg/L	
adulthood	0.07–0.6 mg/L	
SC or gavage dosing of 18 through 21 day old rats with 160 mg/kg bw/day	<b>[93% lower]</b> with oral than sc dosing 2.9 mg/L sc (plasma) 0.2 mg/L oral (plasma)	Yamasaki et al.(125)
Route effects in rats administered 10 or 100 mg/kg bw:		Pottenger et al.(119)
oral	[<2–8%] BLQ (males); 0.04 mg/L (females) (at 10 mg/kg)	
sc	[65–76%] 0.69 (males); 0.87 mg/L (females) (at 10 mg/kg)	
ip	[27–51%] 0.39 (males); 0.34mg/L (females) (at 10 mg/kg)	
Route effects in monkeys:	Percent of dose:	Kurebayashi et al.(124)
iv	5–29%	
oral	0–1%	

2

3 **Table 65. Summary of Elimination Information for Bisphenol A**

Model	Elimination route	Percent dose eliminated	Compound and metabolite profile	Reference
Pregnant or non-pregnant rats orally, ip, or sc dosed with <100 mg/kg bw	Feces	50–83%	Bisphenol A (83–93%); bisphenol A glucuronide (2–3%)	Domoradzki et al, Snyder, et al., Pottenger et al.(119, 126, 129)
	Urine	13–42%	Bisphenol A (3–23%); bisphenol A glucuronide (57–87%); bisphenol A sulfate (2–7%)	
Rats orally or iv dosed with 0.1 mg/kg bw	Feces	64–88%	Not reported	Kurebayashi et al. (138)
	Urine	10–34%		
Rats orally or iv dosed with 0.1 mg/kg bw	Bile	45–66% within 5 hours	Bisphenol A glucuronide (84–88%)	Kurebayashi et al. (138)
Rats orally dosed with 100 mg/kg bw/day	Feces	Not reported	Bisphenol A (61% of dose) Bisphenol A and bisphenol A sulfate ( $\leq$ 1.1% of dose); bisphenol A glucuronide (6.5% of the dose)	Kurebayashi et al. (138)
	Urine			
	Bile			
Pregnant mice injected with 0.025 mg/kg bw bisphenol	Feces Urine	Not reported	Bisphenol A (>95%) Major metabolites: bisphenol A glucuronide	Zalko et al. (135)

## 2.0 General Toxicology and Biological Effects

Model	Elimination route	Percent dose eliminated	Compound and metabolite profile	Reference
A	Bile		and hydroxylated bisphenol A glucuronide Bisphenol A glucuronide (>90%)	
Monkeys orally or iv dosed with 0.1 mg/kg bw	Feces Urine	2–3% 79–86%	Not reported	Kurebayashi et al. (124)

1  
2 Toxicokinetics of bisphenol A were examined in pregnant rats and are summarized in [Table 66](#) for free  
3 bisphenol A and [Table 67](#) for total dose. One study demonstrated similar disposition, metabolism, and  
4 elimination of bisphenol A in pregnant and non-pregnant rats (126). A number of rodent studies  
5 demonstrated distribution of bisphenol A or radioactive dose to fetuses following oral dosing of the dam  
6 (121, 126-128, 131, 134). Bisphenol A distribution to fetus was also demonstrated with iv dosing of rats  
7 (132) and sc dosing of mice or monkeys (135, 136). In a study in which bisphenol A was orally  
8 administered to rats on GD 19, bisphenol A glucuronide was not detected in fetuses (150); study authors  
9 noted the possibilities that bisphenol A glucuronide was not likely transferred from dams to fetuses and  
10 that fetuses do not likely possess glucuronidation ability. Some of the studies demonstrated slower  
11 elimination of bisphenol A from fetuses than maternal blood following oral dosing (121, 128).  
12

13 Toxicokinetics data in lactating rats are summarized in [Table 68](#) for free bisphenol A and [Table 69](#) for  
14 total dose. Distribution of bisphenol A to milk and/or nursing pups was demonstrated in rodent studies  
15 with oral or iv exposures (122, 127, 129). One study reported that most of the bisphenol A dose is present  
16 as bisphenol A glucuronide in milk of lactating rats (129). In a study that compared bisphenol A  
17 concentrations in maternal serum, milk, and offspring after rat dams were administered low oral doses  
18 (0.006 or 6 mg/kg bw/day), a significant increase in bisphenol A concentration was only observed in the  
19 serum of dams from the high dose group on PND 21; no increase was observed in milk or pups (130).  
20 Another study demonstrated higher concentrations of bisphenol A in milk compared to maternal serum  
21 after iv dosing of rat dams (122).  
22

23 A number of in vitro studies compared bisphenol A metabolic velocity rates in microsomes or  
24 hepatocytes from rodents and humans. Generally, faster rates were demonstrated by rodent than human  
25 hepatocytes and microsomes (142, 143) and [reviewed in (2)]. One of the studies noted that adjustment for  
26 total hepatocyte number in vivo resulted in higher predicted rates for humans than rodents (143). The  
27 European Union (2) noted that the interpretation of such studies should included knowledge about in vivo  
28 conditions such as varying metabolic capacity of hepatic cells, relationship of hepatic size to body size,  
29 and possibly important physiological endpoints such as blood flow.  
30

## 2.0 General Toxicology and Biological Effects

1 **Table 66. Toxicokinetic Values for Free Bisphenol A in Pregnant Rats and Fetuses**

Dose	Endpoint	Maternal	Fetal	Reference
1000 mg/kg bw orally on GD 18	$C_{max}$ , $\mu\text{g/L}$	14,700	9220	Takahashi and Oishi (121)
10 mg/kg bw orally on GD 19	Concentration 1 hour post dosing, $\mu\text{g/L}$	34	11	Miyakoda et al. (128)
2 mg/kg bw iv on 1 day between GD 17 and 19	$C_{max}$ , $\mu\text{g/L}$	927.3	794	Shin et al. (132)
1000 mg/kg bw orally on GD 18	$T_{max}$ , minutes	20	20	Takahashi and Oishi (121)
2 mg/kg bw iv on 1 day between GD 17 and 19	$T_{max}$ , hours	No data	$0.6 \pm 0.3$	Shin et al. (132)
1000 mg/kg bw orally on GD 18	AUC, $\mu\text{g}\cdot\text{hour/L}$	13,100	22,600	Takahashi and Oishi (121)
2 mg/kg bw iv on 1 day between GD 17 and 19	AUC, $\mu\text{g}\cdot\text{hour/L}$	905.5	1964.7	Shin et al. (132)
1000 mg/kg bw orally on GD 18	Mean retention time, hours	10.6	20.0	Takahashi and Oishi (121)
1000 mg/kg bw orally on GD 18	Variance in retention time, hours squared	203	419	Takahashi and Oishi (121)
2 mg/kg bw iv on 1 day between GD 17 and 19	Mean residence time, hours	3.0	3.0	Shin et al. (132)
1000 mg/kg bw orally on GD 18	Half-life, hours:			Takahashi and Oishi (121)
	From 20 to 40 minutes	0.0952	0.55	
	From 40 minutes to 6 hours	2.58	1.60	
	From 6 to 48 hours	4.65	173	
2 mg/kg bw iv on 1 day between GD 17 and 19	Elimination half-life, hours	2.5	2.2	Shin et al. (132)

2

3 **Table 67. Toxicokinetic Values for Radioactive Dose in Pregnant Rats (Total Bisphenol A)**

Endpoint	Value
$C_{max1}/C_{max2}$ , $\mu\text{g eq/L}$	370–1006/211–336
$T_{max1}/T_{max2}$ , hours	0.25/12–24
Time to non-quantifiable concentration, hours	72–96
AUC $^{14}\text{C}$ , $\mu\text{g}\cdot\text{eq}\cdot\text{hour/L}$	7100–12,400
AUC Bisphenol A glucuronide, $\mu\text{g}\cdot\text{eq}\cdot\text{hour/L}$	6800–12,300

Dams were gavaged with 10 mg/kg bw/day on GD 6–10, 14–18, or 17–21. From Dormoradzki et al. (126)

4

5 **Table 68. Toxicokinetic Values for Free Bisphenol A in Lactating Rats**

Endpoint	Blood Value	Milk Value
Systemic clearance, mL/minute/kg	119.2 / 142.4 / 154.1 <sup>a</sup>	
Steady state bisphenol A concentration, ng/mL	66.1 / 120.0 / 217.1 <sup>a</sup>	173.1 / 317.4 / 493.9 <sup>a</sup>
Milk/serum ratio		2.7 / 2.6 / 2.4 <sup>a</sup>

Rats were iv injected 0.47, 0.94, or 1.88 mg/kg bw and then infused over a 4 hour time period with 0.13, 0.27, 0.54 mg/hour.

<sup>a</sup>Effect at each dose, from low to high dose. From Yoo et al. (122)

6

1 **Table 69. Toxicokinetic Values for Radioactive Dose in Lactating Rats (Total Bisphenol A)**

Endpoint	Blood Value	Milk Value
C <sub>max</sub> , µg-eq/L	27.2	4.46
T <sub>max</sub> , hours	4	8
Elimination half-life, hours	31	26
AUC (0–48 hours), µg-eq·hour/L	689	156

Rats were orally dosed with 0.5 mg/kg bw on PND 11.

From Kurebayashi et al.(127)

2

3 *2.6.2 General toxicity*

4 Gross signs of toxicity observed in rats acutely exposed to bisphenol A included pale livers and  
5 gastrointestinal hemorrhage [reviewed by the European Union (2)]. Acute effects of inhalation  
6 exposure in rats included transient and slight inflammation of nasal epithelium and ulceration of  
7 the oronasal duct. Based on LD<sub>50</sub>s observed in animals, the European Union (2) concluded that  
8 bisphenol A is of low acute toxicity through all exposure routes relevant to humans. According to  
9 the European Union (2), there is evidence that bisphenol A is irritating and damaging to the eye  
10 and is irritating to the respiratory tract and possibly the skin. Findings regarding sensitization  
11 potential were not clear.

12

13 Possible target organs or systems of toxicity identified in repeat-dose animal studies with oral  
14 dosing included intestine, liver, kidney, and male and female reproductive systems [reviewed in  
15 (2, 157, 158)]. Intestinal findings (effect levels) in rats included cecal enlargement ( $\geq 25$  mg/kg  
16 bw/day) and cecal mucosal hyperplasia ( $\geq 200$  mg/kg bw/day). Hepatic effects included prominent  
17 hepatocyte nuclei or inflammation in rats ( $\geq 500$  mg/kg bw/day), multinucleated giant hepatocytes  
18 in mice ( $\geq 120$  mg/kg bw/day), and increased weight with no evidence of histopathology in dogs  
19 ( $\geq 270$  mg/kg bw/day). Renal tubule degeneration or necrosis was observed in rats dosed with  
20  $\geq 500$  mg/kg bw/day. Reproductive findings are discussed in Section 4.0. Effects in subchronic  
21 inhalation studies in rats included cecal enlargement resulting from distention by food and  
22 transient, slight hyperplasia and inflammation of epithelium in the anterior nasal cavity; both  
23 effects occurred at ( $\geq 50$  mg/m<sup>3</sup>).

24

25 *2.6.3 Estrogenicity*

26 Estrogenicity of bisphenol A has been evaluated using in vitro (Table 52) and in vivo (Table 53)  
27 assays. In those studies estrogenic potency was compared to 17 $\beta$ -estradiol, ethinyl estradiol,  
28 diethylstilbestrol, and, in one study, estrone. There is considerable variability in the results of  
29 these studies with the estrogenic potency of bisphenol A ranging over about 8 orders of  
30 magnitude Figure 2. On the other hand, the average potency only differs by one order of  
31 magnitude and there is very little difference between rat and mouse means.

32

33 Most in vivo estrogenicity studies examined effects on uterine weights of intact weanling or  
34 ovariectomized adult rats or mice. The potency of bisphenol A in increasing uterine weight varied  
35 over ~4 orders of magnitude. Uterine weight findings can be affected by the time period between  
36 dosing and measurement. Most, but not all studies, showed a greater effect on uterine weight with  
37 sc than with oral dosing. The greater activity of sc than oral bisphenol A is presumably due to  
38 glucuronidation of the orally administered compound with consequent loss of estrogenicity (169).  
39 Inter-strain variability in rats has been evaluated as a source of variability in estrogenicity assays.  
40 (see Section 4.0 for additional discussion) Inter-laboratory variability has been noted for  
41 uterotrophic effects in intact weanling mice exposed to bisphenol A (261); one factor that can  
42 result in variability is body weight of the animal. Use of mice with lower body weights results in  
43 lower and less variable control uterine weights and greater likelihood of bisphenol A effect (261,

## 2.0 General Toxicology and Biological Effects

1 277). In in vivo studies examining gene expression profiles, some but not all gene expression  
2 changes were consistent between bisphenol A and reference estrogens (261, 278, 279, 280); ER-  
3 independent activity was suggested by 1 investigator (280). **[Based on one comprehensive study**  
4 **of the effects of bisphenol A orally delivered from 60 to 1000 mg/kg for 3 to 7 days, the**  
5 **Expert Panel concludes that the uterotrophic responses were only found at higher doses**  
6 **(313, 314) whereas sc dosing produced consistent uterine weight increases at lower doses.]**  
7

### 8 2.6.4 Androgenic activity

9 In the majority of in vitro tests conducted, bisphenol A was not demonstrated to have androgenic  
10 activity (196, 213, 219, 291). Anti-androgenic activity was demonstrated in systems using cells  
11 transfected with three different androgen receptor reporting systems (ARE-luc, MMTV-lacZ and  
12 C3-luc) (Table 56). No consistent effects were observed on male accessory reproductive organ  
13 weights in 3 in vivo studies in which rats were dosed with bisphenol A at  $\leq 600$  mg/kg bw/day;  
14 the study authors concluded that bisphenol A does not have anti-androgenic or androgenic  
15 activity (296-298).  
16

### 17 2.6.5 Genetic toxicity

18 In in vitro genetic toxicity studies reviewed by the European Union (2) and/or Haighton et al.  
19 (47), evidence of aneugenic potential, chromosomal aberration, micronuclei formation, and DNA  
20 adducts was observed (Table 57). Because of the lack of chromosomal effects in in vivo studies  
21 (Table 58) and unknown relevance of DNA adduct formation, which only occurred at high doses,  
22 both groups concluded that bisphenol A is not likely to have genotoxic activity in vivo.  
23

### 24 2.6.6 Carcinogenicity

25 Carcinogenic potential of bisphenol A was evaluated in rats and mice by the NTP (157, 307).  
26 NTP concluded that under the conditions of the study, there was no convincing evidence that  
27 bisphenol A was carcinogenic in F344 rats or B6C3F<sub>1</sub> mice. However, NTP stated that there was  
28 suggestive evidence of increased cancer in the hematopoietic system based on marginally  
29 significant increases in leukemia in male rats, non-statistically significant increases in leukemia in  
30 female rats, and a marginally significant increase in combined incidence of lymphoma and  
31 leukemia in male mice. A statistically significant increase in testicular interstitial cell tumors in  
32 aging F344 rats was also considered suggestive evidence of carcinogenesis. The effect was not  
33 considered conclusive evidence because of the high incidence of the testicular neoplasm in aging  
34 F344 rats (88% incidence in historical controls). Both the European Union (2) and Haighton et al.  
35 (47) stated that the evidence does not suggest carcinogenic activity of bisphenol A in rats or mice.  
36 Conclusions by the European Union and Haighton et al. were based on factors such as lack of  
37 statistical significance for leukemia, mammary gland fibroadenoma, and Leydig cell tumors, lack  
38 of activity at noncytotoxic concentrations in both in vitro genetic toxicity tests and an in vivo  
39 mouse micronucleus test, and unlikely formation of reactive intermediates at doses that do not  
40 saturate detoxification pathways.  
41

### 42 2.6.7 Potentially Sensitive Subpopulations

43 Studies in humans and laboratory animals demonstrated developmental changes in UDPGT gene  
44 expression or enzyme activity that could potentially affect the concentration of free bisphenol A  
45 reaching target organs because of a differential capacity for bisphenol A glucuronidation. In  
46 humans, activities for some UDPGT isozymes were reported to be very low at birth but increased  
47 with age (308). No transcripts for UDPGT were detected in samples from 20-week-old human  
48 fetuses and activity for some UDPGT enzymes was lower in children than adults (309).  
49 Compared to adults, human fetal uridine 5'-diphosphoglucuronic acid concentrations were 5-fold  
50 lower in liver and 1.5-fold lower in kidney (310). It is not clear if any of the isozymes examined  
51 are involved in bisphenol A glucuronidation by humans. Human findings were consistent with

## 2.0 General Toxicology and Biological Effects

1 rodent studies that demonstrated no or limited glucuronidation capacity by fetuses (*126, 150, 151*)  
2 and lower glucuronidation capacity in immature than adult rats(*2, 118, 151*).  
3  
4 Some studies suggested possible gender-related differences in sulfation capacity in humans (*98,*  
5 *143*) and laboratory animals (*143*). One study in humans demonstrated no differences in urinary  
6 bisphenol A concentrations in individuals carrying a sulfotransferase genotype associated with  
7 greater activity (*88*).  
8

### 3.0 DEVELOPMENTAL TOXICITY DATA

The Panel attended to multiple design and analysis characteristics in judging the acceptability of individual papers. It was our consensus that for a paper to be acceptable for this review process, several conditions had to be met. First, effects related to litter of origin needed to be accounted for in design and statistical procedures. Second, animals needed to be dosed via the dam or directly under individual housing conditions. Concern that multiple exposures within a cage to different animals could cause cross-animal contamination across cage-mates led to the determination that this design was not acceptable. Third, a minimum of 6 animals per treatment condition needed to be used to provide minimal confidence in results. Fourth, if similar tests were conducted at multiple ages, the statistical analyses needed to account for repeated measurement in order not to inflate degrees of freedom. The Panel carefully considered the merits of each study according to these primary criteria, and the related design characteristics represent the most common reasons for judging a study to be unacceptable for our review process. Our intent was to have our review depend most heavily on studies that would have reduced risks for false negative or false positive findings.

In addition, the Panel carefully considered the value of studies where Bisphenol A was administered anywhere other than to the mouth or stomach of the experimental animal. Human exposure is overwhelmingly oral, and oral exposure produces an internal metabolite profile which is overwhelmingly dominated by the (inactive) glucuronide in both rats and humans. Subcutaneous or parenteral injections result in blood levels of active parent compound which are much higher than those seen after oral exposure. In light of these pharmacokinetic differences, the Panel concluded that injection studies, unless they proved otherwise, would produce irrelevantly high internal doses of the active parent compound, and would tend to produce “false positive” effects from the point of view of the human oral situation. Thus, the Panel viewed those otherwise adequate studies which injected bisphenol A as providing “supplemental” information (i.e., of limited utility), unless they also analyzed the levels of parent compound and metabolites after the injection. The intent of this approach is limit the impact of those studies which produced an unrealistic and irrelevant internal metabolite profile (i.e., one which is significantly different from that experienced by humans). Thus, the closer any given study came to replicating the human situation, the more weight it had in the final analysis.

The report below mentions “dosing procedures” as reasons for limiting the adequacy or utility of various studies. This has been used to mean non-gastric administration (subcutaneous injection, intramuscular injection, intraperitoneal injection, or intracerebroventricular injection).

The Panel also had extensive discussion about dosing vehicles. Dimethyl sulfoxide (DMSO) has significant biological activities of its own (315), and the experience of the Panel is that DMSO can help move solutes into cells. Increasing the DMSO concentration can produce a greater solute effect, even when holding that solute concentration stable. The real impact of this for *in vivo* injections is uncertain, and this effect is likely to be small at the dosing volumes administered in the studies considered here. The use of 100% DMSO as a vehicle for Alzet mini-pump studies is a clear contravention of the directions for mini-pump use<sup>3</sup>, as it accelerates the breakdown of the mini-pumps and produces blood levels which are not predictable and therefore not useful for the Evaluative Process. Various oils each can bring their own

---

<sup>3</sup> Manufacturer instructions specify use of up to 50% DMSO (<http://www.alzet.com/products/checklist.php>). 100% DMSO is completely incompatible with the pump reservoir material and will dissolve reservoirs within 24-36 hours. 80% DMSO causes pinholes to appear in the reservoirs within 4-7 days. Thus, if a high concentration of DMSO is used, one most likely will infuse both degraded reservoir material as well as the salt compound which makes up the osmotic layer. These 2 things, combined with DMSO itself (a strong tissue irritant) will most likely cause tissue inflammation and edema. (Kurt Kemling ALZET Associate Product Manager, personal communication, September 14, 2007)

### 3.0 Developmental Toxicity Data

1 potential issues, such as oxidative damage, but these were considered and discussed by a sub-team of the  
2 Panel and not considered to be consequential for this analysis

3  
4 The Panel also examined the issue of data that would be expected to result when positive controls were  
5 employed. While we did not feel that positive controls were required for studies, when they were used,  
6 expected effects needed to be demonstrated to validate that the experimental model was capable of  
7 responding to a certain stimulus. This is of even more value when there is no response to the main exposure  
8 under study. When looking for estrogenic responses, investigators often use 17 $\beta$  estradiol or  
9 diethylstilbestrol. These must be used at adequate doses to produce the desired response. Inadequate  
10 challenge by the positive control, resulting in no response, leaves the reader uncertain whether the lack of  
11 response is due to the selection of too low a dose, or whether the experimental model is incapable of  
12 responding to a sufficient challenge. Even though the Panel, based on its own scientific experience, might  
13 conclude that inappropriately low doses had been selected and thus a lack of response is not surprising, the  
14 Panel was left with little choice in such situations but to give much less weight to studies where non-  
15 effective doses of a positive control compound were used.

16  
17 The Panel is confident in our assessment of those studies judged adequate and useful, and are focusing our  
18 limited time on the consistency and utilization of these data.

#### 20 **3.1 Human**

21  
22 No studies were located on possible human developmental effects of bisphenol A.

#### 24 **3.2 Experimental Animal**

25  
26 Studies are presented by species (rat, mouse, other), route (oral, parenteral), and by whether exposure was  
27 during pregnancy or the postnatal period. Studies in which exposures were started during pregnancy and  
28 continued after pregnancy are discussed with studies in which exposures occurred postnatally.

##### 30 *3.2.1 Rat—oral exposure only during pregnancy*

###### 32 *3.2.1.1 Evaluation of pre- or perinatal growth and development*

33 **Morrissey et al. (316)**, supported by NTP/NCTR, examined the effects of prenatal bisphenol A exposure in  
34 rats and mice in a study conducted according to GLP. Studies are also available as NTP publications for  
35 rats (317) and mice (318). The study was conducted in two sets of rats and mice, and data were pooled for  
36 each species. **[The data for mice are discussed in Section 3.2.5.1.]** Pregnant CD rats were randomly  
37 assigned to groups of  $\geq 10$  animals in each set of the study, for a total of  $\geq 20$  animals/dose. On GD 6–15  
38 (GD 0 = sperm or plug), rats were gavaged with bisphenol A at 0 (corn oil vehicle), 160, 320, 640, or 1280  
39 mg/kg bw/day. Doses were based on results of preliminary studies and were expected to result in 10%  
40 maternal mortality at the high dose and no toxicity at the low dose. Purity of bisphenol A was >95% and  
41 2,4'-bisphenol A was reported as an impurity. Dosing solution concentrations were verified. Pregnant  
42 animals were weighed during the study. Rats were killed on GD 20. Liver and uterus were weighed, and  
43 corpora lutea and implantation sites were examined. Fetuses were sexed, weighed, and examined for  
44 viability and external, visceral, and skeletal malformations. Data were analyzed by Bartlett test for  
45 homogeneity of variance, ANOVA and/or William multiple comparison, Dunnett, or Fisher exact  
46 probability tests. **[Data were presented and analyzed on a per litter basis.]**

47  
48 An unexpectedly high number of dams (7 of 27) died in the 1280 mg/kg bw/day group, with most deaths  
49 occurring in the second set of animals. Because of the high death rate, the study authors decided not to  
50 evaluate data in the 1280 mg/kg bw/day group. Clinical signs that occurred most frequently in dams from  
51 the 640 mg/kg bw/day group included lethargy, piloerection, pica, rough coat, wet urogenital area, weight

### 3.0 Developmental Toxicity Data

1 loss, and alopecia. Significant and dose-related decreases in maternal body weights were observed during  
2 the entire gestation period and thus were not confined to the GD 6–15 treatment period in rats from the 160,  
3 320, and 640 mg/kg bw/day groups. Body weight corrected for gravid uterine weight was also decreased in  
4 all three dose groups. Effects on maternal body weight were most pronounced during the treatment period.  
5 **[During the treatment period, dam body weights were 35, 53, and 54% lower in the 160, 320, and 640**  
6 **mg/kg bw/day groups than in control groups; estimated benchmark doses<sup>4</sup> in mg/kg bw/day were**  
7 **BMD<sub>10</sub> 113, BMDL<sub>10</sub> 94, BMD<sub>1SD</sub> 416, BMDL<sub>1SD</sub> 321].** Despite this large effect on maternal body weight,  
8 there were no effects on numbers of implantation sites or resorptions, gravid uterine weight, or liver weight.  
9 The numbers of litters available for evaluation in the control and 160, 320, and 640 mg/kg bw/day dose  
10 group were 23, 26, 24, or 29. There were no significant effects on fetal body weight or viability, percentage  
11 males/litter, or malformed fetuses/litter. Study authors concluded that bisphenol A was not teratogenic in  
12 rats at doses that cause maternal toxicity.

13  
14 **Strengths/Weaknesses:** This study used adequate sample sizes to evaluate the effects of GD 6–15  
15 exposure on maternal body weight during gestation and on implantation and resorption sites/dam, fetal  
16 body weight, and fetal viability to GD 20. Strengths are the verification of dosing solutions, use of GLP,  
17 adequate n, sensitive evaluation of soft and hard-tissue structures. Weaknesses include no postnatal  
18 examination, as well as the absence of data from the 1280 mg/kg bw/day group, the absence of a no effect  
19 dose. The absence of effects on fetal endpoints despite marked reductions in maternal body weight  
20 corrected for gravid uterine weight warrants the appropriate conclusion that bisphenol was not teratogenic  
21 when based on GD 20 data. Further, a gross visceral exam is likely insensitive to certain abnormalities of  
22 the reproductive tract and brain, as noted above.

23  
24 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
25 evaluation process.

26  
27 **Kim et al. (319)**, support not indicated, examined the effects of prenatal bisphenol A exposure on  
28 developmental toxicity in rats. Sprague Dawley rats were fed commercial rodent chow (Jeil Feed Co.,  
29 Daejon, Korea) and housed in polycarbonate cages; no information was provided about bedding. Twenty  
30 dams/group were gavaged with 0 (corn oil vehicle), 100, 300, or 1000 mg/kg bw/day bisphenol A [purity  
31 not provided] on GD 1–20 (GD 0 = first 24 hours after detection of vaginal sperm or plug). Dose selection  
32 was based on the results of a preliminary study that demonstrated maternal and developmental toxicity at  
33 doses  $\geq 400$  mg/kg bw/day and a lack of effect at doses  $\leq 200$  mg/kg bw/day. Endpoints examined in dams  
34 during the study were clinical signs, body weight gain, and food intake. Dams were killed on GD 21 and  
35 examined for corpora lutea and implantation sites. Fetuses were sexed, weighed, and examined for viability  
36 and external abnormalities. Anogenital distance was measured and alternate fetuses were examined for  
37 visceral and skeletal malformations. The dam or litter was considered the statistical unit. Data were  
38 analyzed by ANOVA, Scheffé multiple comparison test, Kruskal-Wallis nonparametric ANOVA, Mann-  
39 Whitney *U* test, and Fisher exact probability test.

40  
41 Statistically significant effects are summarized in [Table 70](#). Dose-dependent clinical signs observed in  
42 dams at the 2 highest doses included piloerection, dull fur, reduced locomotor activity, emaciation,  
43 sedation, red-colored tears, soft stool, diarrhea, urination, and perineal soiling. Pregnancy failure, as  
44 observed by lack of implantation sites, was increased in females from the high-dose group. Maternal body  
45 weight, body weight gain, and body weight corrected for gravid uterus weight were reduced at the mid and

---

<sup>4</sup> Benchmark doses are used commonly in a regulatory setting; however, they are used in this report when the underlying data permit their calculation, and are only supplied to provide 1 kind of description of the dose-response relationship in the underlying study. Calculation of a benchmark dose in this report does not mean that regulation based on the underlying data is recommended, or even that the underlying data are suitable for regulatory decision-making. The BMD<sub>10</sub> is the benchmark dose associated with a 10% effect, estimated from a curve fit to the experimental data. The BMDL<sub>10</sub> represents the dose associated with the lower 95% confidence interval around this estimate. Unless otherwise indicated, BMD values in this report were calculated using a power model for continuous data and a probit model for dichotomous data using Environmental Protection Agency (EPA) Benchmark Dose Software version 1.3.2.

### 3.0 Developmental Toxicity Data

1 high dose. GD 4 was the only time period when food intake was significantly reduced at the mid and high  
 2 dose. Expansion and congestion of stomach and/or intestines were observed in dams from the high-dose  
 3 group. Body weights of male fetuses were decreased at the mid and high dose, and body weights of female  
 4 fetuses were reduced at the high dose. Increases in fetal death, early resorption, and postimplantation loss,  
 5 accompanied by reduced number of live fetuses, were observed at the high dose. Anogenital distance was  
 6 significantly reduced in males from the mid- and high-dose groups, but there were no differences in  
 7 anogenital distance of males or females when the values were normalized by the cube root of body weight.  
 8 Significantly reduced ossification was observed in the high-dose group. There were no treatment-related  
 9 differences in fetal sex ratio or external, visceral, or skeletal malformations. Study authors concluded that  
 10 exposure of rats to a maternally toxic dose of bisphenol A during the entire gestation period resulted in  
 11 pregnancy failure, postimplantation loss, reduced fetal body weight, and retarded fetal ossification but not  
 12 dysmorphogenesis.

13  
 14 **Table 70. Maternal and Developmental Effects in Rats Exposed to Bisphenol A**

Endpoint	Dose, mg/kg bw/day						
	100	300	1000	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
<b>Dams</b>							
Number pregnant	↔	↔	↓30%				
Body weight gain	↔	↓35%	↓52%	178	152	379	304
Corrected body weight	↔	↓14%	↓15%	631	490	566	424
Food intake on GD 4	↔	↓24%	↓57%	168	147	313	257
No. fetal deaths	↔	↔	↑6.5-fold	827	13	978	585
No. early resorptions	↔	↔	↑6-fold	821	14	980	584
Postimplantation losses	↔	↔	↑11-fold	1278	394		
<b>Fetuses</b>							
No. live /litter	↔	↔	↓36%	929	348	982	713
Male body weight	↔	↓14%	↓20%	456	339	694	497
Female body weight	↔	↔	↓21%	439	328	682	490
Ossification	↔	↔	↓				

↑,↓ Statistically significant increase, decrease compared to controls; ↔ No statistically significant effect compared to controls.

From Kim et al. (319).

15  
 16 **Strengths/Weaknesses:** This report presents a fairly standard embryo-fetal developmental toxicity study.  
 17 One strength is that the doses utilized incorporated both a no effect dose and a high maternally toxic dose,  
 18 revealing fetal effects only at the high dose that showed marked maternal toxicity. Measurement of  
 19 anogenital distance is another strength. Weaknesses include the absence in all groups of information about  
 20 postnatal viability, and postnatal function. Further, a gross visceral exam is likely insensitive to certain  
 21 abnormalities of the reproductive tract and brain. However, this type of study does report on the ability of  
 22 the exposure to cause structural malformations, which are notably absent.

23  
 24 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
 25 evaluation process.

26  
 27 **Kim et al. (131)**, support not indicated, examined the effects of prenatal bisphenol A exposure on postnatal  
 28 body and organ weights of Sprague Dawley rats. Rats were housed in polycarbonate cages. [No  
 29 information was provided on feed or bedding material.] Rats were grouped according to body weight  
 30 and randomly assigned to dose groups. On GD 7–17 (GD 0 = day of vaginal sperm or plug), at least 10  
 31 rats/dose group were gavaged with bisphenol A (>99.7% purity) at doses of 0 (corn oil vehicle), 0.002,  
 32 0.020, 0.200, 2, or 20 mg/kg bw/day. Dosing solution concentrations were verified. Dams were weighed

### 3.0 Developmental Toxicity Data

1 and observed for clinical signs of toxicity during the study. Dams were killed on the 21<sup>st</sup> day of the  
2 postpartum period. Corpora lutea, implantation sites, resorptions, and fetal viability were assessed.  
3 Maternal liver, kidney, spleen, ovary, and gravid uterus were weighed. Live fetuses were weighed and  
4 examined for external and visceral abnormalities. Fetal liver, kidneys, spleen, and reproductive organs were  
5 weighed in half the fetuses. **[These methods are produced here as written in the original; although  
6 dams were clearly stated to have been killed on PND 21, the “fetal” examinations described appear  
7 more consistent with killing of the dams on GD 21.]** Data were analyzed by ANOVA and Student *t*-test.  
8 **[It was not clear if the litter or fetus was considered the statistical unit in the evaluation of  
9 developmental toxicity data.]**

10  
11 A significant but non-dose-related increase in dam body weight occurred in the 0.2 mg/kg bw/day group on  
12 GD 0–15. Dam body weight was significantly increased on GD 21 in the 2 (by 53%) and 20 (by 43%)  
13 mg/kg bw/day groups. No significant differences in dam body weight were noted during the lactation  
14 period. Significant changes in dam relative organ weights (dose at which effects were observed) were:  
15 increased liver (0.002, 0.020, and 20 mg/kg bw/day); decreased right kidney (0.2 mg/kg bw/day); increased  
16 right kidney (2 mg/kg bw/day), and increased uterine (0.2 mg/kg bw/day). There was no effect on ovary  
17 weight of dams. The majority of dams were in diestrus when killed. One of 7 dams in the 0.2 mg/kg bw/day  
18 group was in proestrus. One of 7 dams in the 0.2 mg/kg bw/day, 1 of 6 dams in the 2 mg/kg bw/day group,  
19 and 2 of 8 dams in the 20 mg/kg bw/day group were in diestrus. Body weight effects in male and female  
20 offspring were reported in most treatment groups when evaluated at various time points between birth and  
21 PND 22. In general, when body weights effects were detected it was an increase in weight of ~12 - 65%.  
22 **[Changes occurred at most dose levels but were not consistent over time and there was little evidence  
23 of dose-response relationships. In general, effects appeared to be most pronounced in the lowest dose  
24 group.]** Relative weights for several tissues attained statistical significance at 1 or more doses in offspring  
25 of both sexes: liver, spleen and right kidney. In addition, relative organ weights for were altered in males  
26 for the left kidney, both testes, right epididymis, left seminal vesicle, and prostate gland. There were no  
27 effects on ovary or uterus weights. **[In most cases, there was little evidence of a dose-response  
28 relationship for organ weights, including male reproductive organs, in offspring.]** Study authors  
29 concluded that bisphenol A had estrogenic effects on rat dams and offspring exposed during the gestation  
30 period.

31  
32 **Strengths/Weaknesses:** While the verification of the dosing solution is a strength, this study is of unclear  
33 quality, to the point that there is real confusion about what was actually done. It is indicated that 10 dams  
34 were assigned to each dose group but numbers at sacrifice were 7, 7, 6, and 8 across the 4 doses. It is  
35 unclear whether fetal data were appropriately analyzed with litter as the unit. It is unclear when the dams  
36 were killed and analyzed. The absence of understandable dose-related effects complicates interpretation at  
37 these low doses; although the possibility of unusual low dose effects cannot be discounted.

38  
39 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for inclusion into the  
40 evaluation process, due to small sample size and poor documentation and communication about what was  
41 done.

#### 42 43 *3.2.1.2 Evaluation of reproductive organ development*

44 **Talsness et al. (320)**, supported by the German Federal Ministry for Environmental Protection and  
45 Radiation Security, examined the effect of prenatal bisphenol A exposure on the reproductive systems of  
46 male and female rats. **[No information was provided about feed, caging, and bedding materials used.]**  
47 On GD 6–21, Sprague Dawley rats (n = 18–20/group) were gavaged with 2% corn starch vehicle or  
48 bisphenol A [purity not indicated] at 0.1 or 50 mg/kg bw/day. A group of 11 dams was gavaged with 0.2  
49 mg/kg bw/day ethinyl estradiol. Litters were weighed during the lactation period. Pups were weaned on  
50 PND 22 (according to Table 1 of the study, PND 1 was apparently the day of birth) and males and females  
51 were separated around PND 30. Vaginal opening was examined in 42–91 female offspring/group, and

### 3.0 Developmental Toxicity Data

1 estrous cyclicity was monitored over a 3-week period in 42–53 females/group. At 4 months of age, 5–10  
2 females/group were killed during diestrus and 20 females/group were killed while in estrus. A  
3 histopathological evaluation of vaginal tissue was conducted in 5 animals [assumed 5/group]. In 44–112  
4 male offspring/group, anogenital distance was measured on PND 3, 15, and 21 and days of testicular  
5 descent and preputial separation were recorded. Males were killed on PND 70 (n = 20/group) or 170 (n =  
6 17–20/group). Blood LH and testosterone concentrations were measured in 14–20 animals/group/time  
7 period. Sperm and spermatid numbers and sperm production and transit rates were determined in all  
8 offspring. Histopathological evaluation of the testis was conducted in 2 animals [assumed/group]. Body,  
9 reproductive organ, and liver weights were measured in all male and female offspring killed. Data from  
10 female rats were analyzed by ANOVA with post hoc Dunnett test or Fisher test. Data from male rats were  
11 analyzed by ANOVA and Dunnett test. **[It appears that offspring were considered the statistical unit.]**  
12

13 Pup body weights at birth were unaffected in the bisphenol A group, but on PND 22, pup body weights  
14 were lower [by 28%] in the low-dose group than in the control group. Study authors noted that the mean  
15 litter size in the low-dose group was larger by 2.6 pups than in the control group. Vaginal opening was  
16 delayed in the low-dose group and accelerated in the high-dose group. When estrous cyclicity data were  
17 evaluated according to total number of cycles, there was an increase in estrous phases lasting more than 1  
18 day and prolongation of the cycle length in the high-dose group. Evaluation of estrous cycles by individual  
19 rat indicated a decrease in the percentage of low-dose females with 3 consecutive 1-day estrus phases. The  
20 only terminal body and organ weight effects occurred in the low-dose group and included decreased  
21 absolute liver weight in females killed in estrus and decreased body and uterus weights in females killed in  
22 diestrus or in estrus. There were no effects on relative organ weights. Histological observations in vaginal  
23 tissue of bisphenol A-exposed rats included less pronounced cornification during estrus and more  
24 pronounced mucification during diestrus, with magnitude of effect greater in the low- than the high-dose  
25 group. Observations in the animals exposed to ethinyl estradiol included decreased pup birth weight,  
26 delayed vaginal opening, near-persistent estrus, decreased absolute and relative uterus weights, and changes  
27 in vaginal histology similar to those described for the low-dose bisphenol A group.  
28

29 Decreased anogenital distances was observed in the bisphenol A groups during all three time periods for  
30 male offspring, but the effect remained statistically significant only in the high-dose group when  
31 normalized for body weight. Testicular descent and preputial separation were delayed in the low-dose  
32 group. Organ weight effects that remained significant following adjustment for body weight included  
33 increased prostate weight in the high-dose group on PND 70 and increased testicular and epididymal  
34 weights in the low-dose group on PND 170. There was no effect on sperm morphology. Blood testosterone  
35 concentration was decreased in the high-dose group on PND 70, and blood LH concentration was increased  
36 in the high-dose group on PND 170. Testicular histopathology observations in the low-dose group on PND  
37 70 included cellular debris in lumens, pyknotic nuclei in spermatids, and apoptotic debris in the region of  
38 the spermatogonia and primary spermatocyte. In testes of 70-day-old animals of the high-dose group, there  
39 were central necrotic masses, low numbers of meiotic figures in spermatocytes, and low spermatozoa  
40 numbers. On PND 170, observations in testes from the low-dose group included low spermatozoa numbers,  
41 a thin layer of spermatocyte meiotic figures, and apoptotic debris in region of spermatids. Low  
42 spermatocyte meiotic figures were the only testicular observation in the high-group on PND 170. Effects  
43 observed in the ethinyl estradiol group included increased anogenital distance, delayed testicular descent,  
44 accelerated preputial separation, decreased testis and prostate weights, decreased sperm counts and  
45 production, increased LH concentrations, increased testosterone concentrations on PND 170, apoptotic  
46 debris, and/or low sperm numbers in testes.  
47

48 Study authors concluded that prenatal exposure to bisphenol A disrupts the reproductive systems of both  
49 male and female rats and that the effects do not occur according to a classic dose-response curve, which is  
50 generally observed in toxicology studies.  
51

### 3.0 Developmental Toxicity Data

1 **Strengths/Weaknesses:** Strengths are the postnatal evaluation of various endpoints to “pup” adulthood and  
2 that the concentration of the dosing solutions was verified. Based on the description of numbers of pups  
3 contributing to various endpoints, however, the authors do not appear to have used the litter as the unit of  
4 analysis. These inflated numbers subjected to analysis complicate the interpretation of findings, especially  
5 for PND 1–21 measures. A weakness also is that only 2 dose levels were examined. The vaginal opening  
6 data for the controls were outside the normal range for Sprague Dawley rats. It is unclear how the estrous  
7 cycle data were analyzed. The F<sub>1</sub> data were not analyzed correctly. Data may be suggestive of  
8 developmental disruptions at both doses, but the magnitudes are likely unreliable, and the authors’  
9 statements about dose-response peculiarities must be viewed with caution until more complete dose-  
10 response assessments are published.

11  
12 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation process.

13  
14 **Tinwell et al. (321)**, support not indicated, examined the effects of in utero exposure to bisphenol A on  
15 sexual development of male rats. The study attempted to duplicate findings from Chahoud and colleagues  
16 that were reported in several abstracts and as a full report (320). Sprague Dawley and Wistar-derived  
17 Alderley Park rats were housed in plastic-bottomed cages containing sawdust and shredded paper bedding.  
18 Rats were assigned to groups based on body weights and 6-7/group/strain were gavaged on GD 6–21 with  
19 bisphenol A (99% purity) at 0 (arachis oil vehicle), 0.020, 0.100, or 50 mg/kg bw/day. A positive control  
20 group initially received 200 µg/kg bw/day ethinyl estradiol, but the dose was reduced to 100 µg/kg bw/day  
21 between GD 11 and 14 due to maternal toxicity. Dosing solution concentrations and stability were verified.  
22 Dams were fed RM3 breeding diet (18.5% soybean protein; Special Diet Services, Ltd.) during gestation  
23 and lactation. At birth, pups were counted, sexed, and weighed. Anogenital distance was measured 24  
24 hours following birth (PND 1). On PND 5, pups were culled to 8/litter, with equal numbers of males and  
25 females when possible. On PND 23, rats were weighed and housed according to sex. Following weaning,  
26 pups were fed RM1 feed (6.5% soybean protein). Pups were weighed throughout the post-lactation period.  
27 Ages at preputial separation, vaginal opening, and first estrus were assessed. Males were killed on PND  
28 90–91 and females on PND 98. Liver and reproductive organs were weighed. Daily sperm production was  
29 determined. Data were analyzed using the litter and grouped individuals as the statistical unit. [**Litter  
30 values are discussed below.**] Data were analyzed by ANOVA, ANCOVA, and Dunnett test.

31  
32 The only significant effect observed in female rats exposed to bisphenol A was a 1.6-day delay in vaginal  
33 opening in Alderley-Park rats of the high-dose group. The study authors stated that effect on vaginal  
34 opening was correlated with body weight. [**Data were not shown by study authors.**] In Alderley Park  
35 males of the high-dose group, significant reductions were observed for total sperm count/testis [**12% lower  
36 than controls**], sperm count/g testis [**10% reduction**], daily sperm count/testis [**12% reduction**], and  
37 daily sperm count/g testis [**10% reduction**]. Benchmark doses for the endpoints with statistically  
38 significant changes are shown in [Table 71](#). In both strains, bisphenol A treatment had no effect on litter  
39 size, sex ratio, birth weight, anogenital distance, first day of estrus, or age of preputial separation. There  
40 were no significant effects on weights of liver, ovary, cervix, uterus, vagina, testis, epididymis, seminal  
41 vesicle, or prostate. Rats treated with ethinyl estradiol also experienced decreased sperm counts, in addition  
42 to decreased weights of male reproductive organs and advanced age of vaginal opening. Several findings  
43 observed by Chahoud and colleagues (320) were not duplicated in this study including: reduced anogenital  
44 distance; altered age of sexual maturation in males and females; variable changes in male reproductive  
45 organ weight, including prostate weight; and reduced sperm production at low doses. Study authors  
46 concluded that this study failed to confirm low-dose endocrine effects.

1 **Table 71. Benchmark Doses for Rat Reproductive Organ Endpoints Affected**  
 2 **by Prenatal Bisphenol A.**

Endpoint	Benchmark dose, mg/kg bw/day			
	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
Delayed vaginal opening	68	51	35	16
Sperm count/testis	55	30	57	31
Sperm count/g testis	81	41	68	34
Daily sperm count/testis	56	31	59	31
Daily sperm count/g testis	83	42	70	34

Calculated from data in Tinwell et al. (321).

3  
 4 **Strengths/Weaknesses:** Strengths of this study are the range and appropriateness of selected measures, the  
 5 use of 2 strains of rat, the verification of dosing solutions, and the use of ethinyl estradiol, which produced  
 6 expected responses. An unfortunate weakness is the small sample size of 6-7 dams/strain/group.  
 7 Nevertheless, data were appropriately analyzed with the litter as the experimental unit, and significance  
 8 judgments were apparently based on 7/group. Modest effects were noted in male and female offspring in  
 9 the 50 mg/kg exposure group. While effects on the lowest doses in this study were not seen, it is important  
 10 to recognize the effects seen at 50 mg/kg bw/day (the high dose in this study) dosing on GD 6–21.

11  
 12 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
 13 evaluation process.

14  
 15 **Schönfelder et al. (89)**, supported by the German Federal Ministry for Education and Research, examined  
 16 the effects of prenatal bisphenol A exposure on the rat vagina. Sprague Dawley rats were gavaged on GD  
 17 6–21 with bisphenol A at 0 [2% corn starch vehicle (Mondamin)], 0.1, or 50 mg/kg bw/day. A positive  
 18 control group was treated with 0.2 mg/kg bw/day 17 $\alpha$ -ethinyl estradiol in a peanut oil vehicle. **[No**  
 19 **information was provided on the number of dams treated, the day of vaginal plug, purity of**  
 20 **bisphenol A, or the type of chow, bedding, and caging materials used.] [According to the author the**  
 21 **number of litters treated were: Mondamin =20, 0.1 mg/kg bw/day bisphenol A= 20, 50 mg/kg bw/day**  
 22 **bisphenol A = 18, and 0.2 mg/kg bw/day 17 $\alpha$  ethinyl estradiol = 11; day of sperm positive smear was**  
 23 **considered to be GD 0 and was used instead of day of vaginal plug; purity of bisphenol A was  $\geq$  98%;**  
 24 **Altromin 1324 rodent chow was used (obtained from Altromin GmbH); bedding was wood shavings**  
 25 **obtained from Altromin GmbH; caging was Type III macrolon cages (G. Schönfelder, personal**  
 26 **communication, July 20, 2007)]. At 3 months of age, estrous cyclicity was evaluated for 3 weeks in 42**  
 27 **female offspring of the control group, 21 offspring of the 0.1 mg/kg bw/day group, 18 offspring of the 50**  
 28 **mg/kg bw/day group, and 24 offspring of the 17 $\beta$ -estradiol group. [The number of litters represented**  
 29 **was not stated.]. At 4 months of age, female offspring were killed in either estrus or diestrus. [Authored**  
 30 **clarified that each estrus group contained 22 offspring from 20 dams in the cornstarch group, 13**  
 31 **offspring from 13 dams in the 0.1 mg /kg / d and 12 offspring from 12 dams in the 50 mg / kg / d**  
 32 **bisphenol A group, as well as 19 offspring from 11 dams in the 0.2 mg / kg / d 17 $\alpha$ -ethinyl estradiol**  
 33 **group (G. Schönfelder, personal communication, July 20, 2007)]. [Exact litter representation for**  
 34 **animals collected during diestrus was not provided]. Vaginas were fixed in Bouin solution and a**  
 35 **histopathological evaluation was conducted. Western blot analyses were conducted to measure expression**  
 36 **of ER $\alpha$  and ER $\beta$ . [It does not appear that statistical evaluations were conducted.]**

37  
 38 Qualitative descriptions of vaginal histopathology changes and ER expression were provided by the study  
 39 authors. Low-dose animals killed during the estrous stage lacked keratinization of the surface epithelium  
 40 and demonstrated reduced thickness of the total epithelium. Similar but less pronounced effects were  
 41 observed in rats of the high-dose bisphenol A group. Vaginal findings were similar in the positive control  
 42 group, and slight desquamation of the superficial layers was also observed. There were no differences in

### 3.0 Developmental Toxicity Data

vaginal histopathology findings in rats killed during the diestrous stage. No ER $\beta$  was observed in vaginas of rats from any treatment group. Full-length ER $\alpha$  expression was not observed in either bisphenol A group during estrus, but ER $\alpha$  in the bisphenol A-exposed groups did not differ from the control group during the diestrous stage. ER $\alpha$  in vaginas obtained from the positive control group was either reduced or was not detected. The study authors concluded that altered vaginal morphology following bisphenol A treatment appears to be due to down-regulation of ER $\alpha$ .

**Strengths/Weaknesses:** Vaginal histopathology of female offspring is of interest but the quality of the study cannot be judged due to unclear methodology. Uncertainty about the numbers of animals, the number of offspring examined and the lack of statistical accounting for litter effects are significant weaknesses.

**Utility (Adequacy) of the CERHR Evaluation Process:** This study is inadequate for the evaluation process for the reasons detailed above.

**Schönfelder et al. (322)**, supported by the German Federal Ministry for Environmental Protection and Radiation Security, examined the effects of prenatal bisphenol A exposure on the rat uterus. **[No information was provided about composition of feed, caging, or bedding.]** Sprague Dawley rats **[number treated not specified]** were gavaged with bisphenol A **[purity not reported]** at 0 (2% corn starch vehicle), 0.1, or 50 mg/kg bw/day on GD 6–21. **[Author clarified that the purity of bisphenol A was  $\geq$  98%; Altromin 1324 rodent chow was used (obtained from Altromin GmbH); bedding was wood shavings obtained from Altromin GmbH; caging was Type III macrolon cages (G. Schönfelder, personal communication, July 20, 2007)].** The high bisphenol A dose was selected because it was reported to be the no observed effect level (NOEL) recommended by the Society of the Plastics Industry. A positive control group was gavaged with 0.2 mg/kg bw/day ethinyl estradiol on GD 6–21. Estrous cyclicity was examined for 3 weeks in 6 female offspring/group beginning at 3 months of age. Six female offspring/group were killed at 4 months of age on the day of estrus. Body and reproductive organ weights were measured. Uteri were fixed in methacarn solution and sectioned. Examinations of uterine morphology were conducted. Immunohistochemistry techniques were used to detect ER $\alpha$  and ER $\beta$  in the uterus, and results were verified by Western blot. Data were analyzed by Mann-Whitney test. **[It was not clear if data were analyzed on a per litter or per offspring basis.] [Author states that each female came from a different litter so the data were analyzed on a per litter basis (G. Schönfelder, personal communication, July 20, 2007)].** Statistically significant findings are summarized in [Table 72](#). Effects observed at both dose levels were increased epithelial cell nuclei, epithelial nuclei with condensed chromatin, and epithelial cells with cavities and reduced ER $\beta$ -positive cells in uterine tissue. Additional effects observed only at the high dose included decreased thickness of luminal epithelium and increased ER $\alpha$ -positive cells in the epithelium. Similar findings were observed following treatment with ethinyl estradiol. The study authors concluded that prenatal bisphenol A exposure causes uterine effects in rat offspring.

**Table 72. Uterine Effects in Rats Exposed to Bisphenol A During Prenatal Development**

Endpoint	Dose, mg/kg bw/day	
	0.1	50
Thickness of luminal epithelium	↔	↓38%
Epithelial nuclei <sup>b</sup>	↑69%	↑89%
Epithelial nuclei with condensed chromatin	↑2.7-fold	↑3.1-fold
Epithelial cells with cavities	↑2.1-fold	↑1.9-fold
ER $\alpha$ positive cells in epithelium	↔	↑67%
ER $\beta$ -positive cells in uterine tissue	↓88%	↓88%

<sup>b</sup>It is unclear if authors were referring to numbers of nuclei.

### 3.0 Developmental Toxicity Data

1 **Strengths/Weaknesses:** A strength is the examination of effects on uterine indices in female offspring. A  
2 slight weakness is the use of only 6 females per group; however, the panel noted that the results appeared to  
3 be consistent across animals and across endpoints, especially in the 50 mg/kg bw/day treatment group.  
4

5 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
6 evaluation process.  
7

8 **Wistuba et al. (323)**, supported by the German Federal Ministry of Education and Science, examined the  
9 effects of prenatal exposure on testicular histology and sperm endpoints in rats. **[No information was  
10 provided about chow, bedding, or caging.]** Sprague Dawley rats were gavaged with 0 (2% corn starch  
11 suspension vehicle), 0.1, or 50 mg/kg bw/day bisphenol A **[purity not reported]** on GD 6–21 (GD 0 = day  
12 of sperm detection). A third group was treated with 0.02 mg/kg bw/day ethinyl estradiol. The high dose  
13 was said to correspond to the current accepted no observed adverse effect level (NOAEL) and the lower  
14 dose was selected to determine if effects occurred at lower doses. It appears that the number of dams  
15 treated was 2 in the control group, 4 in the low-dose group, 1 in the high-dose group, and 4 in the ethinyl  
16 estradiol group. Litters were weighed during the lactation period. Pups were weaned on PND 22 **[day of  
17 birth not defined]**. Male offspring were killed between the ages of ~9 and 12 months. The number of  
18 males killed was 5 from 2 litters in the control group, 15 from 4 litters in the low-dose group, 5 from 1 litter  
19 in the high-dose group, and 10 from 4 litters in the ethinyl estradiol group. Testes were fixed in Bouin  
20 solution, and Sertoli cells were counted. Spermatogenesis was evaluated by examining germinal epithelia  
21 for cell death and distribution of various cell populations. Data were analyzed by ANOVA. **[It appears  
22 that at least some data were analyzed on a per litter basis. In addition, analyses were done to  
23 determine intralitter variability and thus the numbers of animals per group that needed to be  
24 analyzed.]**  
25

26 Examination of tubule cross sections revealed qualitatively normal spermatogenesis in the bisphenol A  
27 groups. A comparison of Sertoli cell numbers in littermates revealed high variability (20–27%) in the 0.1  
28 mg/kg bw/day group. A comparison of Sertoli cell numbers in the 4 litters from the 0.1 mg/kg bw/day  
29 group revealed almost identical results between litters. Sertoli cell numbers/organ were significantly  
30 increased by 19.4% in the low-dose group and 19% in the high-dose group. Bisphenol A had no significant  
31 effect on Sertoli cell numbers/g testis weight. The opposite situation occurred in the ethinyl estradiol group,  
32 with no significant effects on Sertoli cell numbers/organ but a significant increase in Sertoli cell numbers/g  
33 testis weight. Testis weight was not affected by bisphenol A treatment but was decreased in the ethinyl  
34 estradiol group. The study authors concluded that the study does not support the hypothesis of disruption of  
35 the male reproductive system by bisphenol A exposure.  
36

37 **Strengths/Weaknesses:** The conceptual strength is the focus on the male reproductive tract/function.  
38 However, a weakness is that there were too few animals to provide reliable data.  
39

40 **Utility (adequacy) for the CERHR Evaluation Process:** This study is inadequate based on insufficient  
41 sample size (n = 2-4).  
42

43 **Thuillier et al. (324)**, supported by National Institute of Environmental Health Sciences (NIEHS),  
44 examined a possible role for the platelet-derived growth factor system in estrogenic effects induced by  
45 bisphenol A in rats exposed during gestation. The effects of other compounds such as genistein and  
46 coumestrol were also examined but will not be discussed here. Pregnant Sprague Dawley rats were gavaged  
47 with bisphenol A at 0 (corn oil vehicle) or 0.1, 1, 10, or 200 mg/kg bw/day from GD 14 through birth (PND  
48 0). Additional rats were sc injected with diethylstilbestrol at 0.01–2 µg/kg bw/day during the same period.  
49 **[No information was provided about number of rats treated, purity of bisphenol A, feed, or materials  
50 used in bedding and caging.]** Male offspring were killed on GD 21 or PND 3 and testes were collected.  
51 Expression of mRNA or protein for platelet-derived growth factor receptor- $\alpha$  and platelet-derived growth

### 3.0 Developmental Toxicity Data

1 factor receptor- $\beta$  were determined in testes using RT-PCR, in situ hybridization, or immunohistochemistry.  
2 Statistical analyses included unpaired *t*-test with Welch correction. **[It was not clear if the litter or**  
3 **offspring were considered the statistical unit.]**  
4

5 Expression of mRNA for platelet-derived growth factor receptor- $\alpha$  and - $\beta$  was significantly increased at  
6 bisphenol A doses  $\geq 1$  mg/kg bw/day in testes from 3-day-old rats. All other experiments with bisphenol A  
7 were conducted with a single dose of 200 mg/kg bw/day. In situ hybridization examination of testes from  
8 3-day-old rats from the bisphenol A group revealed an increase in expression of platelet-derived growth  
9 factor receptor- $\alpha$  mRNA in testicular interstitium and platelet-derived growth factor receptor- $\beta$  mRNA in  
10 interstitium and seminiferous cords. Exposure to bisphenol A resulted in slightly increased platelet-derived  
11 growth factor receptor- $\alpha$  protein expression and strong expression of platelet-derived growth factor  
12 receptor- $\beta$  in gonocytes from 3-day old rat testes. Immunolocalization studies in testes from 21-day-old  
13 fetuses revealed that exposure to 200 mg/kg bw/day bisphenol A did not affect expression of platelet-  
14 derived growth factor receptor- $\alpha$  protein in gonocytes, but platelet-derived growth factor receptor- $\beta$  protein  
15 appeared to be increased in gonocytes and Sertoli cells. Diethylstilbestrol tended to have a biphasic effect  
16 with increased expression of platelet-derived growth factor receptor- $\alpha$  and - $\beta$  mRNA in 3-day-old rat testis  
17 at low doses and decreased expression at the high dose. Treatment with 1  $\mu$ g/kg bw/day diethylstilbestrol  
18 decreased mRNA expression of platelet-derived growth factor receptor- $\alpha$  in interstitium and increased  
19 platelet-derived growth factor receptor- $\beta$  mRNA expression in seminiferous cords. Immunoreactivity for  
20 platelet-derived growth factor receptor- $\alpha$  protein was maintained but there was a minimal level of platelet-  
21 derived growth factor receptor- $\beta$  protein expression in 3-day-old rat testes following exposure to 1  $\mu$ g/kg  
22 bw/day diethylstilbestrol. In testes obtained from 21-day-old fetuses, expression of platelet-derived growth  
23 factor receptor- $\alpha$  protein was decreased in Sertoli and interstitial cells and expression of platelet-derived  
24 growth factor receptor- $\beta$  protein was apparently increased following exposure to diethylstilbestrol. The  
25 study authors concluded that the platelet-derived growth factor receptor pathway may be a target for  
26 estrogens in the testis, but the findings do not exclude the possibility that effects may have occurred  
27 through an ER-independent mechanism.  
28

29 **Strengths/Weaknesses:** Endpoints are a strength, but inadequate methodological detail (i.e., sample size or  
30 adequate control for litter effects) precludes any informed judgment of study quality.  
31

32 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation process  
33 based on insufficient methodological details.  
34

35 **Wang et al. (325)**, supported by NIEHS, examined the effects of prenatal bisphenol A exposure on  
36 expression of ER-associated proteins in rat testis. The effects of genistein and coumestrol were also  
37 examined but will not be discussed here. Pregnant Sprague Dawley rats [**apparently 3/group**] were  
38 gavaged with corn oil vehicle or bisphenol A at 0.1–200 mg/kg bw/day from GD 14 (14 days post-coitum)  
39 through birth. Additional rats were sc injected with 0.01–2  $\mu$ g/kg bw/day diethylstilbestrol during the same  
40 time period. [**No information was provided about feed, caging and bedding material, or compound**  
41 **purity.**] Male offspring from 3 independent litters were killed on GD 21, PND 3, or PND 21. Western blot,  
42 RT-PCR, and immunohistochemistry techniques were used to measure expression of protein or mRNA for  
43 *Hsp90*, *Hsp90 $\alpha$* , *p23*, *CYP40*, *Hsp70*, and/or *ER $\beta$* . Spermatogonia were quantified in PND 21 rat testis.  
44 Data were analyzed by unpaired *t*-test. The dam was considered the statistical unit.  
45

46 In testes from 3-day-old rats, RT-PCR revealed significant increases in mRNA for *hsp90* at bisphenol A  
47 dose levels of 10 and 200 mg/kg bw/day, and significant decreases in expression of *CYP40* at 200 mg/kg  
48 bw/day and *p23* at 1 mg/kg bw/day. In situ hybridization analyses in 3-day-old rat testes revealed that  
49 bisphenol A tended to increase expression of *hsp90* throughout the testis, with patterns indicating increased  
50 expression in gonocytes and interstitial Leydig cells. Examination of protein in testes from 3-day old rats  
51 exposed to 200 mg/kg bw/day bisphenol A revealed significantly increased levels of *hsp90* and *hsp70*, but

### 3.0 Developmental Toxicity Data

1 no effect on levels of CYP40, p23, or ER $\beta$ . Immunohistochemistry revealed that hsp90 protein in testes  
2 from 3-day-old rats was most increased in gonocytes and less so in interstitium following exposure to 200  
3 mg/kg bw/day bisphenol A. Use of a probe specific for hsp90 $\alpha$  protein revealed that increased protein  
4 expression of hsp90 was due in a large part to the hsp90 $\alpha$  isoform. Examination of testes from GD 21  
5 fetuses and PND 21 pups revealed that the amount of hsp90 protein in the bisphenol A treatment group was  
6 similar to that observed on PND 3 but that the amount of protein did not differ from controls on PND 21. In  
7 21 day-old rats from the bisphenol A group, the number of spermatogonia/tubule was significantly higher  
8 by ~2-fold compared to the control group. **[It is not clear which bisphenol A dose induced an increase in  
9 spermatogonia, but it was most likely 200 mg/kg bw/day, because that dose appeared to be used in all  
10 studies not examining dose-response relationships.]** Effects following diethylstilbestrol exposure  
11 included increased expression of *hsp90* mRNA at 1.0  $\mu$ g/kg bw/day and decreased *CYP40* mRNA  
12 expression at 0.01 and 1  $\mu$ g/kg bw/day, but no effect on protein levels of those compounds was reported in  
13 testes from 3-day-old rats. The number of spermatogonia/tubule was also increased after prenatal exposure  
14 to diethylstilbestrol. The study authors concluded that prenatal exposure to bisphenol A affects *hsp90*  
15 expression in gonocytes of rats, and because hsp90 interacts with several signaling molecules, changes in  
16 its expression could affect gonocyte development.

17  
18 **Strengths/Weaknesses:** This study was generally well conceived, but the small sample size suggests it  
19 presents pilot data only. A full study is needed to provide reliable data.

20  
21 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate based on insufficient  
22 sample size (n=3).

#### 23 3.2.1.3 Neurodevelopmental endpoints

24 **Funabashi et al. (326)**, supported in part by Yokohama City University, examined the effects of bisphenol  
25 A on the numbers of corticotropin-releasing hormone neurons in the preoptic area and bed nucleus of the  
26 stria terminalis of rats exposed during development. **[No information was provided about chow or  
27 composition of bedding and caging.]** Pregnant Wistar rats (n = 8–11/treatment group) were given  
28 drinking water containing the 0.1% ethanol vehicle or 10 mg/L bisphenol A **[purity not reported]** until  
29 their offspring were weaned at 3 weeks of age. **[It is implied but not stated that exposure occurred  
30 during the entire gestation period.]** Bisphenol A intake was estimated by study authors at 2.5 mg/kg  
31 bw/day. Male and female offspring (n = 8–11/group) were killed at 4–7 months of age, and  
32 immunocytochemistry techniques were used to determine the number of corticotropin-releasing hormone  
33 neurons in brain. Female rats were killed during proestrus. **[Although the number of litters represented  
34 in each group was not specified, the number of rats examined suggests that 1 rat/sex/litter was  
35 examined.]** Histological slides of brain were evaluated by an investigator blinded to treatment conditions.  
36 Two series of experiments were conducted, and data from both experiments were combined. Data were  
37 analyzed by ANOVA followed by Fisher protected least significant difference post-hoc test. **[It was not  
38 stated if data were analyzed on a per litter or per offspring basis, but as stated earlier, it appears that  
39 1 rat/sex/litter was examined.]** In the control group, females had more corticotropin-releasing hormone  
40 neurons in the preoptic area and anterior and posterior bed nucleus of the stria terminalis than males.  
41 Bisphenol A treatment did not change the number of corticotropin-releasing hormone neurons in the  
42 preoptic areas of males. A loss in sex difference occurred in the anterior and posterior bed nuclei of the  
43 stria terminalis following bisphenol A treatment because differences in numbers of corticotropin-releasing  
44 hormone neurons between males and females were no longer evident. It appears that bisphenol A treatment  
45 increased the number of corticotropin-releasing hormone neurons in males and decreased the number in  
46 females. The study authors concluded that exposure to bisphenol A during gestation and lactation results in  
47 a loss of sex difference in corticotropin-releasing hormone neurons in the bed nucleus of the stria terminalis  
48 but not in the preoptic area.

49  
50

### 3.0 Developmental Toxicity Data

1 **Strengths/Weaknesses:** This study was appropriately designed to examine effects on the development of  
2 brain areas known to be influenced by hormonal levels. Strengths include the relevance and subtleties of  
3 the endpoints measured; weaknesses include uncertainties about the numbers of animals examined and the  
4 duration of the dosing period. The results suggest a disruption of the normal pattern of sexually dimorphic  
5 neurons, a result of critical importance to concerns about disruptions relevant to reproductive function and  
6 sexually dimorphic behaviors. While the sample size was 8-11/group, the design and statistics appear to be  
7 appropriate. It is a weakness that the control for litter effects was not clear.  
8

9 **Utility (adequacy) for CERHR Evaluation Process:** This study is adequate for inclusion in the  
10 evaluation process, although of limited utility due to uncertainties about the sample size, duration of  
11 dosing, and control for litter effects.  
12

13 **Fujimoto et al. (327)**, supported by the Japanese Ministry of Education, Culture, Sports, Science, and  
14 Technology, examined the effect of prenatal bisphenol A exposure on sexual differentiation of  
15 neurobehavioral development in rats. Wistar rats were fed CE-2 feed (CLEA, Japan). **[Caging and  
16 bedding materials were not described.]** From GD 13 (day of vaginal sperm not defined) to the day of  
17 birth (PND 0), 6 rats/group were given tap water containing bisphenol A **[purity not reported]** at 0 or 0.1  
18 ppm. The study authors estimated the bisphenol A dose at 0.015 mg/kg bw/day. On PND 1, pups were  
19 weighed and litters were culled to 4 pups/sex. Pups were weaned on PND 21. Neurobehavioral evaluations  
20 conducted in 20–24 offspring/sex/group at 6–9 weeks of age included open-field, elevated plus maze,  
21 passive avoidance, and forced swimming tests. Statistical analyses included ANOVA, Fisher protected least  
22 significant difference test, and Mann-Whitney *U* test. **[It appears that offspring were considered the  
23 statistical unit.]**  
24

25 In the control group, rearing frequency and duration were significantly higher in females than males, but  
26 there were no sex-related differences in rearing frequency or duration in the bisphenol A group. Bisphenol  
27 A exposure caused an increase in rearing duration in males when compared to males from the control  
28 group. In the forced swim test, females in the control group struggled more than males but no sex-related  
29 differences in struggling were observed in the bisphenol A group. The duration of immobility in the  
30 swimming test was longer in males from the bisphenol A compared to males from the control group.  
31 Immobility was described as non-significantly increased in females exposed to bisphenol A compared to  
32 control females. Bisphenol A exposure had no effect on performance in passive avoidance and elevated  
33 plus maze test. The study authors concluded that exposure of male offspring to bisphenol A during the final  
34 week of gestation resulted in impaired sexual differentiation in rearing and struggling behaviors and  
35 facilitated depression-like behavior.  
36

37 **Strengths/Weaknesses:** This study utilized a good choice of methods to examine functional disruptions in  
38 sexually dimorphic behaviors. Weaknesses include a lack of clarity about the nature of disruption of  
39 sexually dimorphic behavior patterns that was indicated in the authors' conclusions, the somewhat small  
40 sample size, the use of a single dose level, which was not confirmed, and the lack of clarity of the statistical  
41 methods regarding litter.  
42

43 **Utility (Adequacy) for CERHR Process:** This paper is inadequate for the evaluation process due to  
44 statistical methodology.  
45

#### 46 3.2.2 Rat—parenteral exposure only during pregnancy

47 **Ramos et al. (328)**, supported by the Argentine National Council for Science and Technology, the  
48 Argentine National Agency for the Promotion of Science and Technology, and the Ministry of Health,  
49 examined the effects of bisphenol A exposure on the rat prostate. Wistar rats were housed in stainless steel  
50 cages. **[No information was provided about chow or bedding material.]** Four dams/group were exposed  
51 to bisphenol A **[purity not reported]** at 0 (DMSO vehicle), 0.025, or 0.250 mg/kg bw/day by sc pump on

### 3.0 Developmental Toxicity Data

1 GD 8–23 (GD 1 = day of vaginal sperm). Pups were weighed and sexed at birth. Litters were culled to 8  
2 pups, with 4/sex when possible. Pups were weaned on PND 22 [**day of birth not defined**]. On PND 30,  
3 pups were injected with bromodeoxyuridine and killed 2 hours later. Ventral prostates were dissected and  
4 fixed in 10% neutral buffered formalin. Immunohistochemical techniques were used to measure proteins  
5 associated with cell proliferation and cell phenotypes. Morphometric measurements were taken. [**It was not**  
6 **clear how many rats/treatment group were examined for each endpoint. Although a statement was**  
7 **made that males from a single dam were evaluated, it was later stated that siblings were excluded**  
8 **from the same experimental group. Therefore it appears that different litters were represented.**] Data  
9 were analyzed by Kruskal-Wallis ANOVA and Mann-Whitney *U* test. [**It was not clear if the dam or**  
10 **offspring were considered the statistical unit.**]

11  
12 In the periductal stroma, the fibroblastic layer was increased, the smooth muscle layer was reduced, and  
13 androgen receptor-positive cells were decreased. Prostatic acid phosphatase-positive cells were reduced in  
14 epithelial cells. There were no effects on cell proliferation and ER $\alpha$  was not detected. No changes were  
15 observed in interductal stromal cells.

16  
17 **Strengths/Weaknesses:** This study has an interesting design with respect to choice of endpoints. Certain  
18 design aspects are unclear and statistical approaches are inadequate. The sample size was small (4  
19 dams/group) and there was considerable uncertainty about numbers of offspring examined and accounting  
20 for litter effects. The use of DMSO (% not specified) is of concern, as this can modify the effects of the  
21 solute. Of additional concern is the route of administration (sc pump).

22  
23 **Utility (Adequacy) for CERHR Evaluation Process:** This study is considered inadequate.

24  
25 **Ramos et al. (329)**, supported by the Argentine Ministry of Health, Argentine National Agency for the  
26 Promotion of Science and Technology, and the National University of Litoral, examined the effects of  
27 bisphenol A exposure on the prostate and the hypothalamic-pituitary-gonadal axis in Wistar rats. Rats were  
28 housed in stainless steel cages and 7–9/group were administered DMSO vehicle or bisphenol A at 0.025 or  
29 0.250 mg/kg bw/day by sc pump on GD 8–23 (GD 1 = day of vaginal sperm). [**No information was**  
30 **provided on purity of bisphenol A, the type of feed used, or composition of bedding.**] After birth, pups  
31 were weighed and sexed. Litters were culled to 8 pups with equal numbers of male and female pups when  
32 possible. Pups were weaned on PND 22 [**day of birth not defined**]. During prepuberty (PND 15),  
33 peripuberty (PND 30), and adulthood (PND 120), 6–8 males/group were injected with bromodeoxyuridine  
34 and killed 2 hours later. Serum was collected for measurement of LH and prolactin by RIA.  
35 Immunohistochemistry techniques were used to evaluate markers of cell proliferation, estrogen/androgen  
36 receptors, and prostatic cells. Expression of mRNA for ER $\alpha$  and ER $\beta$  in the preoptic area and medial basal  
37 hypothalamus was determined by RT-PCR. Data were analyzed by Kruskal-Wallis 1-way ANOVA using  
38 Dunn post-test.

39  
40 No significant effects were observed for ventral prostate weight. Numerous transient effects were observed  
41 in both bisphenol A dose groups. On PND 15, cellular proliferation was increased in the periductal stroma  
42 of the prostate, and serum testosterone levels were elevated. On PND 30, the fibroblasts (vimentin-positive  
43 cells) in the prostatic periductal stroma was increased and the area of smooth muscle cells  $\alpha$ -smooth muscle  
44 actin) was decreased. Also observed on PND 30 was a reduction in androgen-receptor positive stromal  
45 cells, a decrease in epithelial cells positive for prostatic acid phosphatase, and an increase in serum  
46 prolactin levels. Expression of ER $\beta$  mRNA was increased in the preoptic areas on PND 30 and 120, and the  
47 study authors considered the effect to be permanent because it occurred on both days. The study authors  
48 concluded that prenatal exposure to environmental concentrations of bisphenol A during gestation results in  
49 transient and permanent changes in the male reproductive axis.

### 3.0 Developmental Toxicity Data

1 **Strengths/Weaknesses:** The design seems reasonable as a means to address the study questions. Like  
2 many of these studies, altered values are given without addressing the normal range of variation or the  
3 likely functional significance of the changes. Weaknesses include use of the sc pump as a route of  
4 administration and use of DMSO as a vehicle.

5  
6 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for inclusion due to the use  
7 of 99.9% DMSO as a vehicle to administer BPA via sc pump. As discussed in earlier, the use of >50%  
8 DMSO as a vehicle for Alzet mini-pump studies is a clear contravention of the directions for mini-pump  
9 use, as it accelerates the breakdown of the mini-pumps

10  
11 **Naciff et al. (278)**, from the Procter and Gamble Company, examined the effects of prenatal bisphenol A  
12 exposure on gene expression and, to a limited extent, development in female rat reproductive organs.  
13 Pregnant Sprague Dawley rats were fed Purina 5K96, a casein-based soy- and alfalfa-free diet.

14 **[Composition of caging and bedding materials was not reported.]** The rats were randomly assigned to  
15 groups ( $\geq 7$  rats/group) sc injected with bisphenol A (~99% purity) in DMSO vehicle at 0, 5, 50, or 400  
16 mg/kg bw/day on GD 11–20 (day of sperm detection = GD 0). Dams were killed on GD 20, and ovaries  
17 and uteri were removed from fetuses. In 4 litters/group, 1 female fetus/litter was examined for ovarian and  
18 uterine histopathology. In 5 litters/group, ovaries and uteri from at least 5 littermates were pooled for a  
19 microarray analysis of gene expression. Changes in gene expression were further quantified using RT-PCR.  
20 Data were analyzed by *t*-test, ANOVA, and Jonkheere-Terpstra test. Comparisons of gene expression  
21 among estrogenic compounds were made by Wilcoxon-Mann-Whitney and Jonkheere-Terpstra tests.  
22 Results of gene expression assays are discussed in Section 2. Vaginal bleeding and early parturition  
23 occurred in 1 of 8 dams in the high-dose group. Bisphenol A treatment had no effect on maternal body  
24 weight or number of live fetuses/litter, and there were no gross or histopathological effects on ovary or  
25 uterus. Prominent nipples and areolas were observed in males and females in the high-dose bisphenol A  
26 group **[number of fetuses and litters affected were not reported]**.

27  
28 **Strengths/Weaknesses:** Strengths are that these endpoints appear appropriate; weaknesses are the limited  
29 nature of the endpoints and the use of neat DMSO as vehicle. The sample sizes are 4-5/endpoint/group and  
30 judged to be inadequate. Of additional concern is the route of administration.

31  
32 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation process.

33  
34 **Naciff et al. (330)**, from The Procter and Gamble Company, examined the effect of prenatal bisphenol A  
35 exposure on male rat reproductive organ histology and gene expression.  
36 Pregnant Sprague Dawley rats were fed Purina 5K96, a casein-based soy- and alfalfa-free diet. Rats were  
37 housed in stainless steel cages prior to mating. Rats were randomly assigned to groups ( $\geq 8$  rats/group) and  
38 sc injected with bisphenol A (~99% purity) in DMSO at 0, 0.002, 0.02, 0.5, 50, or 400 mg/kg bw/day on  
39 GD 11–20 (day of sperm detection = GD 0). Dams were killed on GD 20, and testes and epididymides were  
40 removed from fetuses. In 4 litters/dose group, 1 male fetus/litter was examined for testicular  
41 histopathology. In 5 litters/group, testes and epididymides from 5 littermates were pooled for a microarray  
42 analysis of gene expression. Changes in gene expression were further quantified using RT-PCR. Data were  
43 analyzed by *t*-test, ANOVA, and Jonkheere-Terpstra test. Comparisons of gene expression among  
44 estrogenic compounds were analyzed by Wilcoxon-Mann-Whitney and Jonkheere-Terpstra tests.

45  
46 Bisphenol A treatment had no effect on maternal body weight or number of live fetuses/litter, and there  
47 were no gross or histopathological effects on the testis or epididymis. Prominent nipples/areolas were  
48 observed in male and female fetuses from the high-dose group **[numbers of fetuses and litters affected  
49 were not reported]**. In pooled testis and epididymis samples from the high-dose bisphenol A group,  
50 expression of 15 genes was significantly altered in a dose-related manner. When bisphenol A data were  
51 pooled with data obtained from ethinyl estradiol and genistein and globally analyzed, there were 50 genes

### 3.0 Developmental Toxicity Data

1 that were significantly altered in the same direction by all 3 compounds. The study authors concluded that  
2 transplacental exposure to high doses of bisphenol A alters the expression of certain genes in the testis and  
3 epididymis of fetal rats without causing malformations in those organs. The study authors noted that the  
4 dose response to bisphenol A was monotonic with no evidence of robust quantifiable responses at low  
5 doses.

6  
7 **Strengths/Weaknesses:** Strengths are that these endpoints appear appropriate; weaknesses are the limited  
8 nature of the endpoints and the use of neat DMSO as vehicle. The sample sizes are 4-5/endpoint/group and  
9 judged to be inadequate. Of additional concern is the route of administration.

10  
11 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation process.

12  
13 **Saito et al. (331)**, support not indicated, examined the effect of prenatal bisphenol A exposure on  
14 testosterone production during adulthood in rats. On GD 12–19 (day of vaginal plug not reported), 2 Wistar  
15 rats were sc injected with the corn oil vehicle, 4 rats were sc injected with 0.005 mg/day bisphenol A  
16 [purity not indicated], and 2 rats were injected with 5 µg/day 17β-estradiol. [Assuming a pregnant  
17 Wistar rat weights ~0.33 kg, 0.005 mg/day would be equivalent to 0.015 mg/kg bw/day bisphenol A.]  
18 Other materials found in dental composites were also evaluated but will not be discussed. During the  
19 lactation period, rats were housed in polypropylene cages with synthetic bedding. [No information was  
20 provided on feed.] Offspring were housed separately at 3 weeks of age and killed at 13 weeks of age.  
21 Body and testis weights were measured in all male offspring (22 in the bisphenol A group, 11 in the vehicle  
22 control group, and 5 in the 17β-estradiol group). Plasma testosterone level was measured by RIA, and  
23 plasma cholesterol level was measured using a kit. Activities of testicular enzymes involved in the  
24 production of testosterone from progesterone were also examined in an in vitro assay in which testicular  
25 microsomes were incubated with <sup>14</sup>C-progesterone and <sup>14</sup>C-Δ<sup>4</sup>-androstendione for 20 minutes. Data were  
26 analyzed using unspecified post hoc tests. [Although not clear, it appears that offspring were  
27 considered the statistical unit for some analyses.]

28  
29 Bisphenol A exposure had no effect on pup sex ratio. No effects on body weight or absolute testicular  
30 weight were observed in the bisphenol A group at 13 weeks of age. However, relative (to body weight)  
31 testicular weight was lower [by 6%] in rats of the bisphenol A compared to the control group. Also  
32 observed in the bisphenol A group was a reduction in plasma testosterone level [by ~28%]. No effect was  
33 observed on cholesterol level. In the ex vivo study, prenatal bisphenol A exposure increased activities of  
34 17α-hydroxysteroid dehydrogenase [by ~140%] and 17β-hydroxysteroid dehydrogenase [by ~70%].  
35 Observations in the 17β-estradiol compared to the control group included decreased numbers of offspring  
36 delivered, higher body weight of male offspring at 13 weeks of age, reduced plasma testosterone level, and  
37 increased testicular 17α-hydroxysteroid dehydrogenase activity. The study authors concluded that  
38 bisphenol A had an estrogenic effect on the testis but did not decrease activities of enzymes involved in  
39 testosterone production.

40  
41 **Strengths/Weaknesses:** A strength of this study is the examination of testosterone levels at 13 weeks of  
42 age. This strength is negated by the sample size (n = 2-4), which is too small to draw any firm conclusions.

43  
44 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate based on insufficient  
45 sample size.

46  
47 **Murray et al. (332)**, supported by NIH, examined the effect of prenatal bisphenol A exposure on in situ  
48 induction of mammary tumors. Wistar-Furth rats were fed Harlan Teklad 2018, which was reported to  
49 contain 20 fmol/g estrogen equivalents. Water was supplied in glass bottles. Caging and bedding materials  
50 were not reported, but they were stated that to test negative in the E-SCREEN. From GD 9 (GD 1 = day of

### 3.0 Developmental Toxicity Data

1 vaginal sperm) through PND 1 [**The day of birth was PND 0 (A. Soto, personal communication, March**  
2 **2, 2007)**], rats received the 50% DMSO vehicle or bisphenol A [**purity not reported**] at 0.0025, 0.025,  
3 0.250, or 1 mg/kg bw/day. Dosing solutions were delivered by implanted [**assumed SC**] osmotic pumps.  
4 [**Number of dams treated was not reported. Based on a limited amount of information provided on**  
5 **the number of offspring examined, it appears that  $\leq 6$  dams/group were treated.**] Pup viability was  
6 assessed on PND 1. On PND 2 pups were sexed and litters were culled to 8 pups. Anogenital distance was  
7 measured on PND 4. Litters were weighed during the lactation period. Female offspring were monitored for  
8 body weight and vaginal opening in the post weaning period. Female offspring were killed on PND 50 or  
9 95. Mammary glands were collected and whole-mounted or sectioned for histopathological examination.  
10 Morphometric analyses were conducted to examine possible presence of preneoplastic lesions. Mammary  
11 glands were examined for ER $\alpha$  and Ki-67 protein by an immunohistochemistry technique. Maximal  
12 numbers of “maternal units” were represented in each dose group. One female/litter was included in  
13 histological examinations. [**Apparently  $\leq 6$  offspring/group were examined in histopathological**  
14 **examinations. Number of offspring examined for other endpoints was not reported in the**  
15 **manuscript. According to an author,  $n = 7-21$  for the other endpoints (A. Soto, personal**  
16 **communication, March 2, 2007).**] Statistical analyses included ANOVA followed by post hoc tests  
17 (Bonferroni or *t*-test) when significant effects were observed by ANOVA. [**It was not clear if dams or**  
18 **offspring were considered the statistical unit.**]

19  
20 Bisphenol A exposure did not affect offspring viability, sex ratio, age at vaginal opening, or female  
21 anogenital distance. Anogenital distance was reduced on PND 4 in males from the 0.250 mg/kg bw/day  
22 group. Percent hyperplastic ducts was increased in all dose groups on PND 50 and in the 0.0025 mg/kg  
23 bw/day group on PND 95; the study authors noted that the effect on PND 50 was quantitatively similar in  
24 all dose groups (i.e. 3–4-fold increase). Cribriform structures were observed in the 0.25 and 1 mg/kg  
25 bw/day groups. [**Incidence was not reported for the control and lower dose groups.**] The structures  
26 were classified as carcinomas-in-situ and were characterized by increased ductal size resulting from  
27 luminal epithelium proliferation, enlarged luminal epithelial cells, presence of a nucleolus, variable  
28 chromatin pattern, and rounded luminal spaces consisting of trabecular rods of cells perpendicularly  
29 aligned to the longer duct axis. Numbers of Ki-67- and ER- $\alpha$  positive cells were increased in aberrant  
30 compared to normal tissues, regardless of dose. [**Results in treated compared to control groups were not**  
31 **reported.**] The study authors concluded that fetal bisphenol A exposure in rats is sufficient to induce  
32 development of preneoplastic and neoplastic mammary lesions.

33  
34 **Strengths/Weaknesses:** Relevance of endpoints is a strength, as is the use of multiple dose levels.  
35 Weaknesses include an unstated number of dams (and by inference, a small number of these, and thus,  
36 because of dam-related effects, a small overall  $n$ ), the uncertainty of the response rate of histopathology in  
37 the controls, and the use of 50% DMSO as vehicle.

38  
39 **Utility/Adequacy for CERHR Evaluation:** This study was inadequate due to small sample size, route of  
40 administration, and lack of clarity on statistical analysis.

41  
42 **Durando et al. (333)**, supported by Universidad Nacional del Litoral, Argentine National Agency for the  
43 Promotion of Science and technology, and NIH, examined the effects of prenatal bisphenol A exposure on  
44 susceptibility to mammary tumors in rats. Wistar rats were fed Cooperación (Buenos Aires, Argentina) and  
45 housed in stainless steel cages. [**It was not clear if bedding was used.**] On GD 8–23 (GD 1 = day of  
46 vaginal sperm), 11–14 dams/group were sc dosed by osmotic pump with the DMSO vehicle or 0.025 mg/kg  
47 bw/day bisphenol A [**purity not indicated**]. Pups were delivered on GD 23 and weaned on PND 21. It was  
48 not indicated if day of birth was considered PND 0 or 1. During the study, body weights and day of vaginal  
49 opening were monitored. Offspring were killed before puberty (PND 30), after puberty (PND 50), or in  
50 adulthood (PND 110 and 180). In mammary gland stroma and epithelium, proliferation was assessed by  
51 BrdU incorporation and apoptotic cells were identified by TUNEL method. Morphometric analyses were

### 3.0 Developmental Toxicity Data

1 conducted in sectioned mammary glands. Mast cells were identified by immunostaining for proteinase. At  
2 least 6 offspring/group/time point were evaluated. **[No littermates were used in the evaluation at any**  
3 **given time point (A. Soto, personal communication, March 2, 2007).]** Additional offspring were  
4 examined for responsiveness to chemically-induced mammary preneoplastic or neoplastic lesions. On PND  
5 50, N-nitroso-N-methylurea was administered to 10–16 offspring from the vehicle control group at 25 or 50  
6 mg/kg bw and 21 offspring from the bisphenol A group at 25 mg/kg bw. Based on findings from a pilot  
7 study, 25 mg/kg bw was considered a subcarcinogenic dose and 50 mg/kg bw was considered a positive  
8 control. During the study, rats were palpated for tumors. Rats that received 50 mg/kg bw N-nitroso-N-  
9 methylurea were killed on PND 180 and rats that received 25 mg/kg bw N-nitroso-N-methylurea were  
10 killed on PND 110 or 180. Whole-mounted mammary glands were examined for tumors. Immunostaining  
11 was conducted to identify cytokeratin 8 (an epithelial marker) and p63 (a myoepithelial marker). Data were  
12 statistically analyzed using the Mann-Whitney *U* test.

13  
14 Bisphenol A exposure did not affect successful pregnancies, dam weight gain, pregnancy duration, number  
15 of pups/litter, or percent females/litter. Anogenital distance on PND 1 or 5 and postnatal body weights were  
16 unaffected in pups exposed to bisphenol A. Vaginal opening was accelerated in pups from the bisphenol A  
17 group (mean 34 days of age compared to 39 days of age in controls). On PND 50, the BrdU/apoptosis ratio  
18 was significantly increased and apoptosis was significantly decreased in mammary parenchyma and stroma  
19 of bisphenol A-exposed animals; the effects were not observed on PND 30 or 110. Significantly increased  
20 percentages of hyperplastic ducts, density of stromal nuclei, and numbers of mast cells were observed in  
21 the bisphenol A group on PND 110 and 180. Exposure to bisphenol A resulted in formation of a dense  
22 stroma layer around mammary epithelial structures and replacement of normal adipose tissue with a  
23 fibroblastic stroma. In rats exposed to 25 mg/kg bw N-nitroso-N-methylurea on PND 50, incidence of  
24 hyperplastic lesions on PND 180 was significantly higher in the group with prenatal bisphenol A compared  
25 to DMSO exposure (mean incidence of 35.5% compared to 15.7% in controls). Although statistical  
26 significance was not achieved, exposure to 25 mg/kg bw N-nitroso-N-methylurea resulted in tumors in 2 of  
27 15 rats in the prenatal bisphenol A group and 0 of 10 rats in the prenatal vehicle control group on PND 180.  
28 Cytokeratin 8 immunostaining revealed no invasion by stromal epithelial cells. The study authors  
29 concluded that rats prenatally exposed to environmentally relevant doses of bisphenol A may have an  
30 increased risk of developing mammary tumors.

31  
32 **Strengths/Weaknesses:** Weaknesses include route of administration and the high single dose is a weakness  
33 as is the use of pure DMSO.

34  
35 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for inclusion due to the use  
36 of 99.9% DMSO as a vehicle to administer bisphenol A via sc osmotic pump.

37  
38 **Hong et al. (334)**, sponsored by the Korea Research Foundation, investigated the effects of acute exposures  
39 to bisphenol A during late pregnancy on expression and protein level of calbindin-D<sub>9k</sub>, a putative biomarker  
40 of estrogen activity, in the uteri of offspring and lactating rats on PND 5. Pregnant Sprague Dawley rats  
41 were given free access to water and a diet of soy-free pellets in polycarbonate caging. **[Housing conditions**  
42 **(individual or group) and bedding material were not indicated].** On GD 17–19, pregnant rats were sc  
43 injected daily with 200, 400 or 600 mg/kg bw/day bisphenol A **[purity not provided]** in corn oil (n =  
44 5/group). Negative and positive control groups (n = 10/group) were administered corn oil or 17β-estradiol  
45 40 µg/kg bw/day. On PND 5, lactating dams and female pups were killed and their uteri harvested. Dose  
46 response changes in calbindin-D<sub>9k</sub> expression levels in uteri of lactating dams and female offspring  
47 (3/group) were analyzed by Northern blot and RT-PCR, with appropriate housekeeping gene controls.  
48 Protein levels and localization of calbindin-D<sub>9k</sub> were performed by Western blot and  
49 immunohistochemistry for lactating dams only. Statistical analyses were performed using the Kruskal-  
50 Wallis and Dunnett tests. **[It was not clear if dams or offspring were considered the statistical unit.]**

### 3.0 Developmental Toxicity Data

1 Northern blot analysis revealed a significant increase [**~6.4-fold**] in the level of calbindin-D<sub>9k</sub> expression in  
2 the uteri of lactating dams exposed to 600 mg/kg bw/day bisphenol A compared to oil controls. 17β-  
3 Estradiol treatment produced a significant [**~3.9-fold**] increase in calbindin-D<sub>9k</sub> mRNA expression in the  
4 dam uterus that was not statistically distinct from the effect of the high bisphenol A dose. Uteri of offspring  
5 exposed to the highest dose level of bisphenol A also showed a significant up-regulation [**~4.4-fold**] in  
6 calbindin-D<sub>9k</sub> expression. Expression levels of *ERα* were unaffected in maternal uteri exposed to bisphenol  
7 A. However, *ERα* expression was significantly increased in uteri of pups exposed to 400 and 600 mg/kg  
8 bw bisphenol A [**↑33% and 66%, estimated from a graph**]. Protein levels of calbindin-D<sub>9k</sub> in lactating  
9 dam uteri were significantly elevated at all dose points [**50, 40, and 50%, for 200, 400, and 600 mg/kg**  
10 **bw/day, respectively**]. 17β-Estradiol-treatment was not associated with a significant increase in calbindin-  
11 D<sub>9k</sub> protein. The density of calbindin-D<sub>9k</sub>-immunopositive cells was increased in uterine sections from  
12 lactating dams exposed to all doses of bisphenol A relative to oil controls, correlating with Western blot  
13 results. Authors note insufficient material or low detectability of calbindin-D<sub>9k</sub> protein in offspring tissue,  
14 and protein analyses were not performed.

15  
16 The authors suggest that calbindin-D<sub>9k</sub> can serve as a reliable biomarker of acute estrogenic exposure,  
17 particularly for insight into maternal-fetal metabolic exchange, given that calbindin-D<sub>9k</sub> is tightly regulated  
18 and rapidly induced by 17β-estradiol, diethylstilbestrol, alkylphenols, and now, bisphenol A. They further  
19 point out that calbindin-D<sub>9k</sub> expression is absent in immature rat and ovariectomized rat uteri.

20  
21 **Strengths/Weaknesses:** This study supports the use of calbindin-D<sub>9k</sub> as a uterine biomarker of estrogenic  
22 effect in the perinatal period in the rat, and provides some dose-response information for bisphenol A  
23 induction of an estrogenic response. Limitations are the subcutaneous route of exposure, small sample size,  
24 high doses and uncertain statistical analyses of the F1 data.

25  
26 **Utility (Adequacy) for CERHR Evaluation Process:** While providing some dose-response information  
27 regarding bisphenol A-induced estrogenic effects following exposure of rats in the perinatal period, the lack  
28 of clarity regarding whether the dam or offspring was considered the statistical unit, route of exposure, and  
29 use of high doses render this study inadequate for consideration in the evaluation process.

#### 3.2.3 Rat—oral exposure postnatally with or without prenatal exposure

##### 3.2.3.1 Reproductive studies

34 **The International Research and Development Corporation (335)**, sponsored by General Electric,  
35 examined the effects of bisphenol A exposure on CD rats and their offspring. Male and female F<sub>0</sub> rats were  
36 housed in wire mesh cages and fed Purina Laboratory Chow. Ten rats/sex/group (body weights of 110–170  
37 g for males and 100–151 g for females) were given feed containing bisphenol A [**purity not specified**] at 0,  
38 1000, 3000, or 9000 ppm for 17 weeks. [**It was not clear how long before mating that the dosing was**  
39 **started or if dosing was continued through the gestation and lactation periods.**] The European Union  
40 (2) estimated bisphenol A intake at 0, 70, 200, or 650 mg/kg bw/day in males and 0, 100, 300, or 950  
41 mg/kg bw/day in females. F<sub>0</sub> rats were mated at ~100 days of age and assessed for fertility. F<sub>1</sub> pups were  
42 counted and weighed at birth and on PND 21 (day of birth not defined). Fifteen male and female F<sub>1</sub>  
43 rats/group/sex that were exposed in utero were selected for a 13-week feeding study and were fed diets  
44 containing the same concentration of bisphenol A as their parents. F<sub>1</sub> rats were weighed and observed for  
45 clinical signs. Hematological, clinical chemistry, and urinalysis parameters were examined in 5  
46 rats/sex/group in the control and 2 highest dose groups at 1, 2, and 3 months of F<sub>1</sub> exposure.  
47 Ophthalmoscopic examinations were conducted at 3 months of F<sub>1</sub> exposure. After 13 weeks of dosing, the  
48 F<sub>1</sub> rats were killed and necropsied. Organs were weighed and fixed in 10% neutral buffered formalin.  
49 Included among organs weighed were testis and ovary. Histopathological examinations were conducted in  
50 tissues from 10 rats/sex/group in the control and high dose group. Included among organs histologically

### 3.0 Developmental Toxicity Data

1 examined were prostate, uterus, testis, and ovary. Statistical analyses included chi-squared test with Yates  
2 correction, Fisher exact probability test, Mann-Whitney *U*-test, ANOVA, *t*-test, and Dunnett multiple  
3 comparison test.

4  
5 Fertility was unaffected in F<sub>0</sub> rats. Body weight gain was lower in F<sub>0</sub> rats from the 3000 and 9000 ppm  
6 groups. Body weight at week 17 followed the same patterns as body weight gain [**6–7% decrease in the**  
7 **3000 ppm group and 12–18% decrease in the 9000 ppm group compared to controls**]. There were no  
8 differences in food intake. [**Statistical significance for body weight effects was not reported. It was not**  
9 **clear if statistical analyses were not conducted or if the effects did not attain statistical significance.**]

10  
11 There were no effects on number of F<sub>1</sub> pups/litter or survival of pups. Pup birth weights in the 9000 ppm  
12 group were slightly decreased but were said to be within normal range. Body weight gains on PND 21 were  
13 slightly decreased in pups from the 3000 and 9000 ppm dose groups. Body weights on PND 21 were  
14 significantly lower in pups from the 3000 and 9000 ppm groups [**7 and 12% lower compared to controls;**  
15 **benchmark dose analysis not conducted because variances not reported**]. One male F<sub>1</sub> rat in the control  
16 group and 2 female F<sub>1</sub> rats in each of the 3000 and 9000 ppm group died during the study. Post-weaning  
17 body weight gain was lower in females from all dose group and in males from the 3000 and 9000 ppm dose  
18 groups. Body weight at week 13 followed the same patterns as body weight gain [**13% decrease in the**  
19 **1000 ppm group, 11–17% in the 3000 ppm group, and 22–24% decrease in the 9000 ppm group**  
20 **compared to controls**]. Food intake was decreased in females from all dose groups and in males from the  
21 9000 ppm group. Examination by ophthalmoscopy revealed no treatment-related effects. No treatment  
22 related effects were observed for hematology, biochemistry, or urinalysis. No changes in organ weights or  
23 gross or histopathological lesions were considered treatment related. The study authors noted increases in  
24 mean weights of spleen, brain, thyroid, and adrenals in the treated groups but concluded that the effects  
25 resulted from decreased body weight. [**With the exception of PND 21 pup weights, there was no**  
26 **discussion of statistical significance for effects observed in F<sub>1</sub> rats. It was not clear if statistical**  
27 **analyses were not conducted or if statistical significance was not attained.**]

28  
29 **Strengths/Weaknesses:** This study is a conventional, state-of-the-art-at-the-time 2-generation toxicity  
30 study. The inclusion of a breeding period and a second generation are strengths. Weaknesses are magnified  
31 in hindsight: these include the limited number of animals examined, the lack of close examination of the  
32 reproductive processes in the F<sub>1</sub> animals, and uncertainty about the statistical significances. The study has  
33 not been peer-reviewed.

34  
35 **Utility (Adequacy) for CERHR Evaluation Process:** While this study was not designed to find non-  
36 linear dose-responses, it represents a conventional-for-the-time 2-generation toxicity study, and is adequate  
37 for the evaluation process but of limited utility because the high doses preclude evaluation of low dose  
38 effects and limit its utility in showing a lack of marked organ toxicity or gross reproductive toxicity in a  
39 limited number of animals at very high doses.

40  
41 **The International Research and Development Corporation (336)**, sponsored by General Electric,  
42 examined the effects of bisphenol A exposure on male and female CD rats and their offspring. In the first  
43 part of the experiment, male and female rats were housed in wire mesh cages and were fed Purina  
44 Laboratory Chow containing bisphenol A [**purity not specified**] for 18 weeks. Ten rats/group (body  
45 weights of 135–179 g for males and 114–158 g for females) were assigned to each treatment group based  
46 on even distribution of body weight and litter mates. [**Based on information provided in study tables, it**  
47 **appears that the rats were ~30 days old at the start of dosing.**] Bisphenol A was added to feed at  
48 concentrations of 0, 100, 250, 500, 750, or 1000 ppm. The European Union (2) estimated bisphenol A  
49 intake at 0, 5, 15, 30, 50, and 60 mg/kg bw/day in males and 0, 10, 25, 50, 75, and 100 mg/kg bw/day in  
50 females. Rats were examined for clinical signs, body weight gain, and food intake throughout the study.  
51 Estrous cyclicity was examined in females for 3 weeks prior to breeding and during breeding. At 100 days

### 3.0 Developmental Toxicity Data

1 of age (week 10 of the study), rats were moved to plastic cages with corncob bedding and mated for 3  
2 weeks. GD 0 was defined as the day that vaginal sperm or plug was observed. Rats were assessed for  
3 fertility and gestation length. Day of delivery was designated lactation day 0 (PND 0). Pups were counted,  
4 sexed, and weighed, assessed for viability at birth and through the lactation period. After weaning, 15 male  
5 and female F<sub>1</sub> rats/group that were exposed in utero were selected for a 90-day feeding study. Parental rats  
6 and unselected F<sub>1</sub> rats were killed and discarded.

7  
8 During a 90-day period, F<sub>1</sub> rats were fed diets containing the same concentration of bisphenol A as their  
9 parents. **[Ages at the start of dosing were not reported, but based on body weight ranges reported**  
10 **(64–138 g for males and 57–118 grams for females) it appears that rats were different ages at the**  
11 **start of dosing.]** F<sub>1</sub> rats were weighed and observed for clinical signs. Hematological, clinical chemistry,  
12 and urinalysis parameters were examined at day 30, 60, and 90 of the study. Ophthalmoscopic  
13 examinations were conducted prior to initiation of and following 90 days of dosing. The rats were killed  
14 and organs weighed. Adrenals, pituitary, ovaries, and thyroid were weighed following fixation in 10%  
15 neutral buffered formalin. Histopathological examinations were conducted in tissues from 10 rats/sex/group  
16 in the control and high dose groups. Organs histologically examined included prostate, uterus, testis, and  
17 ovary. Statistical analyses included chi-squared test with Yates correction, Fisher exact probability test,  
18 Mann-Whitney *U*-test, ANOVA, *t*-test, and Dunnett multiple comparison test.

19  
20 In parental rats, bisphenol A exposure did not affect general behavior, appearance, or survival. Mean body  
21 weight of males in the 1000 ppm group was 6% lower than control males. Food intake was increased [**by**  
22 **~7–11%, no dose-response]** in females of all dose groups. Bisphenol A exposure had no effect on estrous  
23 cyclicity or gestation length [**data were not shown**], male or female fertility, number of pups/litter, or pup  
24 survival. Body weights of pups in the 750 ppm group were significantly higher [**by ~10%**] compared to  
25 controls on PND 21, but the study authors did not consider the effect to be treatment related.

26  
27 In the F<sub>1</sub> offspring, a slight decrease in body weight gain was observed for males in the 750 ppm group. [**At**  
28 **the end of the study, body weights of males in the 750 ppm group were ~7% less than controls].** Food  
29 intake was similar in treated and control groups. Ophthalmoscope examinations did not reveal any  
30 treatment-related effects. Although mean blood urea nitrogen levels were slightly lower and mean serum  
31 glutamic-oxaloacetic transaminase values were sporadically increased in treated rats, the study authors  
32 noted that the values were within physiological ranges. There were no effects on hematological or  
33 urinalysis parameters. Some significant organ weight changes were noted by the study authors, but they  
34 stated that the biological significance of the effects was not known. [**There did not appear to be dose-**  
35 **response relationships for any organ weight change.]** The study authors stated that no compound-related  
36 lesions were observed in organs, including reproductive organs.

37  
38 **Strengths/Weaknesses:** The use of multiple dose levels (going down to fairly low exposure levels) is a  
39 plus, as is a breeding phase. Weaknesses include the limited number of animals per group, discarding of the  
40 parental animals without examination, the fact that not all F<sub>1</sub> animals were examined at least for structural  
41 effects, the lack of close examination of F<sub>1</sub> animals for reproductive effects (cyclicity and sperm measures),  
42 and the use of the conventional “top-down” pathology evaluation, wherein the lower dose groups were  
43 examined only if effects were noted in the high dose. The study has not been peer-reviewed.

44  
45 **Utility (Adequacy) for CERHR Evaluation Process:** For what it is, this study is adequate and of limited  
46 utility for the Evaluative Process, as showing no gross changes in the structure of a limited number of  
47 tissues in a limited number of F<sub>1</sub> animals, exposed from pre-conception. This study was not designed to  
48 find unusual effects or non-linear dose-response relationships or to address the issue of low-dose functional  
49 responses or non-linear responses.

50

### 3.0 Developmental Toxicity Data

1 **Ema et al. (337)**, supported by the Japanese Ministry of Health and Welfare, examined developmental  
2 toxicity endpoints, in a 2-generation rats study described in detail in Section 4.2.3.1. Two generations of  
3 rats were gavaged with 0, 0.0002, 0.002, 0.020, or 0.200 mg/kg bw/day bisphenol A (99.9% purity) prior to  
4 and during mating and throughout the gestation and lactation period. These doses were based on previous  
5 studies which found effects at 0.002 and 0.020 mg/kg bw/day. There were some non-dose-related and  
6 sporadic effects, but the study authors concluded that none of the effects were related to bisphenol A  
7 treatment. Bisphenol A exposure did not adversely affect prenatal or postnatal growth or survival,  
8 developmental landmarks, anogenital distance, or age of puberty. In adult animals exposed to bisphenol A  
9 during development, there was no evidence of adverse effects on reproductive endpoints such as fertility,  
10 estrous cyclicity, or sperm counts. Prostate and other male reproductive organ weights were unaffected.

11  
12 **Strengths/Weaknesses:** Strengths of this study were the thoroughness of the evaluation, the size of the  
13 dose range, the large number of animals, the litter-based analysis, and the verification of the dosing  
14 solution. A minor weakness is the lack of a positive control group, which leaves a question about the ability  
15 of this group of rats to respond.

16  
17 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
18 evaluation process.

19  
20 **Tyl et al. (338)**, supported by The Society of the Plastics Industry, Inc., reported some developmental  
21 toxicity effects in a multigeneration bisphenol A study in Sprague Dawley rats that is reported in detail in  
22 Section 4.2.3.1. In that study, F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> rats were exposed to bisphenol A [99.70%-99.76% pure]  
23 indirectly during gestation and lactation and directly through feed after weaning. Dietary doses were 0,  
24 0.015, 0.3, 4.5, 75, 750, or 7500 ppm, and target intakes were ~0.001, 0.02, 0.30, 5, 50, and 500 mg/kg  
25 bw/day. At the 7500 ppm dose there were fewer pups and live pups/litter and body weight gain of pups was  
26 lower during the lactation period. Delayed puberty in both males and females of the 7500 ppm group was  
27 most likely related to reduced body weights according to the study authors. Bisphenol A exposure during  
28 development did not increase the weight of the prostate in adult rats. Although some decreases in  
29 epididymal sperm concentration and daily sperm endpoints were each observed in 1 generation of males  
30 from the high-dose group, the study authors concluded there were no treatment-related effects on sperm  
31 endpoints or reproductive function. The study authors identified an offspring and reproductive NOAEL of  
32 750 ppm (~50 mg/kg bw/day). A systemic NOAEL for adult rats was identified at 75 ppm (~5 mg/kg  
33 bw/day) by the study authors; therefore, bisphenol A was not considered a selective developmental  
34 toxicant.

35  
36 **Strengths/Weaknesses:** This study has numerous strengths, including the quality and number of the  
37 endpoints evaluated, the number of dose groups and generations examined, and the confirmation of dosing  
38 solutions. This study incorporated screening-level endpoints within the context of a multigeneration study.  
39 As such, it addresses gross issues but does provide helpful data regarding the NOAEL.

40  
41 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
42 evaluation process.

#### 43 44 *3.2.3.2 Development of the reproductive or endocrine systems*

45 **Cagen et al. (339)**, support not indicated (but all authors affiliated with industry), conducted a study to  
46 examine the effects of prenatal and lactational bisphenol A exposure on reproductive development of rats.  
47 The study attempted to replicate findings by Sharpe et al. that appeared in an unpublished meeting abstract.  
48 The protocol used by Cagen et al. was the same as that used by Sharpe et al., with the exception that more  
49 dose levels were included, group sizes were larger, and a greater number of reproductive endpoints were  
50 examined. Animals were fed Certified Rodent Chow #5002. Music was played at a low volume to provide  
51 background noise. Female Han-Wistar rats were randomly assigned to groups. For 2 weeks prior to mating,

### 3.0 Developmental Toxicity Data

1 during a 2-week mating period, and during the gestation and lactation periods, 28 rats/group were given  
2 drinking water containing bisphenol A (>99% purity) at 0.01, 0.1, 1.0, or 10 ppm (0.001–0.004, 0.008–  
3 0.038, 0.100–0.391, or 0.775–4.022 mg/kg bw/day). Two negative control groups of 28 rats each were  
4 given undosed drinking water. Because the two control groups were determined to be statistically  
5 equivalent, data from the two groups were pooled. A positive control group of 28 rats was given drinking  
6 water with diethylstilbestrol at 0.1 ppm (0.006–0.036 mg/kg bw/day). Dosing solutions were prepared  
7 weekly, and concentrations were verified. Dams were evaluated for food and water intake, weight gain, and  
8 fertility endpoints. Pups were sexed, weighed, and counted at birth. During the postnatal period, pups were  
9 evaluated for growth and survival. On PND 4, litters were culled to 8 pups with as many male pups retained  
10 as possible. At weaning on PND 22, up to 4 males/litter (86–109 pups/group) were randomly selected to  
11 continue in the study until 90 days of age and were individually housed. At necropsy, brain, liver, kidneys,  
12 and reproductive organs were weighed, daily sperm production was determined, and testes were examined  
13 histologically. Technicians were blinded to treatment group. The litter was considered the experimental unit  
14 in statistical analyses. Data were analyzed by Levene test, ANOVA, Dunnett test, rank transformation, and  
15 Wilcoxon rank sum test with Bonferroni correction.

16  
17 In the bisphenol A groups, there were no significant effects on dam body weight gain or food or water  
18 intake. **[Food and water intake data were not shown.]** There were also no effects on dam fertility,  
19 mating, gestation index and duration, live litter size, or pup survival and body weight gain during the  
20 postnatal period. Male sex ratio was increased in the 0.1 ppm bisphenol A group (56.7% males versus  
21 48.4% in control), but the study authors did not consider the effect to be treatment-related due to the lack of  
22 a dose response relationship. Dams in the diethylstilbestrol group experienced decreased body weight gain  
23 and food intake, increased duration of gestation, smaller litter size at birth, and decreased pup survival in  
24 the postnatal period.

25  
26 In adult offspring from the bisphenol A groups, there were no significant effects on terminal body weight  
27 or organ weights including prostate, epididymis, preputial gland, seminal vesicle, or testis. There were also  
28 no significant effects on epididymal sperm concentration, efficiency of sperm production, or daily sperm  
29 production. No histopathological alterations were observed in the testis. Reproductive development in male  
30 offspring was also unaffected by prenatal exposure to diethylstilbestrol. The study authors noted that the  
31 reduced testis weight and sperm production reported by Sharpe et al. was not confirmed in this study and  
32 that bisphenol A should not be considered a selective reproductive or developmental toxicant.

33  
34 **[The NTP Statistics Subpanel (340) concluded that the statistical methods used by Cagen et al. were**  
35 **appropriate. Although the Subpanel agreed with the study author conclusions, 2 matters were noted.**  
36 **The first was that a significant ANOVA is not a requirement for Dunnett test. The second was that a**  
37 **Bonferroni correction of Wilcoxon-rank sum test was not needed because the authors already**  
38 **required significance by ANOVA, which was sufficient.]**

39  
40 **Strengths/Weaknesses:** Significant strengths of this study include the large number of dose levels and  
41 animals per dose level and the technical care with which the study was performed, as well as the inclusion  
42 of a positive control group and two negative controls. The lack of much effect with diethylstilbestrol  
43 treatment is a weakness.

44  
45 **Utility (Adequacy) for CERHR Evaluation Process:** Although only weak effects were observed for the  
46 diethylstilbestrol positive control the panel considered this study adequate and of high utility.

47  
48 **Elswick et al. (341)**, from the Chemical Industry Institute of Toxicology [CIIT], examined the effects of  
49 sampling design on conclusions made about bisphenol A effects on prostate weight. Two of the 3 studies  
50 discussed in the paper relate to bisphenol A research in Sprague Dawley rats performed at CIIT between  
51 1997 and 1999. One paper is Kwon et al. (342) which is discussed in detail in Section 3.2.3.3. The other

### 3.0 Developmental Toxicity Data

1 paper was unreferenced at the time and remains so. This section discussed the analysis of the unpublished  
2 study. In that study, the litter was considered the experimental unit in statistical analyses. Organ weights  
3 were analyzed using a nested ANOVA with litter within dose as the random effect. Post hoc tests were  
4 conducted when appropriate.

5  
6 Dams were given drinking water containing 0, 0.005, 0.05, 0.5, 5, or 50 mg/L bisphenol A [**purity not**  
7 **indicated**] from GD 2 to PND 21. The study authors estimated bisphenol A intakes at ~0.001–10 mg/kg  
8 bw/day. The lowest doses were reported to be similar to human exposure levels. The study was conducted  
9 in 2 blocks separated by 4 months. A total of 16 dams/group were exposed, and the overall sample size was  
10 ultimately 13–16/group. In the first block, 2 males/litter were most often retained and in the second block, 1  
11 male/litter was retained until 6 months of age. Fresh ventral prostate weights were recorded. Analysis of  
12 data from the first study block revealed no treatment-related effects on ventral prostate weight. Within  
13 litters, ventral prostate weights were observed to be very variable, with weights sometimes differing by  
14 values of 2-fold or more. In the second study block, mean weights in the 0.05, 5, and 50 mg/kg bw/day  
15 groups were significantly higher than those of the control group. It was noted that mean prostate weight in  
16 the control group from block 2 (0.387 g) was much lower than the mean weight observed in block 1 (0.517  
17 g) and that the standard error in block 2 (0.174 g) was almost two times higher than the standard error in  
18 block 1 (0.092 g). When data from the 2 blocks were combined, statistical significance remained. The study  
19 authors noted that no historical control database was available at CIIT at the time of the analysis.

20  
21 **[The NTP Statistics Subpanel (340) reanalyzed these data agreed with its results and conclusions**  
22 **showed a consistent increase in ventral prostate weight in the 2 replicates. Note that the NTP**  
23 **Statistics Subpanel rejected the conclusions in Elswick et al. that use of multiple pups per litter can**  
24 **decrease false positive rates in these studies.]**

25  
26 **Strengths/Weaknesses:** This paper demonstrated an increase in ventral prostate weight. These data argue  
27 for multiple pup/litter sampling, a characteristics that has been uncommon in this literature. The fact that  
28 significant effects were noted in only in 1 block raise the question of a lack of experience or training among  
29 the technicians. The study referred to in Elswick et al. is unpublished and not peer-reviewed.

30  
31 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate because it is primarily a  
32 discussion of previously published results and the new data presented have inconsistencies in block  
33 replicates.

34  
35 **Rubin et al. (241)**, supported by the Tufts Institute of the Environment and NIH, examined the effects of  
36 perinatal bisphenol A exposure on estrous cyclicity and LH levels in rats. Uterotropic responses were  
37 examined in a second group of rats, and those results are listed in [Table 53](#). Sprague Dawley rats were fed  
38 Purina Rodent Chow and provided drinking water in glass bottles. The rats were housed in plastic cages;  
39 estrogenicity testing of ethanol extracts indicated that estrogenic compounds did not leach from cages at  
40 detectable levels. [**No information was provided about bedding.**] Dams were weighed and randomly  
41 assigned to treatment groups of 6 animals given drinking water containing bisphenol A [**purity not**  
42 **reported**] at 0 (1% ethanol vehicle), 1, or 10 mg/L from GD 6 (plug day not indicated) through the  
43 lactation period. Mean bisphenol A doses were estimated by study authors at 0.1 and 1.2 mg/kg bw/day. At  
44 weaning, pups were given untreated water. Dams were examined and weighed during the studies. Offspring  
45 were sexed on PND 2 and weighed beginning in the postnatal period and continuing through adulthood (n =  
46 40–53/group during the neonatal period and 19–27/sex/group during adulthood). Anogenital distance was  
47 examined during the neonatal period. [**It was not clear how many time points and animals were**  
48 **examined. According to 1 study author, anogenital distance was measured on PND 2 (A. Soto,**  
49 **personal communication, March 2, 2007).**] Genital tracts were examined for gross abnormalities in males  
50 killed during the neonatal period, at 3 months, and at 5 months of age and in females killed during the  
51 neonatal period, at 8 months, and at 12–16 months of age. [**The total number of animals examined at**

### 3.0 Developmental Toxicity Data

1 **each time period was reported as 12–34, but it is not known how many/dose group were examined.]**

2 Animals were selected from as many different litters as possible at each time point. Day of vaginal opening  
3 was monitored. Estrous cyclicity was evaluated daily for 18 days at 4 and 6 months of age in 18–28  
4 rats/group. Eight female offspring/group were killed 3 months later following ovariectomy to measure  
5 serum LH levels using an LH assay kit; a total of 6–8 values/group were obtained. Body and uterine  
6 weights and LH levels were analyzed by ANOVA followed by *t*-test, Tukey test, or least significant  
7 difference test. Mammary tumors were analyzed by chi-squared test, and estrous cyclicity data were  
8 analyzed by Kruskal-Wallis test and Mann-Whitney *U* test. **[It appears that offspring were considered**  
9 **the statistical unit.]**

10  
11 On PND 4, 7, and 11, body weights were significantly higher in pups from the bisphenol A groups than in  
12 the control group; body weights were higher in animals of the low compared to the high dose group. Body  
13 weights of low-dose females were higher than body weights of control and high-dose females at PND 28  
14 and beyond. While the percentage of control females with regular estrous cycles was 83% at 4 months of  
15 age and 60% at 6 months of age, the values were significantly reduced in the high dose group to 21% at 4  
16 months of age and 23% at 6 months of age. There were no clear patterns of estrous cycle changes. Periods  
17 of diestrus were extended in some animals and other animals had extended periods of proestrus and/or  
18 estrus. The mean number of 4–5-day estrous cycles was significantly reduced in rats of the high-dose group  
19 at 6 months of age. Serum LH levels in the high-dose group were significantly reduced by ~19% compared  
20 to the control group [**BMD<sub>10</sub> = 0.94, BMDL<sub>10</sub> = 0.48, BMD<sub>1SD</sub> = 1.6, and BMDL<sub>1SD</sub> = 0.78 mg/kg**  
21 **bw/day**]. The treatment group incidences of females with mammary tumors (10% in controls, 20% in the  
22 low-dose group, and 28% in the high-dose group) were not statistically different. The study authors noted  
23 that the study was not designed to detect mammary tumors and that the tumors were detected during routine  
24 handling. No effects were reported for mean number of pups/litter, sex ratio, day of vaginal opening, or  
25 anogenital distance in the neonatal period. **[Data were not shown for anogenital distance.]** In comparing  
26 the effects on estrous cycles and LH levels in animals exposed in the perinatal period to the lack of  
27 uterotrophic effects in animals exposed in the post-pubertal period, the study authors concluded that there  
28 was evidence of increased sensitivity to bisphenol A during the perinatal period.

29  
30 **Strengths/Weaknesses:** This study incorporates a range of basic developmental and gross functional  
31 reproductive endpoints, but the sample sizes are small (6 dams/group) and the statistical approach does not  
32 appear to use litter as the unit. Actual exposures are poorly defined, particularly postnatally. The  
33 plausibility of the estrous cycle changes is a strength.

34  
35 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation process,  
36 based on a lack of adequate control for litter effects

37  
38 **Takashima et al. (343)**, supported by a Grant-in-Aid for Health Sciences Research [**sponsor not**  
39 **indicated**], examined the effect of bisphenol A exposure during development on carcinogenicity induced  
40 by N-nitrosobis (2-hydroxypropyl)amine. **[No information was provided about caging and bedding**  
41 **materials used in this study.]** Female Wistar rats were fed either MF diet or soybean-devoid powder diet  
42 (Oriental Yeast Co.). In each dietary group, 10–11 rats/group received bisphenol A [**purity not indicated**]  
43 at 0 or 1.0% diet. Bisphenol A exposure commenced 10 weeks prior to mating and was continued through  
44 the mating, gestation, and lactation periods. Total intakes of bisphenol A were reported at 21–22 g/rat.  
45 **[Assuming an exposure period of ~16 weeks, mean bisphenol A intake over the course of the study**  
46 **was estimated at ~200 mg/day. Based on reported body weights, bisphenol A intake was ~1600 mg/kg**  
47 **bw/day during the prebreeding stage and 1000 mg/kg bw/day during gestation and at weaning.]** The  
48 rats were mated to males fed CE-2 basal pellet diet (Clea, Inc.), and GD 0 was defined as the day of the  
49 vaginal plug. Endpoints associated with pregnancy, delivery, and nursing were evaluated. Dam body  
50 weight and food intake were measured. Offspring were not culled and were weaned at 3 weeks of age.  
51 Dams were killed following weaning of offspring. Serum levels of thyroid hormones were measured in 2–4

### 3.0 Developmental Toxicity Data

1 dams/group. Implantation sites were evaluated. Weights of several organs, including ovary, were measured.  
2 The organs were fixed in 10% buffered formalin and processed for histopathological evaluation. Offspring  
3 (n = 32–50/group) were evaluated for body weight gain, preputial separation, and vaginal opening.  
4 Beginning at 5 weeks of age and continuing for 12 weeks, offspring in each group were subdivided into 2  
5 groups (n = 17–21/group/sex) that received either undosed tap water or tap water containing 2000 ppm N-  
6 nitrosobis (2-hydroxypropyl)amine. Offspring were killed at 25 weeks of age. Serum thyroid hormone  
7 levels were measured. Organs, including testis, ovary, and uterus were weighed. In 5–19  
8 offspring/sex/group, histopathological examinations were conducted in organs targeted by N-nitrosobis (2-  
9 hydroxypropyl)amine (lungs, thyroid, esophagus, liver, and thymus). Data were analyzed by Dunnett and  
10 chi-squared tests. **[Data for pre-and postnatal survival were presented and apparently analyzed on a  
11 litter basis. The offspring were apparently used as the statistical unit in body weight analyses. It was  
12 not clear if the dam or offspring were considered the statistical unit in other analyses.]**  
13

14 Dam body weight was lower in the 1.0% bisphenol A group fed MF diet compared to the MF diet control  
15 during the gestation period and at weaning. Food intake and maternal serum levels of triiodothyronine,  
16 thyroxine, and thyroid-stimulating hormone were unaffected by bisphenol A exposure. Changes in weights  
17 or histopathological alterations of maternal organs, including uterus and ovary, were not observed in the  
18 bisphenol A groups. **[Data were not shown by the study authors.]** Bisphenol A had no significant effect  
19 on mating, fertility, duration of gestation, live-born pups, implantation loss, or offspring viability through  
20 PND 21. In pups from dams exposed to 1.0% bisphenol A fed MF diet compared to pups from MF  
21 controls, body weights were higher **[by 11%]** in females at 3 days of age and lower in males and females at  
22 10 days and 2 weeks of age **[16–22% decreases in males and 12–19% decreases in females]**. In pups  
23 from dams exposed to 1.0% bisphenol A and fed soybean-free diet compared to pups from the soybean-free  
24 controls, body weights of pups were increased in males at 3 weeks of age **[13% increase]** and in females at  
25 10 days and 3 weeks of age **[13–19% increase]**. Prenatal exposure to bisphenol A did not affect preputial  
26 separation or vaginal opening. In 25-week-old rats that were not exposed to N-nitrosobis (2-  
27 hydroxypropyl)amine, prenatal bisphenol A exposure was associated with some thyroid-stimulating  
28 hormone elevations in males and females from the MF and soybean-free diet groups. According to a  
29 statement in the study abstract, the study authors did not consider the effect on thyroid-stimulating hormone  
30 to be related to bisphenol A exposure. There were no effects of N-nitrosobis (2-hydroxypropyl)amine  
31 exposure on serum thyroid-stimulating hormone, triiodothyronine, or thyroxin levels or on thyroid  
32 histopathology. No effects were observed on offspring organ weights. **[With the exception of uterus and  
33 ovary, no organ weight data were shown.]** Prenatal bisphenol A exposure was not associated with  
34 significant differences in the development of N-nitrosobis (2-hydroxypropyl)amine-induced neoplasms in  
35 the offspring. The study authors concluded that bisphenol A exposure did not induce tissue injury in rat  
36 dams or their offspring or affect the development of tumors in offspring exposed to N-nitrosobis (2-  
37 hydroxypropyl)amine.  
38

39 **Strengths/Weaknesses:** Weaknesses include high doses and inadequate sample sizes. This study seems to  
40 discount the importance of certain effects on body weight and thyroid-stimulating hormone levels that  
41 might have received more attention in a study with a non-tumor focus. Sample size is inadequate to address  
42 neoplasm endpoints. Information is insufficient to judge the appropriateness of the statistical analyses and  
43 hence the reliability of findings.  
44

45 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation process  
46 due to small sample size, high dose levels, and inappropriate statistics.  
47

48 **Kobayashi et al. (344)**, supported by the Japanese Ministry of the Environment, examined the effect of  
49 prenatal and lactational bisphenol A exposure on somatic growth and anogenital distance in Sprague  
50 Dawley rats. The same rats were used to measure plasma hormone levels and testicular testosterone content  
51 in a study by Watanabe et al. (345) and apparently thyroid function in a study by Koybayashi et al. (346).

### 3.0 Developmental Toxicity Data

1 Rats were fed standard laboratory feed (CE-2, CLEA Japan, Inc.). **[No information was provided about**  
2 **caging or bedding materials.]** Rats were randomly assigned to groups and 6 rats/group were gavaged with  
3 bisphenol A (99.8% purity) at 0 (corn oil vehicle), 4, 40, or 400 mg/kg bw/day from GD 6 through PND 20.  
4 GD 0 was defined as the day a vaginal plug was observed, but the day of birth was not defined. Doses were  
5 based on the study by Kwon et al. (342) **[discussed in Section 3.2.3.3]**. On PND 7, litters were culled to 10  
6 pups, with equal numbers of males and females when possible. Offspring were weaned on PND 21. Dams  
7 were weighed during the study. Body weight and anogenital distance were measured in offspring at 1, 3,  
8 and 9 weeks of age. Plasma and testicular testosterone levels were measured at 9 and 36 weeks of age, and  
9 plasma LH and FSH concentration were measured at 9 weeks of age. Weights of liver, kidney, and testis  
10 were examined in offspring at 3 and 9 weeks of age. One to 10 (most often 6–10) offspring/group/sex were  
11 examined for body weight and anogenital distance at 1 week of age and 4–6/sex/group at 3 and 9 weeks of  
12 age. A pair of male and female offspring/litter **[assuming authors meant 1/sex/litter]** was examined for  
13 organ weights, and 4–6 males/group were used in hormone analyses at 3 and 9 weeks of age. Statistical  
14 analyses included ANOVA followed by Dunnett test. **[It was not clear if the dam or litter was**  
15 **considered the statistical unit.]**

16  
17 In the 40 mg/kg bw/day group, all pups from 1 dam were found dead on PND 2. Four of 6 dams of the 400  
18 mg/kg bw/day group died on GD 21, and all pups born to 1 dam in that group died by PND 2. Maternal  
19 body weight gain during pregnancy was reduced in the 400 mg/kg bw/day group. A transient decrease in  
20 body weight gain was observed early in the lactation period in dams of the 40 mg/kg bw/day group. In  
21 offspring from the 4 and 40 mg/kg bw/day group, no statistically significant effects were observed for body  
22 or organ weights, anogenital distance, anogenital distance/g body weight, or anogenital distance/body  
23 weight cubed at any time point in the study. At 9 weeks of age, plasma testosterone levels were  
24 significantly increased by 88% in the 4 mg/kg bw/day group and by 123% in the 40 mg/kg bw/day group.  
25 **[Benchmark dose was not calculated because the SD was provided only graphically.]** The study  
26 authors stated that there was a tendency for plasma testosterone to increase with dose at 36 weeks, but  
27 neither of the values were significantly increased compared to control. Testis testosterone was not  
28 statistically different from control at either dose at 9 or 36 weeks of age. There were no significant effects  
29 on plasma LH and FSH levels at 9 weeks of age. Plasma levels of 17 $\beta$ -estradiol were also unaffected by  
30 bisphenol A exposure. **[Data were not shown.]** The study authors concluded that gestational and  
31 lactational exposure to bisphenol A did not affect somatic growth or anogenital distance but did have a  
32 significant effect on testosterone homeostasis in rat offspring.

33  
34 **Strengths/Weaknesses:** The study appears better able to address maternal toxicity than offspring  
35 outcomes, for which it appears to be best considered a screening study. Sample sizes are too small to  
36 reliably judge postnatal endpoints.

37  
38 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation of  
39 bisphenol A effects on postnatal outcome.

40  
41 **Yoshino et al. (347)**, supported by the Japanese Ministry of Health, Labor, and Welfare, examined the  
42 effects of prenatal and lactational bisphenol A exposure in the prostate and testis of rats. In this study,  
43 pregnant and lactating dams were fed NMF feed and offspring were fed MF feed (Oriental Yeast Co.,  
44 Tokyo). The animals were housed in an unspecified type of cage containing wood chip bedding. F344 rat  
45 dams (n = 19–22/group) were gavaged with bisphenol A (99.9% purity) at 0 (0.5% sodium  
46 carboxymethylcellulose vehicle), 7.5, or 120 mg/kg bw/day during mating, gestation, and lactation periods.  
47 Doses were based on the result of an NTP study (157). Clinical signs, food intake, and body weight were  
48 monitored in dams during the study. After birth, pups were counted and weighed. Pups were randomly  
49 culled to 8/litter on PND 4 (day of birth not defined). On PND 21, weaning occurred and female pups were  
50 killed and discarded. Dams were killed at weaning for examination of implantation sites. Male pups were  
51 weighed during the post-weaning period. On PND 23, 28, and 91, five male offspring/group were killed.

### 3.0 Developmental Toxicity Data

1 Ventral prostate weights were measured during each evaluation period, and anterior and dorsolateral  
2 prostate, testis, and epididymis weight were also measured on PND 91. Reproductive organs were  
3 preserved in 10% buffered formalin and examined histologically. Sperm count, motility, and morphology  
4 were examined on PND 91. The study was repeated with evaluation of 10 male offspring/group. **[The  
5 number of dams treated/group in the repeat study was not reported. Based on body weights reported  
6 for rats in experiment 2, it appears they were evaluated at adulthood, but it was not specified if they  
7 were evaluated on PND 91.]** Data were analyzed by Student *t*-test. **[It appears that offspring were  
8 considered the statistical unit.]**  
9

10 In the first experiment, bisphenol A exposure had no effect on dam body weights during gestation or  
11 lactation, duration of the gestation period, or number of implantation sites. There were no effects on litter  
12 size, pup viability, or sex ratio. On PND 21, relative dorsolateral prostate weight was significantly higher  
13 **[by 23%]** in the low-dose group than in controls. **[It was not stated if organ weights were relative to  
14 body weight.]** There were no effects on final body weight or weights of anterior and ventral prostate, testis,  
15 or epididymis. There were no increases in malformations of reproductive organs. **[Data were not shown  
16 by study authors.]** Testicular sperm counts were significantly lower **[by 22%]** in males of the high-dose  
17 group, but there were no effects on epididymal sperm counts. There were also no effects on sperm motility  
18 or abnormalities. **[Data were not shown by authors.]** In the second experiment examining 10  
19 males/group, exposure to bisphenol A had no effects on final body weights or relative weights of testis,  
20 epididymis, or ventral, anterior, or dorsolateral prostate. There were no adverse effects on testicular or  
21 epididymal sperm count, motility, or morphology. Morphologically abnormal sperm were reduced in rats of  
22 the low-dose group. Study authors concluded that under the conditions of their study, exposure of dams to  
23 bisphenol A during the gestation and lactation periods did not result in adverse effect on the reproductive  
24 system of male offspring.  
25

26 **Strengths/Weaknesses:** The number of dams used in Experiment 1 appears adequate and 10 males/group  
27 were used to examine various organ endpoints at multiple time points. It is unfortunate that these data were  
28 then analyzed by many *t*-tests rather than multivariate analyses.  
29

30 **Utility (Adequacy) for CERHR Evaluation Process:** This study is considered inadequate due to  
31 statistical insufficiencies.  
32

33 **Ichihara et al. (348)**, supported by the Japanese Ministry of Health, Labor, and Welfare, examined the  
34 effects of prenatal and lactational exposure to bisphenol A on the development of prostate cancer in rats.  
35 F344 rat dams were fed NMF feed during pregnancy and lactation and their offspring were fed MF  
36 (Oriental Yeast Co.) following weaning. Rats were housed in cages containing wood chip bedding. **[No  
37 information was provided about caging materials.]** During pregnancy and lactation, ~8–15 dams/group  
38 were gavaged with bisphenol A (99.9% purity) at 0 (0.5% carboxymethyl cellulose sodium salt vehicle),  
39 0.05, 7.5, 30, or 120 mg/kg bw/day. Doses were based on findings from an NTP study **[citation not  
40 provided]**. Dam body weight and food intake were monitored during the study. Gestation period duration  
41 and implantation sites were evaluated. Pups were counted and sexed at birth. Litters were randomly culled  
42 to 8 pups on PND 4, and pups were weaned on PND 21 **[day of birth not defined]**. At 5 weeks of age, 21  
43 male rats/group were injected sc with 50 mg/kg bw 3,2-dimethyl-4-aminobiphenyl 10 times at 2-week  
44 intervals. An additional 12 rats/group in the 0, 0.05, 7.5, and 120 mg/kg bw/day bisphenol A groups were  
45 injected with corn oil during the same time period. Surviving male offspring were killed and necropsied at  
46 65 weeks of age. Blood was collected for analysis of serum testosterone levels in 5 rats/group.  
47 Reproductive organs were examined for gross abnormalities, weighed, and fixed in 10% buffered formalin.  
48 A histopathological examination of the prostate was conducted. Body and organ weight data were analyzed  
49 by Student *t*-test. The incidence of histopathological lesions was evaluated by Fisher exact probability test.  
50 **[It appears that the litter was considered the statistical unit in analyses for numbers and survival of  
51 pups at birth. Offspring were apparently considered the statistical unit for other analyses.]**

### 3.0 Developmental Toxicity Data

1 Body weights of dams in the 120 mg/kg bw/day group were significantly lower than control values from  
2 GD 14 to 20. There were no consistent or dose-related effects on dam body weights during lactation,  
3 although a significant increase in body weight was observed in dams of the 0.05 mg/kg bw/day group on  
4 PND 14. Exposure to bisphenol A had no effect on gestation period duration or number of implantation  
5 sites. In pups exposed to bisphenol A, there were no differences in number of live births, sex ratio, external  
6 anomalies, or body weights during the lactation period. **[Data for pup body weights were not shown by  
7 study authors.]** Terminal body weight of pups exposed to 0.05 mg/kg bw/day bisphenol A prior to  
8 treatment with 3,2-dimethyl-4-aminobiphenyl were significantly higher than controls **[by 12%]**. Exposure  
9 to bisphenol A had no effect on weights of prostate, testis, or epididymis. Incidences of prostatic  
10 intraepithelial neoplasia, carcinoma, and atypical hyperplasia were not increased by bisphenol A treatment,  
11 and there were no increases in tumors found in non-reproductive organs. No effect was observed on serum  
12 testosterone levels. The study authors concluded that exposure of rat dams to bisphenol A during the  
13 gestation and lactation periods does not predispose their offspring to prostate cancer development.

14  
15 **Strengths/Weaknesses:** Strengths are the range of low dose levels, the use of an additional strain (Fischer  
16 344 rat), and the endpoints evaluated. The design is reasonable for some of the endpoints measured, but  
17 sample sizes are inadequate for the prostate cancer endpoint and hormonal endpoints in particular.  
18 Statistical accounting for litter effects is unclear for neonatal measures, body weight, and fertility  
19 endpoints.

20  
21 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate based on insufficient  
22 sample size given the endpoints (i.e., tumors response) and lack of consistently accounting for litter effects.

23  
24 **Yoshida et al. (I30)**, supported by the Japanese Ministry of Health, Labor, and Welfare, examined the  
25 effects of bisphenol A exposure on development of the rat female reproductive tract. Donryu rats (12–  
26 19/group) were gavaged with bisphenol A **[purity not reported]** at 0 (carboxymethylcellulose solution),  
27 0.006, or 6 mg/kg bw/day from GD 2 to the day before weaning of pups at 21 days post delivery. The low  
28 dose was said to represent average daily intake from canned foods and the high dose was reported to  
29 represent the maximum dose level detected in plastic plates for children. **[It is assumed the authors meant  
30 estimated exposure levels for children eating off plastic plates.]** Bisphenol A levels were measured in  
31 maternal and pup tissues, and those values are reported in Section 2.1.2.2.1. After delivery, dams and litters  
32 were housed in plastic cages with wood chip bedding. Tap water was stored in plastic containers. The only  
33 information provided about feed was that it was a commercial pellet diet. Samples of tap water, drinking  
34 water from plastic containers, and feed were measured for bisphenol A content by HPLC. Offspring were  
35 sexed, weighed, and examined for external abnormalities on PND 1 and then weighed weekly through PND  
36 21. Litters were adjusted to 8–10 pups at PND 4 or 6 (day of birth = PND 0). Dams were weighed, and  
37 observed during the study and killed following weaning of litters on PND 21. Implantation sites were  
38 examined and organs including uterus, vagina, and ovaries were fixed in 10% neutral buffered formalin and  
39 examined histologically. **[It does not appear that results of histopathological testing in dams were  
40 reported.]** All female offspring were examined for vaginal opening, and following vaginal opening,  
41 vaginal smears were taken for the remainder of the study. Three to 5 offspring/group from different litters  
42 were killed on PND 10, 14, 21, or 28 and at 8 weeks of age. At most time periods, uteri were weighed,  
43 preserved in 10% neutral buffered formalin, and examined histopathologically to determine development of  
44 uterine glands. Ovaries and vagina were also examined histologically. ER $\alpha$  was determined using an  
45 immunohistochemical method. Serum was collected for measurement of FSH and LH by RIA. Four  
46 offspring/group from different litters were killed at 8 weeks of age on the morning of estrus to examine  
47 ovulation by counting ova in oviducts. Initiation of carcinogenesis following injection of the uterine horn  
48 with N-ethyl-N'-nitro-nitrosoguanidine was examined at 11 weeks of age in 35 or 36 animals/group. At  
49 ~24 weeks following cancer initiation, the 24–30 surviving animals/group were killed and uteri were  
50 examined histologically to determine the presence of tumors and other lesions. Statistical analyses included  
51 ANOVA and Dunnett test. **[Most of the data for endpoints evaluated at birth appeared to be presented**

### 3.0 Developmental Toxicity Data

1 **and apparently analyzed on a litter basis. For other data, it appears that offspring were considered**  
2 **the statistical unit.]**

3  
4 Bisphenol A was not detected in fresh tap water but was detected at ~3 ng/mL following storage in plastic  
5 containers. The bisphenol A concentration in feed was ~40 ng/g. In dams exposed to bisphenol A, there  
6 were no clinical signs of toxicity or effects on body weight, implantation sites, or gestation length.  
7 Bisphenol A exposure had no effect on litter size, pup body weight at birth and through PND 21, external  
8 abnormalities in pups or age of vaginal opening. In uteri of bisphenol A-exposed offspring, there were no  
9 effects on weight, gland development, ER $\alpha$ , or cell proliferation. No increase in lesions was reported in  
10 organs of the alimentary, urinary, respiratory, or nervous system. **[Data were not shown by study**  
11 **authors.]** Bisphenol A exposure had no effect on ovulation, estrous cyclicity, or serum FSH or LH levels.  
12 There were no effects on uterine preneoplastic or neoplastic lesions or ovarian histopathology following  
13 bisphenol A treatment. The study authors concluded that perinatal exposure to bisphenol A at levels  
14 comparable to human exposure did not affect the reproductive system of female rats.

15  
16 **Strengths/Weaknesses:** Strengths of this study were the bisphenol A determinations that were made and  
17 the anchoring of animal exposure levels to human exposures. The design appears sound with a good range  
18 of endpoints measured. Small numbers of animals were sacrificed at several time points and cellular  
19 analyses were performed; these numbers were too small for a definitive cancer evaluation and were, in fact,  
20 too small for definitive conclusions to be reached for most of the adult reproductive endpoints. Statistics are  
21 not described in enough detail to determine how data from multiple sampling points were evaluated. This  
22 experiment represents a thorough screening study.

23  
24 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate based on insufficient  
25 sample size (3-5/group).

26  
27 **Takagi et al. (349)**, supported by the Japanese Ministry of Health, Labor, and Welfare, examined the effect  
28 of perinatal bisphenol A exposure on the reproductive and endocrine systems of rats. Nonylphenol was also  
29 examined but will not be discussed. Sprague Dawley rat dams were fed a soy-free diet (Oriental Yeast Co.,  
30 Tokyo) prepared according to the formula for NIH-07. At weaning, the offspring were fed CRF-1 diet  
31 (Oriental Yeast Co., Tokyo), which contains soybean and alfalfa-derived proteins. Rats were housed in  
32 polycarbonate cages containing wood chip bedding. Dams were randomly assigned to groups, and 5–6  
33 dams/group were fed diets containing bisphenol A (96.5% purity) at 0, 60, 600, or 3000 ppm from GD 15  
34 (GD 0 = day of vaginal plug) to PND10 (PND 1 = day of birth). The study authors estimated bisphenol A  
35 intake at ~5, 49, and 232 mg/kg bw/day during the gestation period and ~9, 80, and 384 mg/kg bw/day  
36 during the lactation period. Dose levels were based on results of preliminary studies, and selected with a  
37 goal of achieving weak to moderate toxicity in dams at the highest dose. In a separate study, rats were fed  
38 diets containing ethinyl estradiol at 0 or 0.5 ppm from GD 15 to PND 10. On PND 2, offspring were  
39 counted, sexed, and weighed and anogenital distance was measured. Litters were culled to 6 pups on PND  
40 10, and pups were weaned on PND 21. Five pups/sex/group (1/sex/litter) were selected for necropsy on  
41 PND 21 and brain, adrenals, testis, ovary, and uterus were weighed. Eight offspring/sex/group (at least  
42 1/sex/litter) were selected for evaluation in adulthood, and these rats were observed for age and body  
43 weight at puberty. Estrous cyclicity was observed from 8 to 11 weeks of age. Offspring were killed at 11  
44 weeks of age, on the day of diestrus for cycling female rats. Brain, pituitary, thyroid, adrenal mammary  
45 gland, epididymis, prostate, seminal vesicles, ovary, uterus, and vagina were weighed and examined  
46 histologically. The testis was fixed in Bouin solution, and other organs were fixed in 10% neutral buffered  
47 formalin. The volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) was measured. It  
48 appears that endpoints were assessed in 8 adult rats/sex/group, with the exception of histopathological  
49 evaluations, which were conducted in 5 rats/sex/group. The litter was considered the experimental unit in  
50 statistical analyses of data from PND 21 offspring, and the individual animal was considered the statistical  
51 unit for data obtained from adult offspring. Homogenous numerical data were analyzed by ANOVA and

### 3.0 Developmental Toxicity Data

1 Dunnett test, and heterogeneous numerical data were analyzed by Kruskal-Wallis *H* test and Dunnett-type  
2 rank sum test. Data for histopathological lesions and vaginal cyclicity were analyzed by Fisher exact  
3 probability test or Mann-Whitney *U* test.

4  
5 Maternal body weight gain was significantly decreased the high-dose bisphenol A group during gestation,  
6 but there were no effects on body weight gain during lactation or food intake. In offspring evaluated on  
7 PND 2, there were significant decreases in body weight in low- and high-dose males [**13 and 22%**] and in  
8 high-dose females [**20%**], but there were no effects on number of live offspring or anogenital distance.  
9 Body weight gain was lower in high-dose males [**21%**] and females [**29%**] from PND 2 to 10. Increased  
10 relative brain weight as a result of growth retardation was reported in high-dose offspring evaluated on  
11 PND 21. [**Data were not shown by study authors.**] Exposure to bisphenol A did not affect onset of  
12 vaginal opening, preputial separation, or estrous cyclicity. Body weight of males was significantly lower  
13 [**by 9.3%**] at adult necropsy. Weights and histopathology of brain, pituitary, thyroid, adrenal mammary  
14 gland, epididymis, prostate, seminal vesicles, ovary uterus, and vagina in adulthood were unaffected in rats  
15 from the bisphenol A group. [**Organ weight data were not shown by study authors.**] Bisphenol A did not  
16 affect SDN-POA volume. Effects observed in offspring from the ethinyl estradiol study included reduced  
17 numbers of live offspring, increased male:female ratio, decreased body weight and body weight gain,  
18 accelerated vaginal opening, delayed preputial separation, increased estrous cycle irregularities, and  
19 histopathological alterations in pituitary, ovary, uterus, vagina, and mammary gland. The study authors  
20 concluded that bisphenol A did not affect endocrine or reproductive system development of rats at doses  
21 that induced maternal toxicity.

22  
23 **Strengths/Weaknesses:** Strengths include the range of doses and endpoints measured and the use of the  
24 ethinyl estradiol comparator group. The study used small sample sizes of dams (n=5-6/group) and  
25 inadequate statistical procedures to control for litter effects.

26  
27 **Utility (Adequacy) for CERHR Evaluation Process:** This study is considered inadequate for the  
28 evaluative process, based on sample size and statistical procedures.

29  
30 **Akingbemi et al. (350)**, supported by NIEHS, US EPA, NICHHD, and NIH, conducted a series of studies  
31 in Long Evans rats to determine the effects of postweaning and perinatal exposure to bisphenol A on  
32 testicular steroidogenesis. In vitro studies were also conducted and are described in Section 4 because cells  
33 used in the studies were obtained from adult animals. Rats were fed Purina chow, which contains soybean  
34 meal, and given drinking water in polycarbonate bottles. Pregnant and nursing dams were housed in  
35 polycarbonate cages lined with wood bedding, but no information was provided on caging used at the other  
36 life stages. To reduce leaching of bisphenol A, the cages were washed, rinsed, and dried at least twice/week  
37 and were discarded once they began getting cloudy; water bottles were cleaned daily. Corn oil vehicle was  
38 used for bisphenol A and was administered to control animals. Rats were stratified according to body  
39 weight and randomly assigned to treatment groups. RIA methods were used to measure steroid hormone  
40 concentrations in serum or testicular fluid. RT/PCR methods were used to examine changes in mRNA  
41 expression. Statistical analyses included ANOVA with multiple comparisons conducted by the Duncan  
42 multiple range test. [**In the part of the study in which dams were dosed, it appears that offspring were  
43 considered the statistical unit.**]

44  
45 In the first study, rats were gavaged with bisphenol A [**purity not given**] at 0, 0.0024, 0.010, 100, or 200  
46 mg/kg bw/day from PND 21 through 35. The two lowest doses were selected to represent environmental  
47 exposures, and the higher doses were selected to compare the effects between low and high doses. Rats  
48 were killed at the end of treatment and blood was collected for measurement of serum LH, testosterone, and  
49 17 $\beta$ -estradiol levels. Leydig cell cultures were prepared for measurement of ex vivo testosterone  
50 production with and without the addition of LH, testosterone precursors, or metabolizing enzymes.  
51 Additional weanling rats were exposed to bisphenol A at 0 or 0.0024 mg/kg bw/day on PND 21–35. At the

### 3.0 Developmental Toxicity Data

1 end of treatment, mRNA for *LHβ*, *ERβ* and *ERα* was measured in pituitary using an RT-PCR technique.  
2 All endpoints were reported for 7–12 rats/group. Compared to rats in the control group, rats exposed to  
3 bisphenol A at 0.0024 mg/kg bw/day had significantly lower levels of serum LH [**by 62%**] and testosterone  
4 [**by 40%**]. Serum 17β-estradiol levels were decreased in rats exposed to 0.0024, 0.010, and 100 mg/kg  
5 bw/day bisphenol A [**by ~30, 40, and 25% in each respective dose group**]. There were no effects on basal  
6 ex vivo testosterone production by Leydig cells. In Leydig cells obtained from rats exposed to 0.0024  
7 mg/kg bw/day bisphenol A, testosterone production was significantly reduced when cells were exposed to  
8 LH or CYP450 17α-hydroxylase/17–20 lyase. In Leydig cells obtained from rats exposed to 0.0024 or  
9 0.010 mg/kg bw/day bisphenol A, testosterone production was significantly reduced following exposure of  
10 the cells to pregnenolone or progesterone. No effects on blood hormone levels or ex vivo testosterone  
11 production were observed at higher doses. Significant effects observed in pituitaries obtained from rats  
12 exposed to 0.0024 mg/kg bw/day bisphenol A were decreased *LHβ* mRNA and increased *ERβ* mRNA. The  
13 study authors concluded that the decreased serum LH level resulted from bisphenol A effects on the  
14 pituitary and that decreased LH stimulation of Leydig cells was the cause of reduced serum testosterone  
15 levels.

16  
17 In the second experiment, 7 dams/group were gavaged with bisphenol A at 0 or 0.0024 mg/kg bw/day from  
18 GD 12 through PND 21. Male offspring received no further treatment following weaning. Males were  
19 randomly selected from each dam and killed on PND 90. Endpoints evaluated in 10–12 male  
20 offspring/group included serum LH and testosterone levels, ex vivo testosterone production by Leydig  
21 cells, testosterone levels in testicular interstitial fluid, and seminal vesicle and prostate weight. Significant  
22 ( $P < 0.01$  or 0.05) effects observed in 90-day-old males that had been perinatally exposed to bisphenol A  
23 compared to the control group included increased body weight [**10%**], decreased relative weight (to body  
24 weight) of paired testes [**17%**] and seminal vesicles [**17%**], reduced testicular testosterone level [**~39%**],  
25 and reduced basal and LH-induced ex vivo testosterone production.

26  
27 In the third experiment, 10–12 rats/group were gavaged with bisphenol A at 0 or 0.0024 mg/kg bw/day  
28 from PND 21 through 90. Within 24 hours following treatment, rats were killed and examined for the same  
29 endpoints described for the second experiment. Significant ( $P < 0.01$  or 0.05) effects compared to the  
30 control group included increased serum LH level [**117%**], decreased seminal vesicles weight [**absolute:**  
31 **15%, relative: 16%**], reduced testicular testosterone level [**~24%**], and decreased basal and LH-induced  
32 ex vivo testosterone production. For the second and third experiments, the study authors concluded that  
33 bisphenol A exposure inhibits androgen production by Leydig cells.

34  
35 **Strengths/Weaknesses:** Significant strengths of this report were the sequential nature of the work, in  
36 which later studies built on the previous data, and the clear expertise that the authors brought to this  
37 endeavor. Experiment 1 provided a helpful examination of postnatal effects following adolescent exposure  
38 and examined hormonal levels under stimulated and unstimulated conditions, thus separating pituitary from  
39 target organ contributions to serum levels. In Experiment 2, the sample size of 7 dams/prenatal treatment  
40 group and the examination of 10–12 offspring/group raise questions about the adequacy of the sample size  
41 with respect to the number of litters represented and the number of offspring used to represent each litter. In  
42 Experiment 3, 10–12 rats/group were treated from postnatal days 21–90 (through adolescence and into early  
43 adulthood) and then examined according to endpoints common to Experiments 1 and 2. Weaknesses  
44 include an inadequate number of animals to obtain confidence about the hormonal changes (indeed, LH  
45 was decreased in the first experiment and increased in the third), the lack of histopathology evaluation, and  
46 lack of an estrogenic positive control.

47  
48 **Utility (Adequacy) for CERHR Evaluation Process:** Experiments 1 and 3 are adequate but of limited  
49 utility because of the mechanistic nature of the endpoints examined. Experiment 2 is inadequate for  
50 consideration due to inappropriate statistics that failed to account for litter effects.

51

### 3.0 Developmental Toxicity Data

1 **Masutomi et al. (351)**, supported by the Japanese Ministry of Health, Labour, and Welfare, examined the  
2 potential effects in rats of neonatal bisphenol A exposure through maternal dietary intake on the number of  
3 offspring pituitary cells positive for LH, FSH, and prolactin. The authors exposed 5–8 pregnant  
4 CD(SG)IGS dams from GD 15 to PND 10 to soy-free diet containing: 1) genistein 20, 200 or 1000 ppm, 2)  
5 diisononyl phthalate 400, 4000, or 20,000 ppm, 3) methoxychlor 24, 240, or 1200 ppm, 4) 4-nonylphenol 60,  
6 600, or 3000 ppm, or 5) bisphenol A [**96.5% purity**] 60, 600, or 3000 ppm. Ethinyl estradiol at 0.5 ppm  
7 was also administered to a positive control group and the regular soy-free diet to a control group. [**Only the**  
8 **bisphenol A-treated group will be considered here. Feed consumption and dam body weight were not**  
9 **reported, but would be expected to have changed dramatically over the treatment period, making it**  
10 **difficult to estimate the bisphenol A doses received by the rats.**] After weaning, offspring were placed  
11 on CRF-1 rodent chow. Animals were housed in polycarbonate cages with wood-chip bedding.

12  
13 During postnatal week 3 or 11, offspring were killed and anterior pituitary glands from 5 male and 5 female  
14 offspring/group were harvested. Immunohistochemistry using paraffin-embedded sections for LH, FSH,  
15 and prolactin was conducted and the percentage of cells positive for LH, FSH, and prolactin was  
16 determined in 2 sections/gland. Statistical analyses were performed by Student or Welch *t*-test using values  
17 from the highest bisphenol A dose group and the control. There was no effect of bisphenol A treatment on  
18 relative pituitary weight or on cell counts for LH, FSH, or prolactin. There was an increase in cells staining  
19 for prolactin in female offspring from the ethinyl estradiol-treated dams at 3 weeks.

20  
21 **Strengths/Weaknesses:** This hypothesis-driven study was carefully designed with respect to exposure  
22 during established periods relevant to the sexual differentiation of the brain and with respect to assessment  
23 of appropriate parameters related to reproductive function. A large number of dose levels were examined  
24 across 5 compounds, one being bisphenol A with evaluation of 4 dose levels, including controls. Five to  
25 eight animals/sex/dose were used in evaluations and animals were selected as 1 male and 1 female/litter.  
26 Findings were judged against an incorporated positive control that resulted in predicted findings.

27  
28 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is adequate but of limited utility for the  
29 evaluation because of the secondary nature of the endpoints for a human health evaluation.

30  
31 **Tan et al. (352)**, supported by the University of Malaya and the Ministry of Science, Technology, and  
32 Environment, examined the effects of bisphenol A exposure on pubertal development of male rats. Sprague  
33 Dawley rats were fed soy-free feed (Gold Coin Feedmills) and housed in aluminum cages containing  
34 shredded recycled paper as bedding. On PND 23–53, twelve rats/group were gavaged with 100 mg/kg  
35 bw/day bisphenol A [**purity not reported**] in a Tween-80/water solution (1:9 v/v), 100 mg/kg bw/day  
36 nonylphenol in corn oil, or a mixture of 100 mg/kg bw/day bisphenol A and nonylphenol. A control group  
37 of 12 rats was gavaged with Tween 80 in corn oil. Dosage selection was based upon published studies  
38 reporting NOAELs of 50 mg/kg bw/day for both compounds. Rats were examined for preputial separation  
39 during the study. Six rats/group were killed on PND 52, and the other 6/group were killed on PND 53.  
40 Testes, epididymides, liver, kidney, adrenal, seminal vesicles plus coagulation gland, and thyroid were  
41 weighed. [**The Expert Panel assumes that by coagulation gland, the authors mean the anterior**  
42 **prostate or coagulating gland.**] Thyroid, testis, kidney, and liver were fixed in 10% formalin and  
43 examined histologically. Statistical analyses included ANOVA and Fisher protected least significant  
44 difference test.

45  
46 There was no significant effect on weight gain in rats treated with bisphenol A. In the bisphenol A group,  
47 preputial separation occurred by PND 53 in 66.7% of rats compared to 100% of rats in the control group. In  
48 the bisphenol A group, significant increases were observed in absolute and relative (to body weight) kidney  
49 and thyroid weights and significant decreases were observed for absolute and relative liver weight. Cortical  
50 thickness of the kidney was significantly decreased [**by ~13% compared to controls according to**  
51 **CERHR calculations and ~30% according to study authors**]. There was no effect on testicular weight or

### 3.0 Developmental Toxicity Data

1 tubule diameter. Normal patterns of spermatogenesis were observed in rats from the control group.  
2 Multinucleated giant cells were observed in seminiferous tubules and there was no indication of  
3 spermatogenesis in 4 of 12 rats of the bisphenol A group. Giant cells were observed and spermatogenesis  
4 was found to occur in only some seminiferous tubules of the remaining rats treated with bisphenol A.  
5 Moderate-to-severe hydronephrosis was observed in 50% of rats and mild hydronephrosis was observed in  
6 the other 50% of rats from the bisphenol A group.

7  
8 Preputial separation occurred by PND 53 in 33.3% of rats in the nonylphenol group and 58.3% of rats  
9 exposed to the bisphenol A/nonylphenol mixture. In animals treated with nonylphenol, relative liver weight  
10 was increased, absolute and relative seminal vesicle weights were decreased, and the diameter of testicular  
11 tubules was reduced. A decrease in relative seminal vesicle weight was the only significant organ weight  
12 effect observed in the group treated with both bisphenol A and nonylphenol. Moderate hydronephrosis was  
13 observed in 25% of rats exposed to the bisphenol A/nonylphenol mixture and mild hydronephrosis was  
14 observed in the other rats from that exposure group. No spermatogenesis was observed in 3–5 of 12  
15 rats/group treated with nonylphenol or the mixture of bisphenol A/nonylphenol. The study authors  
16 concluded that both bisphenol A and nonylphenol affected the reproductive system of rats, while only  
17 bisphenol A affected the kidneys. They also noted a less-than-additive effect with administration of the  
18 bisphenol A/nonylphenol mixture.

19  
20 **Strengths/Weaknesses:** This study was apparently well performed and documents the endpoints tested. A  
21 weakness is the use of a single high dose level of bisphenol A.

22  
23 **Utility (Adequacy) for CERHR Process:** This study is adequate and of high utility for the evaluation  
24 process.

25  
26 **Kobayashi et al. (346)**, supported by the Japanese Ministry of Health, Labor, and Welfare, examined the  
27 effects of developmental exposure to bisphenol A on thyroid status in rats. Rats used in this study were fed  
28 standard laboratory chow (CE-2, Clea Japan). **[No information was provided about caging or bedding**  
29 **materials.]** From GD 6 (day of copulatory plug = GD 0) through PND 20 (day of birth not defined), 6  
30 maternal CD rats/group were gavaged with bisphenol A (>99.8% purity) at 0 (corn oil vehicle), 4, 40, or  
31 400 mg/kg bw/day. The 400 mg/kg bw/day maternal group was excluded from analysis because of  
32 excessive maternal toxicity. Details about maternal toxicity and additional aspects of this study are  
33 available in the summary for the study by Kobayashi et al. (344). On PND 7, litters were culled to 5  
34 pups/sex when possible. It appears that culled pups may have been used in analyses conducted at 1 week of  
35 age. Pups were weaned on PND 21. Approximately 1 male and female pup/litter were killed at 3 and 9  
36 weeks of age. Plasma thyroxin levels were measured by chemiluminescence immunoassay in 1–9  
37 offspring/group/sex at 1 week of age and 3–6 offspring/sex/group at 3 and 9 weeks of age. At 9 weeks of  
38 age, thyroid stimulating hormone test was conducted in 2–7 rats/sex/group by measuring thyroxin levels  
39 after injection with bovine thyroid stimulating hormone. Statistical analyses included ANOVA followed by  
40 Dunnet test or Student or Welch *t*-test. **[It was not clear if the litter or offspring were considered the**  
41 **statistical unit.]**

42  
43 In the 4 and 40 mg/kg bw/day groups, there were no significant differences in thyroxine levels at 1, 3, or 9  
44 weeks of age or in thyroid stimulating hormone-induced increases in thyroxine levels at 9 weeks of age.  
45 Based on the findings of this study, the study authors concluded that prenatal and lactational exposure of  
46 rats to bisphenol A does not appear to affect thyroid function.

47  
48 **Strengths/Weaknesses:** Strengths of this study include the use of a range of dose levels of bisphenol A.  
49 Weaknesses include the limited endpoints addressed (thyroid function), concern that the number of animals  
50 used (6 dams per treatment group) may not provide adequate statistical power to assess changes in hormone

### 3.0 Developmental Toxicity Data

1 levels and response given the variability inherent in these measures, and failure to account for litter in the  
2 analyses.

3  
4 **Utility (Adequacy) for CERHR Evaluation Process:** As presented, this publication is inadequate due to  
5 uncertainty on whether litter effects were adequately controlled for.

6  
7 **Zoeller et al. (353)**, supported in part by NIH, examined the effect of bisphenol A exposure on the thyroid  
8 of developing rats. Sprague Dawley rats were housed in plastic cages. **[No information was provided**  
9 **about composition of feed or bedding materials.]** Prior to initiation of dosing, rats were trained to eat an  
10 untreated wafer each day. On GD 6 (day of vaginal plug not defined) through the remainder of the  
11 experiment (the remainder of the gestation and lactation periods), 9 rats/group were given a wafer dosed  
12 with bisphenol A **[purity not reported]** at levels resulting in exposure to 0 (methanol vehicle), 1, 10, or 50  
13 mg/kg bw/day. Doses were based on those used in the study by Tyl et al. (338). Pups (n = 7–  
14 9/group/sex/time period) were weighed and killed on PND 4, 8, 15, or 35 (day of birth not defined). During  
15 each of those time periods, serum thyroxin was measured by RIA. On PND 15, brains of male pups were  
16 sectioned and examined for presence of RC3/neurogranin mRNA, a thyroid hormone-responsive gene,  
17 using an in situ hybridization and autoradiography technique. Serum thyroid-stimulating hormone was  
18 measured using an unspecified method in 6–8 male pups/group (1/litter) on PND 15. Statistical analyses  
19 included ANOVA and Bonferroni *t*-test.

20  
21 The text of the study indicated a significant reduction in maternal body weight gain during pregnancy in the  
22 high dose group, while Figure 1 of the study indicated a significant reduction in maternal body weight gain  
23 during pregnancy at all dose levels. Maternal body weight gain during the lactation period was unaffected  
24 by bisphenol A treatment. Bisphenol A exposure had no effect on litter size at birth. **[Data were not shown**  
25 **by study authors.]** Bisphenol A had no effect on pup body weights on PND 4, 8, or 15. On PND 15, but at  
26 no other time period, there was a significant increase in serum thyroxin levels in all dose groups of male  
27 and female pups **[percent increases compared to controls were ~11, 35, and 37% in each respective**  
28 **dose group.]** Significant increases in expression of RC3/neurogranin mRNA were observed in the upper  
29 and lower dentate gyrus in males from each treatment group **[with no apparent dose-response**  
30 **relationship]**. Expression of RC3/neurogranin mRNA in cortex was unaffected by bisphenol A treatment.  
31 No significant effects were observed for thyroid-stimulating hormone levels in males on PND 15. The  
32 study authors concluded that bisphenol A acts as a thyroid antagonist at these concentrations.

33  
34 **Strengths/Weaknesses:** Strengths of the study include use of a range of doses and examination of a role of  
35 bisphenol A as a thyroid hormone antagonist. Weaknesses include the lack of litter-based analysis.

36  
37 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate based on inappropriate  
38 statistics (i.e., not accounting for repeated measures over time or the use of more than one pup per litter for  
39 a given endpoint).

#### 40 41 3.2.3.3 *Studies with neurobehavioral endpoints*

42 **Kwon et al. (342)**, from CIIT, examined the effects of bisphenol A exposure during pre- and postnatal  
43 development on reproductive endpoints and the SDN-POA of rats. Sprague Dawley rats were fed NIH-07  
44 feed and housed in polycarbonate cages with cellulose fiber-chip bedding. Water was provided in glass  
45 bottles with Teflon-lined caps. Pregnant rats were randomly assigned to groups according to body weight.  
46 Rats (n = 8/group) were gavaged with bisphenol A (~99% purity) at 0 (corn oil vehicle), 3.2, 32, or 320  
47 mg/kg bw/day from GD 11 (GD 0 = day of sperm detection) through PND 20, excluding the day of  
48 parturition. It was not stated if the day of parturition was considered PND 0 or 1. A positive control group  
49 was treated with 15 µg/kg bw/day diethylstilbestrol. Rats were examined for clinical signs of toxicity and  
50 weighed during the study. Pups were weighed on PND 1 and 7. Pups were weaned on PND 21. After pups  
51 were weaned, dams were killed for assessment of body and organ weights. On PND 10, brains were

### 3.0 Developmental Toxicity Data

1 collected from 1–3 female offspring/litter from 6–8 litters/group for measurement of SDN-POA volume.  
2 All remaining female pups were examined for age of vaginal opening and day of first estrus, and estrous  
3 cyclicity was monitored for 22 days, beginning at ~4 months of age. Lordosis behavior was examined at 6  
4 months of age in 1–2 female offspring/litter from 7–9 litters/group that had been ovariectomized 2 weeks  
5 prior to reproductive behavior testing and primed with estradiol benzoate and progesterone. Male offspring  
6 were killed on PND 180 for measurement of body and reproductive organ weights and histopathological  
7 evaluation of ventral prostates fixed in 10% neutral buffered formalin. **[Based on information presented  
8 in the results section, it also appears that ovaries and uteri were examined in an unspecified number  
9 of female offspring at 6 months of age.]** Statistical analyses included ANOVA, Dunnett test, ANCOVA,  
10 and pair-wise comparison of least square means. The litter was considered the experimental unit.

11  
12 Bisphenol A treatment had no significant effect on maternal body weight during pregnancy or lactation or  
13 on maternal liver, kidney, adrenal, ovary, or uterus weights. There was no effect on number of live  
14 pups/litter at birth or pup weight on PND 1 or 7. In female offspring, bisphenol A exposure had no  
15 significant effect on volume of SDN-POA, age or weight at vaginal opening or first estrus, estrous  
16 cyclicity, or mean lordosis intensity. In male offspring, there were no significant effects on body weight or  
17 weights of testis, epididymis, seminal vesicle, or prostate. The study authors noted that a 23% increase in  
18 ventral prostate weight in the high-dose group did not reach statistical significance. No treatment-related  
19 histopathological alterations were reported for ventral prostate, ovary, or uterus. Effects observed in the  
20 diethylstilbestrol group included increased maternal liver weight, increased SDN-POA volume in female  
21 offspring, and disrupted estrous cycles. The study authors concluded that indirect exposure of offspring to  
22 bisphenol A at these levels during gestation and lactation did not affect estrogen-mediated reproductive  
23 endpoints. A similar study with comparable findings in females was reported in abstract form (354).

24  
25 **Strengths/Weaknesses:** This study was well performed and presented. The wide coverage of the dose  
26 range (across a three log range) is a major strength. The use of diethylstilbestrol as a positive control is a  
27 strength, as is the number of reproductive organs and endpoints evaluated. A weakness was the limited  
28 analysis of those reproductive organs (wet weight only; histopathology was only performed on the prostate)  
29 and a lack of determination of pup exposure during lactation.

30  
31 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
32 evaluation process.

33  
34 **Kubo et al. (355)**, supported by the Japanese Ministry of Education, Science, and Culture, examined the  
35 effect of prenatal bisphenol A exposure on sexually dimorphic behavior and brain development in rats. **[No  
36 information was provided about the type of chow, bedding, or caging materials used.]** Throughout the  
37 gestation (from the day that sperm were detected in the vagina) and lactation periods, 5 Wistar rats/group  
38 were administered bisphenol A through drinking water at 0 or 5 mg/L. **[No information was provided on  
39 bisphenol A purity or use of a vehicle.]** The study authors estimated the bisphenol A dose at 1.5 mg/kg  
40 bw/day. **[It is not clear whether this is an estimate based upon water consumption or dosing by a  
41 separate, unspecified route. If based upon drinking, this estimate is suspect because it implies a daily  
42 consumption of approximately 70 mL water (because the weight of the rats is not supplied this must  
43 of necessity be a guess), which is well in excess of published intakes for post partum rats (generally  
44 noted as around 20 mL/day). It is also noted that water consumption varies widely in non-lactating  
45 rats and throughout the period of lactation in rats, reflecting milk production, so any such estimate  
46 would of necessity be suspect, and doses will vary with time post partum.]** Litters were adjusted to 5  
47 pups/sex on the day following birth. Pups were weaned on PND 21 **[day of birth not defined]** and housed  
48 according to sex and litter. Behavior was tested for 10 minutes in an open-field apparatus at 6 weeks of age  
49 (n = 11–14) **[It was not clear if the number of animals examined included total animals, total/group,  
50 or total/sex/group. Litter distribution was not indicated.]** A passive avoidance test was conducted at 7  
51 weeks of age (n = 11–14); the test included a habituation period and testing of retention 24 hours later. An

### 3.0 Developmental Toxicity Data

1 unspecified number of rats were killed and necropsied at 12 weeks of age, with females killed in diestrus.  
2 Reproductive organs were weighed (n = 12–14) and sperm endpoints were evaluated in an unspecified  
3 number of rats. Serum hormone levels were measured by RIA (n = 5–10/group). At 20 weeks of age, 6  
4 rats/sex from the control group and 7 rats/sex from the treated group were killed to measure the volume of  
5 the SDN-POA and the locus ceruleus. Behavioral data were analyzed by Student *t*-test or Mann-Whitney *U*  
6 test, and brain morphology data were analyzed by Student *t*-test. **[It was not clear if the litter or offspring**  
7 **was considered the statistical unit.] No information was provided on data analyses for reproductive**  
8 **organ weight, serum hormone levels, or sperm endpoints.]**  
9

10 In open-field testing of controls, females moved significantly greater distances, reared more times, and  
11 stayed in the center of the apparatus for a longer period of time than males. In passive avoidance testing of  
12 controls, latency to enter the dark chamber following electric shock was significantly longer in male than  
13 female rats. In rats exposed to bisphenol A, there were no significant differences in the behaviors of males  
14 compared to females. Study authors attributed the loss of sexually dimorphic behaviors to  
15 demasculinization of male behavior and defeminization of female behavior. Bisphenol A treatment did not  
16 affect brain weight, which was higher in male than female controls. The larger size of SDN-POA in male  
17 compared to female controls was retained following bisphenol A treatment. The volume of the locus  
18 ceruleus was significantly larger in females than males of the control group. In the bisphenol A group, the  
19 volume of the locus ceruleus was described as larger in males than females, but the stated increase was not  
20 statistically significant ( $P = 0.12$ ). **[Graphically, there is an estimated 14% difference between male**  
21 **and female locus ceruleus volume in controls and in bisphenol A-exposed animals, with the direction**  
22 **of the difference apparently reversed by treatment.]** Bisphenol A treatment had no effect on absolute  
23 weight of the testis or epididymis or relative weights of the ventral prostate, ovaries, or uterus. There were  
24 no significant effects on serum levels of LH, FSH, testosterone, or 17 $\beta$ -estradiol. Sperm count and motility  
25 were also unaffected by bisphenol A exposure. The study authors concluded that current methods for  
26 establishing NOAELs may not be sufficient to detect disrupted sexual dimorphism in the brain.  
27

28 **Strengths/Weaknesses:** A strength is the variety of biological and behavioral endpoints assessed. The  
29 major weakness of the study is the lack of experimental detail, which makes it difficult to determine  
30 whether litter effects were adequately controlled for and how much bisphenol A was received by the  
31 animals.  
32

33 **Utility (Adequacy) for CERHR Evaluation Process:** Given the lack of methodological data provided in  
34 the paper, this communication is inadequate for the evaluation process.  
35

36 **Kubo et al. (356)** examined the effect of prenatal bisphenol A exposure on sexually dimorphic behavior  
37 and brain structure of rats. **[No information was provided on the type of feed or materials used in**  
38 **bedding or caging.]** Wistar rats were dosed with the 0.1% ethanol in distilled water vehicle (n = 5  
39 dams/group) or bisphenol A **[purity not reported]** at 0.1 or 1 mg/L (n = 6 dams/group). The study authors  
40 estimated bisphenol A intake at 0.030 and 0.3 mg/kg bw/day and noted that the levels were below the  
41 tolerated daily intake. **[Though not clearly stated, it appears that as in the previous study by Kubo et**  
42 **al. (355), exposures occurred through drinking water during the entire gestation and lactation**  
43 **period.]** Five dams/group were exposed to *trans*-resveratrol, an estrogenic compound found in grapes, at 5  
44 mg/L or diethylstilbestrol at 50  $\mu$ g/L. Body weight and anogenital distance were measured in pups on PND  
45 1 (the day following birth). **[All litters were examined and although the number of pups examined in**  
46 **each litter was not clearly stated, it was implied that all pups were analyzed.]** Anogenital distance was  
47 adjusted by the cube root of body weight. Following the evaluations on PND 1, litters were standardized to  
48 5 pups/sex. Pups were weaned on PND 21 and housed according to sex and litter. Day of testicular descent  
49 or vaginal opening was monitored in all remaining offspring (n = 25/sex in the control group and 30–31/sex  
50 in the treated group). Open-field testing was conducted in 20–24 animals/group at 6 weeks of age. **[It is not**  
51 **clear if the authors meant 20–24 animals/group or animals/group/sex].** Sexual behavior of 7–13 male

### 3.0 Developmental Toxicity Data

1 and female rats/sex/group was tested at 11–12 weeks of age. Males and females (n = 11–15/sex/group)  
2 were killed at 12 weeks of age, females during proestrus. Reproductive organs were weighed. Serum  
3 hormone levels were measured by RIA. Sperm from one testis and cauda epididymis were counted.  
4 Histological examinations were conducted on testis fixed in Bouin solution and ovary fixed in 10% neutral  
5 buffered formalin. Rats were killed at 14 weeks of age for measurement of SDN-POA and locus ceruleus  
6 volume in 7–8 males and females/group.

7  
8 Because of the large number of animals used, the experiment was conducted in 3 blocks representing  
9 identical experiments. All data were collected and analyzed following completion of the third block of the  
10 study. The litter was considered the statistical unit in analyses of data collected prior to weaning of animals.  
11 Individual animals were considered the statistical unit in analyses of data collected subsequent to weaning.  
12

13 Behavior and brain structure data were analyzed by ANOVA and differences between sexes were analyzed  
14 by Student *t*-test. Reproductive data were analyzed by ANOVA followed by Fisher protected least  
15 significant difference test for each sex.  
16

17 Bisphenol A exposure had no significant effect on body weight on PND 1, anogenital distance in males and  
18 females, day of testicular descent, or day of vaginal opening. Body weight at vaginal opening was  
19 significantly higher [**by 7%**] in the high-dose bisphenol A group. In sexual behavior testing of males, a  
20 non-dose-related decrease in the intromission rate observed in the low-dose group was the only significant  
21 effect reported following bisphenol A exposure. There were no effects on mounting or ejaculation.  
22 Bisphenol A exposure had no significant effects on female sexual behavior as measured by ear wiggle,  
23 lordosis quotient, and rejection of males. The study authors concluded that bisphenol A exposure had no  
24 remarkable effects on male or female sexual behavior. The only significant effect on organ weights was an  
25 [**9%**] increase in testis weight in the high-dose bisphenol group. There were no significant effects on  
26 absolute weight or relative (to body weight) weights of ventral prostate, seminal vesicle, uterus, or ovary.  
27 Bisphenol A treatment did not affect sperm count or motility or estrous cycles. Serum levels of LH, FSH,  
28 prolactin, testosterone, and 17 $\beta$ -estradiol were also unaffected by treatment. No histopathological findings  
29 were observed in testis or ovary. [**Data were not shown.**]  
30

31 In open-field testing of control rats, females moved greater distances, reared more often, and spent more  
32 time in the center of the testing apparatus. Following treatment with the low or high dose of bisphenol A,  
33 there were no longer significant differences between males and females in frequency of rearing and or  
34 duration of time spent in the center of the apparatus. Differences in distances moved by males versus  
35 females were no longer significant following exposure to the high bisphenol A dose. Males in the low  
36 bisphenol A group reared significantly more times than males in the control group. Bisphenol A treatment  
37 had no significant effect on the sex-related difference in size of the SDN-POA, which was significantly  
38 larger in males than females in the control and treatment groups. Although the volume of the locus ceruleus  
39 was significantly greater in females than males of the control group, locus ceruleus volume was  
40 significantly larger in males than females of both bisphenol A groups. The change was due to a significant  
41 increase in volume in males at the low dose and significant decrease in volume in females at both dose  
42 levels of bisphenol A. [**Magnitude of locus ceruleus volume changes in males and females was ~12–**  
43 **17% compared to controls, as estimated from a graph.**] The numbers of neurons in the locus ceruleus  
44 was affected in the same manner as volume by bisphenol A treatment, except that increases in neuron  
45 numbers following bisphenol A treatment were also significant in males of the high-dose group.  
46

47 Diethylstilbestrol mainly affected open-field behavior, locus ceruleus volume, and the reproductive system.  
48 *Trans*-resveratrol mainly affected locus ceruleus volume and the reproductive system. The study authors  
49 concluded that the brain is highly sensitive to bisphenol A at levels below the tolerable daily intake and  
50 disruptions in sexual differentiation may differ from effects observed with diethylstilbestrol and *trans*-  
51 resveratrol.

### 3.0 Developmental Toxicity Data

1 **Strengths/Weaknesses:** As with the previous study by this group (355) the main weakness of the paper  
2 lies in the failure to accurately describe the methods to allow a reader to determine how much bisphenol A  
3 the dams received during the experiment. Despite well-selected endpoints, the sample size of 5 dams/group  
4 and lack of clarity on the number of pups analyzed per litter are weaknesses.

5  
6 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
7 due to insufficient sample size and lack of experimental detail.

8  
9 **Facciolo et al. (357)**, supported in part by the Italian Ministry of University Education and Research,  
10 examined the effects of developmental exposure to bisphenol A on the somatostatin receptor subtype  $sst_2$  in  
11 the limbic circuit of rats. Sprague Dawley dams were exposed orally to bisphenol A at 0 (arachis oil  
12 vehicle), 0.040, or 0.400 mg/kg bw/day. **[No information was provided on the specific method of oral  
13 dosing, the purity of bisphenol A, or the number of dams treated/group. There was no information  
14 on the type of chow used or composition of cage and bedding materials.] [Author states that 32 dams  
15 were subdivided into three treatment subgroups: controls (n= 8); low bisphenol A and high  
16 bisphenol A (n=12 per group) (R. Facciolo, personal communication, July 17, 2007)].** The authors  
17 stated that the doses selected were relevant to human exposures from can linings and dental sealants and  
18 had been reported to induce morphometric changes in offspring. The rats were mated for 5 days during the  
19 treatment period, and treatment was continued through gestation and lactation. Litters (minimum 8/group)  
20 were culled to 8 pups at birth and 1 pup/litter was randomly assigned to a dam in the same treatment group  
21 for postnatal rearing. Pups were weaned on PND 23 (day of birth not defined). On PND 10 and 23, 4–7  
22 rats/group **[10–11/group according to figures in the study]** were killed and their brains were removed to  
23 examine effects on  $sst_2$  receptors in the limbic region. Receptor binding was assessed using  $^{125}\text{I-Tyr}^0$ -  
24 somatostatin-14 as a ligand. At the same ages, interactions of  $sst_2$  with  $\alpha$ -containing  $\gamma$ -aminobutyric acid  
25 (GABA) receptors, using the agonists zolpidem and Ro 15-4513, were examined in 12–13 rats/group.  
26 Results were reported for only the high dose of bisphenol A (0.400 mg/kg bw/day) because higher affinity  
27 was obtained for receptor ligand binding. Statistical analyses included Student *t*-test, ANOVA, and  
28 Newman-Keuls multiple range test. Analyses did not account for litter of origin.

29  
30 **Strengths/Weaknesses:** Strengths of this study are the fact that it appears to have been carefully performed  
31 and used biologically-relevant concentrations delivered orally. A weakness is the purposeful confounding  
32 of litter of origin through cross-fostering

33  
34 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate due to experimental design  
35 which did not sufficiently account for litter effects.

36  
37 **Facciollo et al. (358)**, supported by the Italian Ministry of University Education and Research, examined  
38 the effects of bisphenol A on expression of somatostatin subtype 3 ( $sst_3$ ) receptor mRNA in brains of  
39 female rats exposed during development and investigated whether the  $\alpha\text{GABA}_A$  receptor is also involved in  
40 this effect. Sprague Dawley rats were housed in stainless steel cages. **[No information was provided  
41 about the type of feed or bedding used.]** Beginning 8 days before mating and continuing through the  
42 mating period (5 or 8 days) and during pregnancy and lactation (42 days), 8 rats received the arachis oil  
43 vehicle and 12 rats/group received bisphenol A **[purity not reported]** at 0.040 or 0.400 mg/kg bw/day.  
44 Vehicle or bisphenol A were orally administered by pipette. To minimize litter effects, 1 female pup from  
45 each litter was fostered to a dam from the same treatment group (8 pups/dam). Pups were weaned on PND  
46 23. On PND 7 and at 55 days of age, 4 rats/group/time period were killed. Brains were sectioned and a  $^{32}\text{S}$ -  
47 labeled probe was used in an in situ hybridization method to measure  $sst_3$  mRNA expression. The effects of  
48  $\alpha\text{GABA}_A$  receptor subunits on expression of  $sst_3$  mRNA was examined by incubating the brain sections in 1  
49 nM–100  $\mu\text{M}$  of  $\alpha\text{GABA}_A$  receptor agonists (zolpidem, flunitrazepam, RY 080, and RO 15-4513).  
50 Additional brain sections from high-dose rats were used to determine interactions between  $sst_3$  with  $\alpha_1$  and  
51  $\alpha_5$  subunits with or without addition of 5–500 nM zolpidem or RY 080. Statistical analyses included

### 3.0 Developmental Toxicity Data

1 ANOVA followed by Dunnett *t*-test or Neuman-Keuls multiple range post hoc test, when analysis by  
2 ANOVA indicated statistical significance.

3  
4 Changes in *sst<sub>3</sub>* expression varied with dose and age. Expression patterns were changed in the presence of  
5  $\alpha$ GABA<sub>A</sub> receptor agonists. Based on their findings, the study authors concluded that bisphenol A exposure  
6 can affect cross-talking mechanisms involved in the plasticity of neural circuits with resulting influences on  
7 neuroendocrine/sociosexual behaviors.

8  
9 **Strengths/Weaknesses:** Strengths of this study are the fact that it appears to have been carefully performed  
10 and used biologically-relevant concentrations. A weakness is the purposeful confounding of litter of origin  
11 through cross-fostering.

12  
13 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate due to experimental  
14 design.

15  
16 **Aloisi et al. (359)**, supported in part by the Italian Ministry for Universities and Scientific and  
17 Technological Research (MURST), examined the effects of prenatal or postnatal bisphenol A exposure on  
18 the pain response of rats. **[No information was provided in the manuscript on chow or composition of**  
19 **caging and bedding. The Expert Panel has been informed that Harlan Teklad 2018 feed, Lignocel**  
20 **bedding, and polysulfone cages were used (F. Faraboli et al., personal communication, March 1,**  
21 **2007).]** Sprague Dawley rats were fed peanut oil vehicle (n = 13) or 0.040 mg/kg bw/day bisphenol A  
22 **[purity not given in the manuscript;  $\geq$ 95% according to the authors (F. Faraboli et al., personal**  
23 **communication, March 1, 2007)]** (n = 7/group) by pipette during pregnancy and lactation. Within 48  
24 hours after birth, the offspring were sexed and cross-fostered to form the following groups:

- 25  
26
  - Prenatal exposure group—born to dams receiving bisphenol A and nursed by dams receiving the  
27 peanut oil vehicle (n = 11 males; 9 females)
  - Postnatal exposure group—born to vehicle control dams but fostered to bisphenol A treated dams  
28 (n = 11 males; 9 females)
  - Vehicle control group—born to and nursed by dams exposed to the vehicle control (n=16 males  
29 and 11 females)

30  
31  
32  
33 At 22 weeks of age, the rats were randomly assigned to sham or formalin treatment groups, but the sham  
34 group was not analyzed. The formalin group was sc injected with 10% formalin on the dorsal surface of the  
35 right hind paw. Pain behaviors, such as licking, flexing, and jerking of the paw were recorded for 60  
36 minutes. Following testing, the phase of the estrous cycle was determined and blood was drawn to measure  
37 plasma levels of testosterone in males and corticosterone and 17 $\beta$ -estradiol in both sexes by RIA. Data  
38 were analyzed by ANOVA followed by post hoc least significant difference test.

39  
40 The frequency of paw jerking was decreased at 30–60 minutes following formalin injection in postnatally  
41 exposed rats. **[The study abstract and results section indicate that the effect occurred in males and**  
42 **females, but according to data presented in figures of the study, the effect only appeared to have**  
43 **occurred in males.]** Duration of flexion was increased 0–30 minutes following formalin injection in both  
44 sexes exposed prenatally to bisphenol A. Although statistical significance was not attained, the study  
45 authors noted an increase in licking duration at 0–30 minutes following formalin injection in females  
46 exposed to bisphenol A during prenatal development. No effects were observed on open-field behaviors or  
47 plasma levels of testosterone, 17 $\beta$ -estradiol, or corticosterone. The study authors concluded that their  
48 findings indicated sex- and exposure-related modifications of neural pathway activity or nociception  
49 centers following exposure to bisphenol A.

50

### 3.0 Developmental Toxicity Data

1 **Strengths/Weaknesses:** A strength of this study is the added dimension being investigated (pain response).  
2 A weaknesses, however, are use of a single dose and the purposeful confounding of litter of origin during  
3 the cross-fostering process. In addition, the sample size of 7 dams in the 0.040 mg/kg bw/day bisphenol A  
4 group and the examination of n=11 male and n=9 female offspring in the prenatal exposure group raise  
5 questions about experimental or statistical accounting for litter effects.  
6

7 **Utility (Adequacy) for CERHR Evaluation Process:** The data presented are inadequate due to the  
8 methodological design and lack of clarity on accounting for litter effects  
9

10 **Negishi et al. (360)**, support not indicated, examined the effect of perinatal bisphenol A exposure on  
11 behavior of rats. F344/N rats (n = 8–9/group) were orally exposed to bisphenol A at 0 (olive oil vehicle), 4,  
12 40, or 400 mg/kg bw/day from GD 10 through PND 20. GD 0 was defined as the day that vaginal sperm  
13 were detected and PND 0 was defined as the day of parturition. **[No information was provided on purity  
14 of bisphenol A, the specific method of oral dosing, type of chow used, or composition of bedding or  
15 caging materials.]** Dams were observed and weighed throughout the study. On PND 0, pups were counted,  
16 weighed, and culled to 8/litter with equal numbers/sex when possible. Pups were weighed periodically from  
17 PND 7 through 84. Pups were housed as same-sex littermates following weaning on PND 21. Upon  
18 weaning of pups, dams were killed and body and organ weights were recorded. Behavioral testing of  
19 offspring consisted of spontaneous motor activity measured at 28–34 days of age (n = 12–27/group), active  
20 avoidance testing conducted at 28–34 and 56–62 days of age (n = 8–9/group), and open-field behavior  
21 evaluations at 56–62 days of age (n= 9–18/group). Litter was not accounted for in the analyses. On PND  
22 62, offspring were randomly selected (8/sex/group) and killed for evaluation of body and organ weights.  
23 Statistical analyses included ANOVA, nested ANCOVA, and post hoc Fisher protected least significant  
24 difference test. **[Data analyzed at birth were presented and analyzed on a per litter basis. Postnatal  
25 data were apparently analyzed on a pup basis.]**  
26

27 Maternal body weight gain was reduced during the gestation and lactation period in dams exposed to the  
28 mid or high dose. The only organ weight effects in dams were reduced absolute and relative (to body  
29 weight) thymus weight. There were no effects on weights of liver, kidney, or spleen in dams. Bisphenol A  
30 treatment did not affect the number of pups/litter or sex ratio. In male offspring, body weights were lower  
31 than control values on PND 7 and 28 at the mid dose, and PND 7, 21, 28, and 56 at the high dose. Body  
32 weights of female offspring were lower than controls at PND 7 and 28 at the low and mid dose and PND 7,  
33 21, and 28 at the high dose. On PND 62, there were no effects on body weight or liver, kidney, spleen,  
34 thymus, brain, or testis weights. There were no effects on spontaneous activity, but total immobile time was  
35 increased in females of the mid-dose group. Performance of males in avoidance testing improved in the  
36 mid- and high-dose group at 4 weeks of age but decreased in the low-dose group at 8 weeks of age.  
37 Increased grooming by males of the low-dose group was observed in open-field testing. The study authors  
38 concluded that perinatal bisphenol A exposure caused behavioral alterations that differed by sex.  
39

40 **Strengths/Weaknesses:** Doses were sufficiently high to produce gross body weight changes, and 3  
41 different measures of behavior were collected, as well as organ weights at necropsy from the same animals.  
42 Weaknesses include a lack of statistical accounting for possible litter effects in the postnatal analysis, the  
43 lack of an evaluation of hormone-dependent behaviors, and the lack of assessment of more hormone-  
44 dependent tissues (prostate, levator ani muscle, etc.) or processes (age at balanopreputial separation,  
45 postnatal anogenital distance).  
46

47 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
48 due to a failure to account for litter effects.  
49

50 **Negishi et al. (361)**, support not indicated, examined the effect of perinatal bisphenol A **[purity not  
51 indicated]** exposure on the behavior of rats. The effects of nonylphenol were also examined but will not be

### 3.0 Developmental Toxicity Data

1 discussed. F344/N rats (10 or 11/group) were gavaged with bisphenol A at 0 (corn oil vehicle) or 0.1 mg/kg  
2 bw/day from GD 3 to PND 20. GD 0 was defined as the day that vaginal sperm was detected, and PND 0  
3 was the day of parturition. At birth, pups were counted and weighed. Litters were culled to 6 pups, with  
4 equal numbers of each sex when possible. Pups were weighed throughout the postnatal period. At weaning,  
5 dams were killed and organ weights were measured. One male pup/litter (n = 8–10/group) was subjected to  
6 a series of behavioral tests. The remaining male pups were killed for measurement of organ weights at 21  
7 days or 8 weeks of age. Neurobehavioral endpoints evaluated included open-field behavior at 8 weeks of  
8 age, spontaneous motor activity at 12 weeks of age, passive avoidance at 13 weeks of age, performance in  
9 the elevated-plus maze at 14 weeks of age, and active avoidance at 15 weeks of age. At 22–24 weeks of  
10 age, a monoamine reduction test was performed: rats were injected with the monoamine oxidase inhibitor  
11 trans-2-phenylcyclopropylamine hydrochloride or with saline, and behavior was then evaluated. Data were  
12 analyzed by ANOVA, and if statistical significance was obtained, Fisher protected least significant  
13 difference test was conducted. Behavioral endpoints were measured on 1 male pup/litter, thus accounting  
14 for litter issues.

15  
16 Bisphenol A exposure did not affect dam body weights during gestation or lactation, gestation duration,  
17 litter size, number of male and female pups, or final dam body and organ weights. **[Data were not shown.]**  
18 Body and organ weights of male offspring at 21 days and 8 weeks of age, behavior in open-field testing,  
19 spontaneous motor activity, and performance in the elevated-plus maze were also unaffected by bisphenol  
20 A exposure. **[Data were not shown by study authors.]** Bisphenol A had no significant effect on  
21 performance in the passive avoidance test, although tendencies for increased latency were observed. In  
22 active avoidance testing, rats from the bisphenol A group had significantly ( $P < 0.01$ ) fewer correct  
23 avoidance responses during the first, second, and third of 5 sessions, and failure of avoidance was  
24 significantly increased [**~2.5% in the bisphenol A group compared to 0.2% in controls**]. In contrast to  
25 control rats, bisphenol A-treated rats did not show an increase in locomotion following a challenge with  
26 trans-2-phenylcyclopropylamine hydrochloride. The number of rearings following 2-  
27 phenylcyclopropylamine hydrochloride exposure did not differ significantly between rats from the  
28 bisphenol A and control groups. The study authors concluded that perinatal exposure of rat dams to  
29 bisphenol A at concentrations slightly higher than environmental exposures irreversibly affected perception  
30 of fear-provoking stimuli and monoaminergic neural pathways in male offspring.

31  
32 **Strengths/Weaknesses:** The use of a single dose level is a weakness. Strengths include the variety of  
33 endpoints used to provide data, which point to effects that are not gross structural changes but relatively  
34 subtle behavioral effects.

35  
36 **Utility (Adequacy) for CERHR Evaluation Process:** These data are adequate and of high utility for the  
37 evaluation process.

38  
39 **Farabollini et al. (362)**, supported by the University of Siena, University of Firenze, MURST, and the  
40 Italian National Research Council, examined the effects of perinatal bisphenol A exposure on behavior in  
41 male and female rats. **[No information was provided in the manuscript about chow or composition of**  
42 **cage and bedding materials. The Expert Panel has been informed that Morini MIL chow, lignocel**  
43 **bedding, and polysulfone cages were used (F. Faraboli et al., personal communication, March 1,**  
44 **2007).]** Three groups of Sprague Dawley rats were orally dosed with the arachis oil vehicle or bisphenol A  
45 **[purity not reported in the manuscript; ≥95% according to the authors (F. Faraboli et al., personal**  
46 **communication, March 1, 2007)]** by micropipette. One group of 11 rats was administered 0.040 mg/kg  
47 bw/day bisphenol A from 10 days prior to conception until weaning of pups at 21 days of age. A second  
48 group of 11 rats was given arachis oil from 10 days prior to conception through GD 13, 0.400 mg/kg  
49 bw/day bisphenol A from GD 14 **[day of vaginal plug not stated]** through 6 days following delivery of  
50 pups, and arachis oil until weaning of pups. A control group of 9 rats was given arachis oil from 10 days  
51 prior to conception until weaning of pups. Beginning at 85 days of age and continuing for 3 days,

### 3.0 Developmental Toxicity Data

1 behavioral testing was conducted using a hole board and elevated-plus maze in 15 offspring/sex from the  
2 low-dose group, 11–12 offspring/sex from the high-dose group, and 14 pups/sex from the control group.  
3 **[Litter distribution was not reported.]** Separate sessions were conducted for each sex and treatment  
4 group. Data were analyzed by ANOVA and Fisher least significant difference test. A factor analysis was  
5 conducted using the principal components method with an orthogonal rotation of the factor matrix. **[It**  
6 **appears that offspring were considered the statistical unit.]**  
7

8 In general, head dipping (extending head over edge of apparatus) and arm entries were reduced and self-  
9 grooming was increased in exposed females. Head dipping and stretched-attend posture (moving body  
10 forward without moving paws and then returning to original position) were inhibited and arm entries were  
11 increased in exposed males. A factor analysis indicated reduced anxiety and motivation to explore in  
12 treated males and reduced activity and motivation to explore in treated females. The study authors  
13 concluded that although sex-related differences in behavior were noted following bisphenol A treatment,  
14 there was no clear masculinization of behavior in females. The authors also noted the lack of substantial  
15 differences in results between the two exposure protocols.  
16

17 **Strengths/Weaknesses:** The unusual exposure scenario in this paper is both a strength and a weakness;  
18 however, the use of 11-15 pups from 9-11 litters raises concern for possible litter effects which were  
19 unaccounted for in the statistical analysis.  
20

21 **Utility (adequacy) for CERHR Evaluation Process:** This study is inadequate due to insufficient control  
22 for possible litter effects.  
23

24 **Farabolini et al. (363)**, supported by the University of Siena, University of Firenze, and MURST,  
25 examined the effects of perinatal bisphenol A exposure on sociosexual behavior in rats. Sprague Dawley  
26 rats were housed in polysulfone cages. **[No information was provided in the manuscript on type of feed**  
27 **or composition of bedding materials. The Expert Panel has been informed that Harlan Teklad 2018**  
28 **chow and Lignocel bedding were used (F. Faraboli et al., personal communication, March 1, 2007).]**  
29 Dams received arachis oil vehicle (n = 13) or 0.040 mg/kg bw/day bisphenol A **[purity not indicated]** (n =  
30 7) through a micropipette from mating through weaning of pups. On the 2<sup>nd</sup> day following delivery, litters  
31 were culled to 4 pups/sex and cross-fostered to obtain the following exposure groups of 12 animals/sex:  
32

- 33 • Prenatal exposure group: born to bisphenol A-treated dams and nursed by vehicle-treated dams
- 34 • Postnatal group: born to vehicle-treated dams and nursed by bisphenol A-treated dams
- 35 • Control group: born to and nursed by vehicle-treated dams  
36

37 Litters were weaned on PND 21 (day of birth not defined). On day 45 **[assumed to be PND 45]**, animals of  
38 the same sex were randomly chosen and housed 4/cage, with no siblings in any cage. At 100 days of age,  
39 behavior in the presence of an intruder rat was observed. In female rats, vaginal smears were taken at the  
40 end of intruder testing and only females in diestrus were considered (n = 8 – 9/group). One week later,  
41 sexual orientation was tested in 12 rats/sex/group by placing a rat between cages containing a sexually  
42 receptive female and sexually mature male and recording the number of visits to each rat. Sexual  
43 performance was tested next in males; evaluation was restricted to only males that ejaculated (n = 10–12  
44 group). One week later, sexual behavior was tested in females during the diestrus or proestrus phase. **[It**  
45 **is not clear how many females were evaluated for sexual behavior.]** Behavior testing sessions were  
46 video recorded and later evaluated by a blinded observer. Data were analyzed by ANOVA followed by post  
47 hoc Fisher least significant difference test. Litter effects were purposely confounded through cross-  
48 fostering.  
49

50 In intruder testing, statistically significant effects observed in males exposed prenatally to bisphenol A  
51 included an increased number showing defensive behavior (9 of 10 versus 4 of 10 in the control group), a

### 3.0 Developmental Toxicity Data

1 decreased number showing ambivalent behavior (3/10 versus 8/10 in the control group), and increased ratio  
2 of defensive/agonistic behaviors [**by 280% compared to controls**]. No significant effects were observed in  
3 intruder testing of female rats. There was no effect on sexual preference of males or females. For sexual  
4 behavior testing of females, data from the pre- and postnatal exposure groups were pooled because there  
5 were no significant differences between groups. Bisphenol A exposure significantly decreased exit latency  
6 in females in diestrus [**by ~66%**] and proestrus [**by ~83%**] and significantly ( $P < 0.05$ ) increased lordosis  
7 frequency in females in proestrus [**~11.75 versus 3.75 times in controls**]. Statistically significant effects on  
8 sexual performance of treated males included an increased number of intromissions [**~15 compared to 11**  
9 **in controls**] in the postnatal exposure group and increased duration of intromission latency [**~115 versus**  
10 **40 seconds in controls**] and genital sniffing [**~40 versus 16 seconds in controls**] in the prenatal exposure  
11 group. The study authors stated that the results suggested a slight intensification of sexual behavior in  
12 females, slightly reduced performance in a limited number of endpoints in males, but no effect on other  
13 important sexual endpoints in males (e.g., latency of ejaculation and refractory period). It was concluded  
14 that pre- or postnatal exposure to bisphenol A potentiated female behavior and depotentiated male  
15 behavior.

16  
17 **Strengths/Weaknesses:** The work was carefully performed. The use of a single dose level of bisphenol A  
18 is a weakness; however, this dosing paradigm is consistent with many other papers by this group making  
19 comparisons between the papers relevant. Addressing aggressive/defensive behavior as well as sexual  
20 performance and interest in both male and female offspring is a strength. The failure to address underlying  
21 biological mechanisms is a weakness. Further weaknesses include the inability to account for litter effects  
22 as the use of multiple pups from some litters without appropriate statistical control raises concern for  
23 possible litter effects due to unequal litter representation

24  
25 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for evaluation purposes  
26 due to the inability to fully account for possible litter effects.

27  
28 **Dessi-Fulgheri et al. (364)**, supported by the University of Firenze, University of Siena, and MURST,  
29 examined the effect of perinatal bisphenol A exposure on play behavior in rats. Sprague Dawley rats were  
30 housed in polysulfone cages. [**No information was provided in the manuscript on chow or bedding**  
31 **material. The Expert Panel has been informed that Morini MIL chow and Lignocel bedding were**  
32 **used (F. Faraboli et al., personal communication, March 1, 2007).**] Using a pipette, rats were fed  
33 solutions containing the arachis oil vehicle and/or bisphenol A according to 1 of 3 exposure scenarios. A  
34 control group of 9 rats was given arachis oil from 10 days prior to mating until weaning of pups on PND 21  
35 [**day of birth not defined**]. Eleven rats in the low-dose group were given 0.040 mg/kg bw/day bisphenol A  
36 [**purity not provided**] from 10 days prior to mating until weaning of pups. Eleven rats in the high-dose  
37 group received arachis oil vehicle from 10 days prior to mating until GD 13 [**day of vaginal plug not**  
38 **defined**], 0.400 mg/kg bw/day bisphenol A from GD 14 to PND 6, and arachis oil from PND 7 until  
39 weaning. Both doses were considered to be within the range of human exposure. The low dose was said to  
40 represent exposures through food occurring over a long period of time. The high dose was said to represent  
41 exposures occurring through dental procedures occurring over a short period of time. Litters were culled to  
42 8 pups at birth. [**No information was provided in the manuscript on the sex distribution of the retained**  
43 **pups; the Expert Panel was advised that there were 4 males and 4 females/litter (F. Faraboli et al.,**  
44 **personal communication, March 1, 2007).**] After pups were weaned, 3 male and 3 female pups were  
45 randomly caged together, with no siblings co-housed in any cage. Behavioral testing was conducted on  
46 PND 35, 45, and 55. For the behavioral testing, rats from the same cage were individually identified by  
47 marking them with dye. On each day of testing, the 6 cage mates were transferred to a neutral arena that  
48 was covered in clean sawdust and video recorded for 6 minutes. Behaviors recorded during the 2<sup>nd</sup> and 3<sup>rd</sup>  
49 minute of each testing session were evaluated. There were 12–15rats/sex/group. [**The methods section**  
50 **indicates that 15 rats/sex were tested at the high dose, 12 rats/sex at the low dose, and 15 rats/sex in**  
51 **the control group. According to Table 4 of the study, which gives the pooled number of rats tested for**

### 3.0 Developmental Toxicity Data

1 **3 age periods, it appears that 12/sex were tested in the high-dose group, 15/sex in the low-dose group,**  
2 **and 15/sex in the control group. The Expert Panel has been informed that Table 4 is correct (F.**  
3 **Faraboli et al., personal communication, March 1, 2007).]** For statistical analyses, individual factor  
4 scores were used as independent variables in a 3-way ANOVA that considered treatment, sex, and age.  
5 Fisher least significant difference test was used when appropriate. At weaning, housing conditions  
6 confounded litter of origin which was not then accounted for in statistical analyses.

7  
8 Behavioral elements were categorized under 8 general factors. The authors first presented results that were  
9 pooled for the 3 different age groups. In females of the low-dose group, bisphenol A treatment was found  
10 to significantly increase factors addressing play directed towards females. Factors affecting low-intensity  
11 mating elements (e.g. crawling-under behavior) were significantly reduced in high-dose males and females.  
12 Factors of sociosexual exploration (e.g., genital and body sniffing) were significantly reduced in high-dose  
13 females and in males from both dose groups. Factors of social interest (e.g., approaching) were  
14 significantly reduced in both sexes at the high dose but increased in low-dose males. The authors next  
15 discussed results for PND 35, because it is the approximate time period of vaginal opening in females.  
16 Factors that were significantly affected at PND 35 included increased social interest by males and females  
17 of the low-dose group, decreased low-intensity mating elements by females of both dose groups, and  
18 decreased sociosexual exploration by males of both dose groups. The study authors concluded that 2 factors  
19 of female behavior were masculinized by treatment: play with females and sociosexual exploration.

20  
21 **Strengths/Weaknesses:** A strength of this work is that it evaluated the socio-sexual consequences of  
22 exposure, and specifically at a young age. Weaknesses include absence of accounting for litter influences  
23 and inadequate statistical procedures (i.e., failure to consider the repeated measures design). In addition, the  
24 hypothesis is not biologically plausible (i.e., consistent with expected effects of a chemical with an  
25 estrogenic mode of action) and the factor analysis does not necessarily cluster the play behaviors that are  
26 known to be sexually dimorphic.

27  
28 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for evaluation process due  
29 to faulty statistical procedures.

30  
31 **Porrini et al. (365)**, supported by MURST, the University of Firenze, and the University of Siena,  
32 examined the effects of perinatal bisphenol A exposure on play behavior of female rats. **[No information**  
33 **was provided in the manuscript about the type of feed or bedding and caging materials. The Expert**  
34 **Panel has been informed that Harlan Teklad 2018 chow, polysulfone cages, and Lignocel bedding**  
35 **were used (F. Faraboli et al., personal communication, March 1, 2007).]** Female Sprague Dawley rats  
36 were co-housed with males for 36 hours and then fed the peanut oil vehicle (n = 10) or 0.040 mg/kg bw/day  
37 bisphenol A **[purity not stated]** (n = 12) by micropipette during the gestation and lactation period. Two  
38 days following delivery, litters were adjusted to 4 pups/sex and pups were fostered by a dam from the same  
39 treatment group. Pups were weaned on day 21 **[assumed to be PND 21; day of birth not defined]**.  
40 Offspring were housed in cages containing 3 pairs of male-female siblings, with no siblings of the same sex  
41 in the same cage. Each group contained 18 female pups. Prior to examination of behavior in rats from the  
42 same cages at 35, 45, and 55 days of age, animals were individually identified with dye. Behavior was  
43 observed in a neutral arena in which the floor was covered with clean sawdust. Animals were allowed to  
44 familiarize themselves to the new environment for 1 minute and then behavior was video recorded for 6  
45 minutes. Video recordings were analyzed by an investigator blinded to treatment conditions. Only behavior  
46 of female rats was considered. Data were analyzed by ANOVA for repeated measures. The cross fostering  
47 design precluded the ability to examine litter effects.

48  
49 Factors were defined by study authors based on groups of behaviors. Significant effects were reported for 3  
50 factors. Social and non-social exploration was increased **[by ~34%]** at 35 days of age and **[by ~25%]** at 45  
51 days of age. Frequency of play behavior with males was decreased **[by ~100%]** at 45 days of age.

### 3.0 Developmental Toxicity Data

1 Grooming behavior was also decreased [**by ~63%**] at 45 days of age. The study authors concluded that  
2 bisphenol A does not clearly induce masculinization of female behavior, but some aspects of female  
3 behavior were defeminized.

4  
5 **Strengths/Weakness:** This paper reports a well-performed study with a poorly researched endpoint  
6 (juvenile play behavior) that has implications for reproductive behavior later in life. The use of only a  
7 single dose level of bisphenol A is a weakness. The fostering of pups within treatment group prevents the  
8 evaluation of litter effects and the use of multiple pups from some litters without appropriate statistical  
9 correction raises concern for possible litter effects.

10  
11 **Utility (Adequacy) for CERHR Evaluation Process:** This work is inadequate for the evaluation process  
12 due to insufficient control for possible litter effects.

13  
14 **Adriani et al. (366)**, supported by the Nervous and Mental Disorders Research Area, Istituto Superiore di  
15 Sanità, Italy, and by MURST, examined the effects of perinatal exposure to bisphenol A on behavior in  
16 rats. Sprague Dawley rats were housed in Plexiglass cages with sawdust bedding. [**No information was  
17 provided in the manuscript about feed. The Expert Panel has been informed that Morini MIL feed  
18 was used (F. Faraboli et al., personal communication, March 1, 2007).**] Nine dams/group were dosed  
19 with bisphenol A [**purity not reported**] orally by micropipette at doses of 0 (arachis oil vehicle) or 0.040  
20 mg/kg bw/day from the day of mating to the day pups were weaned. Pups were weaned on PND 25 (PND 0  
21 = day of birth) and housed in groups of 3 according to sex. One male and 1 female/litter were observed in  
22 testing that included novelty-seeking behavior during adolescence (PND 30–45), impulsivity during  
23 adulthood (PND 70), and open-field behavior following injection with 1 mg/kg bw *d*-amphetamine during  
24 adulthood. It appears that the same animals were tested at each time period. Data were analyzed by Tukey  
25 HSD test and ANOVA.

26  
27 In novelty testing, the time spent in a new area of the testing apparatus was lower in females exposed to  
28 bisphenol A [**~45–55% compared to vehicle control,  $P < 0.05$** ]. Males and females of the bisphenol A  
29 group exhibited increased activity in the novel area [**increases of ~75% in males and 35–55% in females,  
30  $P < 0.05$** ]. The study authors interpreted the effects of novelty testing as suggesting a less pronounced  
31 habituation profile and increased stress in a novel situation. In the impulsivity testing, food-restricted  
32 animals were placed in an apparatus that involved nose poking in a small hole to immediately deliver 1  
33 pellet of feed or a larger hole to deliver 5 pellets of feed following a delay that was increased over the time  
34 of the study. Lights were turned on during the delay periods following nose poking and for 25 seconds after  
35 delivery of feed, time periods in which no feed could be delivered. Both groups of rats preferred the larger  
36 hole with delayed delivery, but treatment with bisphenol A resulted in a more marked preference for the  
37 larger hole ( $P < 0.05$ ), thus indicating reduced impulsivity. When the length of the delay was increased for  
38 the large hole, the frequency of inadequate responding (i.e., nose poking during the delay) was decreased in  
39 males from the bisphenol A group; the study authors interpreted the effect as indicating a demasculinization  
40 of the restlessness profile. [**The study report originally mislabeled the control and bisphenol A-treated  
41 groups in Figure 3a. A corrected version of the figure was included in an erratum statement released  
42 by the study authors (367).**] In open-field testing, vehicle control males displayed significantly more  
43 rearing and crossing behaviors following injection with *d*-amphetamine, but an increase in rearing and  
44 crossing behavior following *d*-amphetamine injection did not occur in males exposed perinatally to  
45 bisphenol A. The study authors concluded that perinatal exposure of rats to bisphenol A resulted in altered  
46 behavior in rats.

47  
48 **Strengths/Weakness:** This study used protocols that are well established by this group. The use of only a  
49 single exposure level of bisphenol A is a weakness, with the proviso that the dose used is directly  
50 comparable to other studies. The degrees of freedom reported for behavioral measures suggest inflation of  
51 sample size due to failure to account for multiple time sampling.

### 3.0 Developmental Toxicity Data

1 **Utility (Adequacy) for CERHR Evaluation Process:** The paper is inadequate for evaluation due to  
2 inappropriate statistical procedures.

3  
4 **Carr et al. (368)**, supported by the National Science Foundation, the Mississippi Agricultural and Forestry  
5 Experiment Station, and the College of Veterinary Medicine at Mississippi State University, examined the  
6 effects of bisphenol A exposure on performance of rats in the Morris water maze. In this study, F344 rat  
7 dams and pups were fed Purina Test Diet 8117, a casein-based rodent chow. **[No information was**  
8 **provided about caging or bedding materials.]** Treatment groups were assembled by including pups from  
9 different litters such that there was a member of each treatment group from each sex from each litter: a  
10 control animal was always present in each litter. Ten pups/sex/group were gavaged from PND 1 (day  
11 of birth = PND 0) through PND 14 with bisphenol A (>99% purity) at 0 (safflower oil vehicle), 0.1, and  
12 0.25 mg/kg bw/day. An additional group of rats was gavaged with 17 $\beta$ -estradiol 72  $\mu$ g/kg bw/day during  
13 the same time period. Straight channel swimming was tested on PND 33. Spatial learning and memory  
14 were tested by Morris water maze for 4 days beginning on PND 34. In the test, acquisition of maze solution  
15 occurred when the rat found a platform. A probe trial measuring the amount of time spent in an escape  
16 quadrant from which the platform had been removed was conducted on PND 40. Data were analyzed by  
17 ANOVA followed by means separation by least squared means or Greenhouse-Geisser adjusted F ratios.

18  
19 There were no significant effects of bisphenol A treatment on straight channel swimming or time to  
20 acquisition of maze solution in the Morris maze test. Time spent in the escape quadrant was significantly  
21 lower in females of the high-dose group **[by ~38%]** than in controls. The study authors noted that  
22 acquisition of maze performance was significantly better in control males than control females. However,  
23 no sex-related difference was observed following treatment with the low bisphenol A dose. Increased time  
24 to acquisition in males on the third day of testing, and no sex-related differences in performance were  
25 reported for the 17 $\beta$ -estradiol group. The study authors concluded “These data indicate that [17 $\beta$ -estradiol]  
26 and low dosages of [bisphenol A] can alter the normal sex-dependent pattern of acquisition, while higher  
27 dosages of [bisphenol A] alter the retention of spatial information without significantly affecting  
28 acquisition.”

29  
30 **Strengths/Weaknesses:** Strengths are the additional behavioral dimensions captured by this paper and the  
31 use of a positive control. The analyses appeared appropriate. The within litter dosing design raises concerns  
32 about cross-contamination which would decrease differences between groups and challenge interpretation  
33 of results of non-standard dose-response curves. Analyses did not account for the repeated measures  
34 design, thus inflating degrees of freedom. A weakness is the limited number of endpoints investigated.

35  
36 **Utility (Adequacy) for CERHR Evaluation Process:** This study is considered inadequate because of the  
37 limitations noted.

38  
39 **Della Seta et al. (369)**, supported by MURST and the University of Siena, examined the effects of pubertal  
40 bisphenol A exposure on behavior of male rats. **[No information was provided in the manuscript about**  
41 **feed, caging, or bedding. The Expert Panel has been informed that Harlan Teklad 2018 chow,**  
42 **Lignocel bedding, and polysulfone cages were used (F. Faraboli et al., personal communication,**  
43 **March 1, 2007).]** Seventy-eight Sprague Dawley males were obtained from 16 dams and housed in groups  
44 of 4 with each from a different litter. On PND 23–30 (day of birth not defined), the rats were fed (by  
45 micropipette) peanut oil vehicle, 0.040 mg/kg bw/day bisphenol A **[purity not reported in the**  
46 **manuscript;  $\geq$ 95% according to the authors (F. Faraboli et al., personal communication, March 1,**  
47 **2007)],** or 0.4  $\mu$ g/kg bw/day ethinyl estradiol. **[The number of rats treated in each group was not**  
48 **specifically indicated, but can be inferred to be 24–26/group.]** On PND 45, 12 males/group were tested  
49 for social and non-social behavior in response to a black PVC tube introduced into the cage. Behaviors  
50 were examined according to factor clusters of play and social interaction, environmental exploration and  
51 social investigation, and elements directed to the object. Twelve adults/group (> 90 days of age) were

### 3.0 Developmental Toxicity Data

1 tested for sexual behavior with a sexually receptive female. Males that were not used in behavioral testing  
2 were killed on PND 37 (n = 7 or 8/group) and 105 (n = 5 or 6/group) to measure plasma 17 $\beta$ -estradiol and  
3 testosterone levels by RIA. Data were assessed by ANOVA and Fisher least significant difference test.  
4

5 Around the time of treatment, bisphenol A effects on juvenile behavior were not found on factors  
6 associated with environmental exploration and social investigation or with play and social interaction.  
7 However, juvenile behaviors directed to the object (biting, sniffing, climbing) occurred at a significantly  
8 lower frequency in the bisphenol A than control group. Compared to the vehicle controls, the ethinyl  
9 estradiol group exhibited lower frequencies of behaviors associated with environmental exploration or  
10 social investigation and with behaviors directed to the object. With respect to adult sexual behavior, data  
11 from the 9 or 10 of 12 animals/group that were sexually active were analyzed. Decreased intromission  
12 latency was significantly affected in males from the bisphenol A group. Significant effects in the ethinyl  
13 estradiol compared to the control group included decreased intromission latency as well as decreased  
14 latency to mount, increased frequency of intromission, increased ratio of intromissions/mount, and  
15 decreased duration of genital sniffing. On PND 37, the plasma testosterone level was significantly lower in  
16 the bisphenol A and ethinyl estradiol group than in controls. The plasma testosterone level was also  
17 significantly lower in the bisphenol A than control group on PND 105. No effects were observed on plasma  
18 17 $\beta$ -estradiol levels. The study authors concluded that the behavioral effects observed in the bisphenol A-  
19 exposed rats occurred in the same direction as those observed in the ethinyl estradiol group and could be  
20 interpreted as consistent with estrogenic mediation.  
21

22 **Strengths/Weaknesses:** This study was well-conceived and executed. Appropriate dosing periods, design,  
23 and testing methods and timeframes were used to capture developmental effects of pubertal bisphenol A  
24 exposure of a short-term (juvenile period) and long term (into adulthood) nature. Sample sizes were  
25 adequate.  
26

27 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is adequate and of high utility for use in  
28 the evaluation process.  
29

30 **Ceccarelli et al. (370)**, supported by the University of Siena and MIUR, investigated the effects of orally  
31 administered bisphenol A and ethinyl estradiol during puberty in Sprague-Dawley rats. Sixteen pregnant  
32 Sprague Dawley rats gave birth to offspring that were cross-fostered on PND 1, weaned on PND 21, and  
33 housed in groups of 4 males and 4 females. **[No details of housing conditions during gestation were  
34 provided, including individual or group residency, bedding or cage material, or diet.]** On PND 31,  
35 male and female offspring were separately housed in groups of 4 in Plexiglas cages with free access to  
36 water and food and maintained under a reversed light cycle. On PND 23–30, rats (n = 14/group) were given  
37 bisphenol A 40  $\mu$ g/kg bw/day, ethinyl estradiol 0.4  $\mu$ g /kg bw/day, or peanut oil vehicle. Half the offspring  
38 (n = 7/group) were killed on PND 37 and half on PND 90. Females killed on PND 90 were killed in estrus.  
39 Blood samples were taken and animals were formalin perfused. Brains were harvested, post-fixed, and  
40 cryopreserved. Immuno-histochemistry was performed on frozen sections for comparative ER $\alpha$  level  
41 analysis, with a focus on sexually dimorphic regions of the hypothalamus: the arcuate nucleus,  
42 ventromedial nucleus, and medial preoptic area. Two or three sections/rat were stained, equivalent field  
43 areas outlined, and ER $\alpha$ -positively stained nuclei counted under light microscopy by an evaluator blinded  
44 to all experimental parameters. Serum testosterone and 17 $\beta$ -estradiol were determined by RIA. Statistical  
45 analyses were performed using ANOVA and post-hoc least significant difference test.  
46

47 The results for ER $\alpha$  are shown in [Table 73](#). There were few statistically significant difference between  
48 controls and treated rats. Effects identified for ethinyl estradiol were not seen with bisphenol A with the  
49 exception of an increase in bisphenol A-treated females compared to males in ER $\alpha$  at 90 days in the medial  
50 preoptic area. On PND 37, testosterone was significantly reduced [**~40%**] in bisphenol A treated males

### 3.0 Developmental Toxicity Data

1 compared to control males. There were no significant effects of bisphenol A treatment on 17β-estradiol or  
2 on testosterone/17β-estradiol ratio.

3  
4 The authors conclude that exposures to bisphenol A at 40 μg/kg bw/day during early puberty can induce  
5 both short-term and long-term changes in sexually dimorphic regions of the brain and circulating  
6 testosterone/17β-estradiol ratio.

7  
8 **Table 73. Effects of Pubertal Exposure to Bisphenol A on ERα Levels in Sexually Dimorphic**  
9 **Hypothalamic regions in the Rat**

Region	PND	Comparison, % change						
		To oil control				Males to females		
		Bisphenol A		Ethinyl estradiol		Control	Bisphenol A	Ethinyl estradiol
		Males	Females	Males	Females			
Arcuate nucleus	37	↔	↔	↔	↔	↔	↔	↔
	90	↔	↔	↔	↔	↔	↔	↔
Ventromedial nucleus	37	↔	[↑50]	[↑112]	↔	↔	↔	[↑70 in males]
	90	↔	↔	↔	↔	↔	↔	↔
Medial preoptic area	37	↔	↔	↔	↔	↔	↔	↔
	90	↔	↔	↔	[↑85]	↔	[↑50 in females]	[↑118 in females]

↑,↓,↔ Statistically significant increase, decrease, or no change compared to vehicle-treated, orchietomized control.  
Comparisons estimated from a graph.

From Ceccarelli et al. (370)

10  
11 **Strengths/Weaknesses:** Strengths: This interesting and novel manuscript examined the potential for the  
12 ethinyl estradiol positive control and bisphenol A administered prior to puberty, but after the most sensitive  
13 period (i.e., PND 3–10), to modulate ER and steroid hormones during puberty and sexual maturity. It  
14 appears that the authors tried to remove the potential for bias by blinded quantification of ER-positive  
15 neurons. The oral route of exposure was relevant. These data must be linked functionally to the results of  
16 Della-Seta et al., 2006 (369). A weakness is that hormonal measurements were taken at single time points.

17  
18 **Utility (Adequacy) for CERHR Evaluation Process:** These data are adequate and of high utility for the  
19 evaluation process.

#### 20 21 3.2.4 Rat—parenteral exposure postnatally

##### 22 23 3.2.4.1 Reproductive endpoints

24 **Fisher et al. (371)**, supported by the European Centre for Ecotoxicology of Chemicals and Zeneca,  
25 examined the effect of neonatal bisphenol A exposure on excurrent ducts of the rat testis. On PND 2–12  
26 (PND 1 = day of birth), Wistar rat pups were sc injected with the corn oil vehicle or 37 mg/kg bw/day  
27 bisphenol A [purity not given]. The dose was based on the solubility limit in oil. [The number of rats  
28 treated was not indicated nor was relationship to litter, but based on the number of rats examined in  
29 each time period (~3–7 in treated group and 5–20 in control group), it appears that there were  
30 ~25/group in the bisphenol A group and ~48 in the vehicle control group. No information was  
31 provided about caging or bedding materials.] Seven other compounds were also examined but will not  
32 be discussed, with the exception of a brief explanation of results obtained with 0.0037–0.37 mg/kg bw/day  
33 diethylstilbestrol. Rats were killed at 10, 18, 25, 35, and 75 days of age. Testes and epididymides were  
34 removed and fixed in Bouin solution. Immunohistochemistry techniques were used to examine water  
35 channel aquaporin-1 levels. Morphology of rete testis and efferent duct were examined. Data were analyzed  
36 by ANOVA.

37

### 3.0 Developmental Toxicity Data

1 In the bisphenol A group, the only effect on testis weight was a significant decrease [~40%] at 35 days of  
2 age. Epithelial cell height in the efferent ducts was significantly reduced [by ~15%] at 18 and 25 days of  
3 age, but not at later time periods. There was no effect on expression of water channel aquaporin-1 protein  
4 or morphology of the rete testis. Treatment with most diethylstilbestrol doses resulted in reduced testicular  
5 weights at all ages, decreased expression of water channel aquaporin-1 protein, and decreased epithelial  
6 cell height in efferent ducts at 25 days of age and younger, and fluid retention and enlargement of rete  
7 testis, which was most severe at PND 18 and 25. The study authors concluded that the magnitude and  
8 duration of adverse effects induced by estrogenic compounds were broadly similar to the estrogenic  
9 potencies of the compounds.

10  
11 **Strengths/Weaknesses:** This is a carefully performed study, although the inclusion of many  
12 methodological details (*vide supra*) would have improved it. Strengths include the use of a wide range of  
13 estrogenic compounds to alter testicular development. A limitation for the present purpose is that only a  
14 single dose level of bisphenol A was administered subcutaneously. A weakness is that tissues other than the  
15 testis were not examined. Other weaknesses include sample sizes ranging from 3-20 examined pups across  
16 groups and sc administration.

17  
18 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for evaluation due to lack  
19 of clarity about experimental or statistical control for litter effects.

20  
21 **Nagao et al. (372)**, supported by the Japanese Ministry of Health and Welfare, examined the effects of  
22 neonatal bisphenol A exposure on reproductive function of male and female Sprague Dawley given CE-2  
23 feed (Clea Japan). **[No information was provided about caging or bedding materials.]** From PND 1 to 5  
24 (birth by 16:00 considered PND 0), 28–31 pups/sex/group were sc injected with corn oil vehicle, 300  
25 mg/kg bw/day bisphenol A **[purity not reported]**, or 2 mg/kg bw/day estradiol benzoate. Pups within  
26 litters were treated with the same dose. Doses were based on results of preliminary studies that  
27 demonstrated no effect on growth or viability at bisphenol A doses up to 300 mg/kg bw/day administered  
28 by sc injection in the neonatal period. Pups were examined for viability from PND 6 to 21. On PND 21, 5  
29 pups/sex/group were randomly selected and killed. Pups were transcardially perfused, and reproductive  
30 organs were collected for histopathological evaluation. At 12 weeks of age, 22–25 rats/sex were mated with  
31 untreated rats. Females were killed on GD 13 for an evaluation of implant number and viability of embryos.  
32 After fertility evaluation, sexual behavior with a sexually receptive female was assessed in 10 males/group.  
33 Following evaluation of sexual behavior, 15 male rats/group were killed for measurement of reproductive  
34 organ and brain weight. Histopathology of reproductive organs and SDN-POA volume were measured in 5  
35 males/group. Copulation and fertility indices were analyzed by chi-squared and Fisher exact 1-tailed test.  
36 Data for other endpoints were analyzed by Student *t*-test.

37  
38 In rats treated with bisphenol A, there were no clinical signs of toxicity or effects on pup viability or body  
39 weight gain during or following the lactation period **[data for pup viability not shown by study authors]**.  
40 There were no effects on age of vaginal opening or preputial separation. Copulation and fertility indices  
41 and numbers of live embryos/litter were not affected in male or female rats treated with bisphenol A.  
42 Bisphenol A treatment did not affect sexual behaviors of males, as determined by number of mounts,  
43 intromissions, and ejaculations. No histopathological alterations were observed in the ovaries of treated  
44 females at 21 days of age or in the epididymis, prostate, or seminal vesicles of treated male rats at 21 days  
45 or 14 weeks of age. **[The prostatic lobe not specified; based on the figure provided, the lobe seems to**  
46 **have been ventral prostate. The Expert Panel notes that the number of apically located nuclei may be**  
47 **elevated by 14 weeks of age over what would normally be expected; however, this observation cannot**  
48 **be determined definitively based on a single high power field and in the absence of a matched**  
49 **control.]** No effect of treatment was observed on the SDN-POA of males. In contrast to the bisphenol A  
50 groups, rats treated with estradiol benzoate experienced decreased body weight gain, compromised male  
51 sexual behavior, infertility, lesions in reproductive organs, and reduced volume of the SDN-POA. The

### 3.0 Developmental Toxicity Data

1 study authors concluded that neonatal exposure to a relatively high dose of bisphenol A had no effect on  
2 morphological development or function of the reproductive system.

3  
4 **Strengths/Weaknesses:** Strengths include a well performed and documented study that compared effects  
5 of bisphenol A and estradiol benzoate. Additional strengths include documentation of both behavioral  
6 (mating behavior) and biological (genital tract development) endpoints in both male and female rats.  
7 Weaknesses include the use of only a single dose level of bisphenol A via subcutaneous injection, and no  
8 accounting for litter effects within the context of individual animal treatments within litters.

9  
10 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is adequate for evaluation, however  
11 utility is limited by subcutaneous administration.

12  
13 **Stoker et al. (373)**, support not indicated, examined the effects of prepubertal bisphenol A exposure on  
14 prolactin secretion and prostate size in rats. **[No information was provided about feed, bedding, or**  
15 **caging materials.]** On PND 22–32 (day of birth = PND 0), 15–17 male Wistar rats from different  
16 litters/group were sc injected with bisphenol A **[purity not reported]** at 0 (sesame oil vehicle) or 50 mg/kg  
17 bw **[assumed to be 50 mg/kg bw/day]**. Another group of rats was administered 17 $\beta$ -estradiol through a sc  
18 Silastic tube implant **[dose administered not clear]**. On PND 29, 6 animals/dose were killed and blood  
19 was collected for measurement of serum prolactin concentration. The remaining rats (n = 9–11/group) were  
20 killed at 120 days of age. Prolactin levels were measured in serum and anterior pituitary by RIA.  
21 Inflammation was visually examined in the ventral and lateral prostate. Left lateral and ventral prostates  
22 were weighed and lateral prostate was analyzed for myeloperoxidase (an indicator of neutrophil numbers)  
23 and DNA. The right lateral prostate was subjected to histological examination. Statistical analyses included  
24 ANOVA, Dunnet *t*-test for multiple comparison, and Fisher exact probability test.

25  
26 On PND 29, serum prolactin levels were significantly increased by ~210% in rats of the bisphenol A group  
27 compared to the control group. On PND 120, there was no effect on prolactin levels in serum or pituitary in  
28 the bisphenol A group. Ventral prostate weight was unaffected but lateral prostate weight was increased  
29 **[by ~25%]** in the bisphenol A group. Exposure to bisphenol A had no effect on body or testis weight.  
30 **[Data were not shown by study authors.]** The myeloperoxidase assay was reported to show a “trend” for  
31 lateral prostate inflammation in the bisphenol A group. **[Trend was not defined; there was no statistical**  
32 **difference between the bisphenol A group and the control in the myeloperoxidase assay.]** No  
33 histological evidence of inflammation was observed in prostates from the control group. In the bisphenol A  
34 group, histopathological analyses revealed that 44.4% of prostates contained increased a focal luminal  
35 polymorphonuclear cellular infiltrate that was milder in severity compared to prostates from the 17 $\beta$ -  
36 estradiol group. The study authors noted the discrepancy between the results obtained by myeloperoxidase  
37 assay and histological observation in the bisphenol A group and stated that the discrepancy may have been  
38 due to evaluation of the whole tissue by myeloperoxidase assay versus only one section of the tissue by  
39 histological evaluation. Bisphenol A had no effect on prostate DNA content. In addition to prostate  
40 inflammation, effects observed in the 17 $\beta$ -estradiol group were increased serum prolactin levels on PND 29  
41 and elevated myeloperoxidase and DNA content in lateral prostate on PND 120. Based on these findings,  
42 the study authors concluded that chemically induced, transient increases in prolactin secretion in the  
43 prepubertal period can lead to increased incidence of lateral prostate inflammation in 120-day-old rats.

44  
45 **Strengths/Weaknesses:** Comparison with other agents is a strength. Weaknesses include low to moderate  
46 sample sizes and the use of a single high dose level of bisphenol A through subcutaneous administration.

47  
48 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate for the evaluation process  
49 but has limited utility due to concerns about sample sizes and route of administration of treatment.

50

### 3.0 Developmental Toxicity Data

1 **Atanassova et al. (374)**, supported by the European Center for the Ecotoxicology of Chemicals and  
2 AstraZeneca, examined the effects of neonatal bisphenol A exposure on the reproductive system of male  
3 rats. Wistar rats were fed rat and mouse breeding diet No. 3, which contains 15.5% soy meal flour. **[No**  
4 **information was provided about caging and bedding materials.]** Litters of 8–12 male rats from  
5 randomized litter origin were assembled by cross-fostering pups on PND 1 (day of birth). On PND 2–12,  
6 rats were sc injected with corn oil vehicle or bisphenol A **[purity not given]** 0.5 mg/day. **[Assuming a 5–**  
7 **25 g body weight during this interval, this dose would be ~100 mg/kg bw/day at the beginning of the**  
8 **interval and ~20 mg/kg bw/day at the end of the interval.]** Other groups of rats were sc injected with  
9 0.01–10 µg diethylstilbestrol every other day between PND 2 and 12 or 2 mg 4-tert-octylphenol/day during  
10 PND 2–12. Rats were killed on PND 18, 25, and 90–100. At PND 18 and 25, testes were weighed and fixed  
11 in Bouin solution. Testicular cell numbers and seminiferous tubule lumen formation were determined by  
12 standard point counting of cell nuclei. Apoptosis was assessed by DNA fragmentation detected by in situ  
13 DNA 32-end labeling. Spermatocyte nuclear volume as a fraction of Sertoli cell nuclear volume was  
14 calculated as “an index of spermatogenic efficiency.” Plasma FSH and inhibin B were measured by RIA  
15 and ELISA methods, respectively. Fertility was assessed at 80–90 days of age; rats were mated for 7 days  
16 and number of pups was counted at birth. The number of rats/group examined was 7–14 at 18 days of age,  
17 4–12 at 25 days of age, and 6 in fertility testing. Data were analyzed by ANOVA.

18  
19 Significant effects observed on PND 18 were advanced testicular lumen formation and increases in testis  
20 weight, Sertoli cell volume/testis, and spermatocyte nuclear volume/unit Sertoli cell. A decrease in germ  
21 cell apoptosis was also described on PND 18 but was not statistically significant. Plasma FSH levels were  
22 significantly increased on PND 18, but there was no effect on plasma inhibin B concentration. The only  
23 significant effect observed on PND 25 was increased plasma FSH levels. Testis weight was increased in  
24 adulthood, but there were no effects on fertility or litter size. Effects observed with octylphenol were  
25 similar to those observed with bisphenol A. In contrast, exposure to one or more doses of diethylstilbestrol  
26 resulted in increased apoptosis, decreased plasma inhibin levels, decreased Sertoli cell nuclear volume, and  
27 changes in spermatocyte/Sertoli cell ratios. The study authors concluded that the effect of bisphenol A on  
28 spermatogenic processes is benign.

29  
30 **Strengths/Weaknesses:** Comparison with other agents is a strength. Weaknesses include low to moderate  
31 sample sizes, the use of a single high dose level of bisphenol A through subcutaneous administration, and  
32 no accounting for litter effects within the context of individual animal treatments within litters.

33  
34 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate for the evaluation process  
35 but has limited utility due to concerns about sample sizes and route of administration of treatment.

36  
37 **Williams et al. (375)**, supported by the European Centre for Ecotoxicology, examined the effect of  
38 neonatal bisphenol A exposure on seminal vesicle structure and expression of sex steroid receptors in rats.  
39 On PND 2 (day of birth = PND 1), litters consisting of 8–14 male Wistar rat pups were derived through  
40 cross-fostering. Rats were sc injected with corn oil vehicle or 0.5 mg/day bisphenol A on PND 2–12.  
41 **[Assuming a 5–25 g body weight during this interval, the dose would be ~100 mg/kg/day at the**  
42 **beginning of the interval and ~20 mg/kg bw/day at the end of the interval.]** The dose was based on the  
43 highest amount that could remain in solution. A positive control group was injected with diethylstilbestrol  
44 at 0.1, 1, or 10 µg/day on PND 2, 4, 6, 8, 10, and 12. Ethinyl estradiol was administered at 10 µg/day,  
45 according to the protocol for diethylstilbestrol. Control animals for each compound were dosed with  
46 vehicle on the appropriate days, and because no differences were noted for controls, data were pooled. The  
47 effects of 4-tert-octylphenol, genistein, Antarelix, flutamide, and tamoxifen were also examined but will  
48 not be discussed. **[No information was provided about feed, caging or bedding materials, or purity of**  
49 **compounds.]** Animals were killed on PND 18, and seminal vesicles from 11–15 animals/group were  
50 collected and stored in Bouin solution. Seminal vesicles were examined for gross abnormalities in stroma  
51 and epithelium. Immunolocalization studies were conducted to assess ERβ, ERα androgen receptor, and

### 3.0 Developmental Toxicity Data

1 progesterone receptor proteins in the seminal vesicle. Studies were replicated 3–5 times using samples from  
2 at least 6 animals/group. Results were subjectively scored.

3  
4 The gross structure of the seminal vesicles from bisphenol A-treated rats appeared normal, and there were  
5 no changes in ER $\beta$ , ER $\alpha$ , androgen receptor, or progesterone receptor proteins in the seminal vesicle. In  
6 contrast, diethylstilbestrol induced changes in seminal vesicle morphology, increased ER $\alpha$  and  
7 progesterone receptor, and decreased androgen receptor. Effects of ethinyl estradiol were similar to those  
8 observed with diethylstilbestrol. The study authors concluded that the lack of bisphenol A effects suggested  
9 that only high doses of potent estrogens induce gross abnormalities in the male reproductive system; and  
10 that only agents that suppress androgen receptor while increasing ER $\alpha$  and progesterone receptor are likely  
11 to cause gross developmental abnormalities in the male reproductive system.

12  
13 **Strengths/Weaknesses:** Strengths include expertise of the group coupled to well-performed experiments,  
14 data recording, and interpretation. Bisphenol A was not a primary target in this study but was one of a  
15 series of estrogenic compounds, allowing comparison with other similar compounds. However, a  
16 significant weakness are the sc route of administration, only a single varying dose level of bisphenol A was  
17 used and there was no accounting for litter effects within the context of individual animal treatments within  
18 litters.

19  
20 **Utility (Adequacy) for CERHR Evaluation:** This work is inadequate for the evaluation process, based on  
21 lack of clarity for experimental or statistical control for litter effects.

22  
23 **Rivas et al. (376)**, supported by the European Union and the Spanish Ministry of Education, examined the  
24 effects of bisphenol A exposure on reproductive tract development of male rats. The main focus of the  
25 study was determining the effects of decreased androgen production in combination with a low dose of  
26 diethylstilbestrol. Effects of flutamide were also examined but will not be discussed. Wistar rats were fed a  
27 soy-free diet (rat and mouse soya-free breeding diet, SDS, Dundee, Scotland). **[No information was**  
28 **provided about caging and bedding materials.]** Litters of 8–12 male pups were assembled by cross-  
29 fostering on PND 1 (day of birth). Male rats were sc injected with the corn oil vehicle or 0.1 mg bisphenol  
30 A **[purity not indicated]** on PND 2, 4, 6, 8, 10, and 12 with and without co-administration of 10 mg/kg  
31 GnRH antagonist (a suppressor of androgen production). **[Assuming a 5–25 g body weight during this**  
32 **interval, the bisphenol A dose would be ~20 mg/kg bw/day at the beginning of the interval and ~4**  
33 **mg/kg bw/day at the end of the interval.]** Additional rats were sc injected with diethylstilbestrol at doses  
34 of 0.1 or 10  $\mu$ g on PND 2, 4, 6, 8, 10, and 12 with and without administration of GnRH antagonist. Rats  
35 were killed on PND 15. The testis was fixed in Bouin solution and testicular structures were measured.  
36 Plasma testosterone levels were measured using an ELISA technique. From 3 to 10 animals/group were  
37 examined for each endpoint. Data were analyzed by ANOVA.

38  
39 Treatment with bisphenol A alone did not affect plasma testosterone levels but treatment with GnRH  
40 antagonist alone and in combination with bisphenol A significantly lowered plasma testosterone levels.  
41 Treatment of rats with bisphenol A alone or in combination with GnRH antagonist had no significant effect  
42 on rete testis luminal area, efferent duct luminal area, efferent duct epithelial cell height, or vas deferens  
43 epithelial cell height. Exposure to the high diethylstilbestrol dose increased rete area, and both doses of  
44 diethylstilbestrol decreased plasma testosterone levels, increased efferent duct luminal area, and decreased  
45 epithelial cell height in efferent duct and vas deferens. The study authors concluded that the estrogenicity of  
46 bisphenol A when injected at a moderately high dose was insufficient for disrupting the estrogen-androgen  
47 balance in rats.

48  
49 **Strengths/Weaknesses:** This study was carefully performed and well documented. Weaknesses include:  
50 the dose of bisphenol A was high and only a single dose level administered subcutaneously was examined,  
51 and litter effects were not addressed in the context within litter dosing of cross-fostered litters.

### 3.0 Developmental Toxicity Data

1  
2 **Utility (Adequacy) for CERHR Evaluation Process:** This work is inadequate for the evaluation process,  
3 based on lack of clarity on control for litter effects.  
4

5 **Sharpe et al. (377)**, supported in part by the European Union and the Spanish Ministry of Education,  
6 examined the effects of neonatal exposure of rats to bisphenol A on Leydig cell development and function.  
7 Wistar rat dams were fed a standard soy-containing feed (rat and mouse breeding diet, SDS, Dundee, UK).  
8 **[No information was provided on feed given to male offspring following weaning or bedding and**  
9 **caging materials.]** Litters of 9–12 male pups were created by cross fostering pups on PND 1 (day of birth).  
10 Male pups were sc injected with the corn oil vehicle or 0.5 mg/day bisphenol A **[purity not reported]** on  
11 PND 2–12. **[Assuming 5–25 g body weight during this interval, the dose would be ~100 mg/kg bw/day**  
12 **at the beginning of the interval and ~20 mg/kg bw/day at the end of the interval.]** Other groups of rats  
13 received diethylstilbestrol at 0.1–10 µg/day on PND 2, 4, 6, 8, 10, and 12. Additional rats were treated with  
14 GnRH antagonist Antarelix or 4-tert-octylphenol, but those results will not be discussed. Rats were killed  
15 on PND 18, 25, 35, or 90. Testes were weighed and fixed in Bouin solution. Sections of testes were  
16 immunostained with the Leydig cell marker 3β-hydroxysteroid dehydrogenase to evaluate Leydig cell  
17 development in 5–7 animals/group. Plasma testosterone levels were measured by ELISA. Group sizes for  
18 evaluation of testes weight and plasma testosterone were 2–23, with most groups containing at least 8  
19 animals. Data were analyzed by ANOVA.  
20

21 The only significant effect on plasma testosterone level following exposure to bisphenol A was an increase  
22 on PND 18 (n = 4). In rats of the bisphenol A group examined at each time period, there were no significant  
23 effects on testis weight, percent Leydig cell nuclear volume/testis, Leydig cell nuclear volume/testis, or  
24 total Leydig cell volume (nuclear + cytoplasmic volume/testis). Significant results in rats exposed to  
25 diethylstilbestrol included decreased Leydig nuclear cell volume at the mid and/or high dose on or before  
26 PND 35 and reduced plasma testosterone level and testis weight at all doses and most time points of  
27 evaluation. The study authors concluded that there were no consistent changes in Leydig cell development  
28 following exposure to bisphenol A.  
29

30 **Strengths/Weaknesses:** A strength is that bisphenol A was one of a number of compounds examined  
31 enabling internal comparison with other similar molecules. Limitations include use of a single high but  
32 variable dose of bisphenol A and small sample sizes for critical endpoints.  
33

34 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate due to small or uncertain  
35 sample sizes for key endpoints.  
36

37 **Khurana et al. (378)**, supported by NIH, March of Dimes, and Pardee Foundation, examined the effects of  
38 neonatal bisphenol A exposure on prolactin levels in rats. **[The type of chow used and composition of**  
39 **bedding and caging materials were not reported.]** On PND 1–5 (day of birth = PND 0), 8–10 Fischer  
40 344 rat pups/sex/group (litter relationships are unclear) were sc injected with the tocopherol-stripped corn  
41 oil vehicle, bisphenol A **[purity not indicated]** at 0.1 or 0.5 mg/day, diethylstilbestrol at 5 µg/day, or  
42 octylphenol at 0.1 or 0.5 mg/day. **[Assuming a pup body weight of 5 g, bisphenol A intakes were**  
43 **estimated at 20 and 100 mg/kg bw/day.]** On PND 15, 20, and 25, blood was collected for measurement of  
44 serum prolactin level by RIA. A final sample for prolactin analysis was obtained when animals were killed  
45 on PND 30. Medial basal hypothalamus, anterior pituitary, uterus, and prostate were collected for  
46 measurement of *ERα* and *ERβ* mRNA expression by RT-PCR in animals of the low-dose group. Statistical  
47 analyses included ANOVA followed by Student-Newman-Keuls test.  
48

49 In male and female rats, hyperprolactemia was observed on PND 25 and 30. **[On PND 30, prolactin levels**  
50 **in the low- and high-dose groups compared to the control group were ~ 150 and 95% higher in**  
51 **females and 120 and 80% higher in males].** In females exposed to the low dose, *ERα* mRNA in the

### 3.0 Developmental Toxicity Data

1 medial basal hypothalamus was higher [by 25%] than control levels. In anterior pituitary of low-dose  
2 males, *ERα* mRNA was higher [by ~80%] and *ERβ* mRNA was higher by 35–40% compared to control  
3 levels. There were no effects on *ERβ* mRNA in female tissues. Most effects observed with octylphenol  
4 exposure were similar to those observed with bisphenol A exposure. Diethylstilbestrol induced transient  
5 increases in prolactin levels, decreased expression of *ERα* in medial basal hypothalamus of males,  
6 upregulated *ERα* and *ERβ* expression in the pituitary of males, decreased expression of *ERα* in the uterus,  
7 and upregulated *ERβ* expression in prostate. The study authors concluded that exposure of neonatal rats to  
8 bisphenol A resulted in delayed and sustained hyperprolactemia and changes in *ER* mRNA expression.  
9

10 **Strengths/Weaknesses:** A strength is that both male and female animals were assessed following  
11 administration of two dose levels. Weaknesses include small treatment groups consisting of unclear  
12 numbers of litters and composition and limited experimental details regarding design.  
13

14 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
15 due to lack of design clarity.  
16

17 **Fukumori et al. (379)**, support not indicated, examined the effect of postnatal bisphenol A exposure on  
18 ultrastructure of the prostate in rats. [The study was published in Japanese; a translation was provided  
19 by the American Plastics Council.] On day 1–21 following birth, F344 rats were sc injected with  
20 bisphenol A 5 days/week at doses of 0 (DMSO vehicle), 0.0008, 0.004, 0.020, and 0.500 mg/kg bw/day. A  
21 positive control group received 100 µg/kg bw 17β-estradiol by sc injection during the same time period.  
22 Rats were killed at 22 days of age. Ventral prostates were fixed in glutaraldehyde, sectioned, and examined  
23 by electron microscopy. [The number of rats treated and examined/group and the number of litters  
24 represented were not reported. No information was provided on purity of bisphenol A, type of feed,  
25 or composition of bedding and caging. The translated version of the report did not include figures  
26 from the original report.]  
27

28 In ventral prostates obtained from rats exposed to 17β-estradiol, there was an increase in secretory granules  
29 accompanied by reductions in microvilli on the surface of the glandular epithelium. Proliferation of  
30 fibroblasts was observed in the fibromuscular layer of the stroma in rats from the 17β-estradiol group. In  
31 the 0.020 and 0.500 mg/kg bw/day bisphenol A groups, a slight increase in secretory granules and slight  
32 decrease in microvilli was observed in glandular epithelium. Effects in stroma were described as  
33 unremarkable for the bisphenol A groups. The study authors concluded that bisphenol A may have  
34 ultrastructural effects on the ventral prostates of suckling rats.  
35

36 **Strengths/Weaknesses:** This is a translation of an apparently carefully performed study to assess the  
37 effects of low doses of perinatal bisphenol A on prostatic structure. A major weakness is that the original  
38 figures were not provided in the translated version of the report, and the route is subcutaneous injection in  
39 DMSO. The young age at which the animals were sacrificed is also a concern because prostatic  
40 development is not complete at 22 days of age making comparisons with the bulk of established data  
41 problematic. The lack of data specifics raise the level of uncertainty about this study.  
42

43 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is considered inadequate for inclusion in  
44 the evaluative process because of the lack of detail on study design (i.e., litter representation, number of  
45 animals per group).  
46

47 **Kato et al. (380)**, supported by the Japanese Ministry of Education, Culture, Sports, Science, and  
48 Technology and the Ministry of Health, Labor, and Welfare, examined the effects of neonatal bisphenol A  
49 exposure on the reproductive organs of rats. Sprague Dawley rats were fed CRF-1 diet. [No information  
50 was provided on caging or bedding materials.] Female offspring from 8 dams were grouped to achieve

### 3.0 Developmental Toxicity Data

1 equal distribution of body weight. At least 8 female offspring/group were sc injected with 0 (ethanol/corn  
 2 oil vehicle), 0.25, 1, or 4 mg/day bisphenol A [purity not reported] from PND 0 to 9 (day of delivery =  
 3 PND 0). [Based on body weights reported on PND 0 and 9, CERHR calculated mean bisphenol A  
 4 intakes of ~26, 105, and 427 mg/kg bw/day.] A positive control group was given 10 µg/day 17β-estradiol  
 5 [~3 mg/kg bw/day] during the same time period. Rats were weighed during and following the lactation  
 6 period and examined for day of vaginal opening. External reproductive organs were examined on PND 60,  
 7 and estrous cycles were assessed from PND 61 to 94. One group of rats was ovariectomized on PND 80;  
 8 ovaries were weighed, and fixed in 10% neutral buffered formalin for evaluation of corpora lutea and  
 9 polyovular follicles. Another group of bisphenol A-exposed and the vehicle-treated control females were  
 10 given 1 µg/kg 17β-estradiol from PND 94 to 96 and killed the day following final injection; uterus and  
 11 vagina were weighed, and fixed in 10% formalin. For all endpoints, 5–8 rats/group were examined.  
 12 Statistical analyses included Student *t*-test and Fisher exact probability test.

13  
 14 Treatment-related results are summarized in Table 74. Two rats of the high-dose group died. Body weights  
 15 of rats in the high-dose group were lower than controls on PND 9–30 but higher than controls on PND 61–  
 16 97. Effects observed at the mid and high dose included accelerated vaginal opening, increased incidence of  
 17 polycystic ovaries, decreased area of corpora lutea, and decreased uterine fluid weight. All rats of the mid-  
 18 dose group had partial clefts in the clitoris, and all rats of the high-dose group had deep clefts in the clitoris.  
 19 Additional effects observed in rats of the high-dose group included disrupted estrous cycles (e.g., irregular  
 20 cycles or persistent estrous) and decreased relative (to body weight) ovary and wet or blotted uterus  
 21 weights. Absolute weights of wet uterus and ovary were also reduced in the high-dose group. No corpora  
 22 lutea were observed in rats of the high-dose group. Qualitatively similar effects were observed in the group  
 23 treated with 17β-estradiol. The study authors concluded that exposure of rats to bisphenol A during the  
 24 neonatal period resulted in changes in female reproductive organs.

25  
 26 **Table 74. Effects in Female Rats Exposed to Bisphenol A During the Neonatal Period**

Endpoint	Dose, mg/kg bw/day [CERHR estimate]						
	26	105	427	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
Body weight gain							
PND 9	↔	↔	↓16%	286	200	233	156
PND 97	↔	↔	↑10%	432	261	430	253
Day of vaginal opening	↔	↓2.9 days	↓4.1 days	345	267	159	116
No. with normal estrous cycles <sup>a</sup>	↔ (8/8)	↔ (2/8)	↓ (0/6)	81	28		
No. with cleft clitoris <sup>b</sup>	↔ (0/8)	↑ (0/8)	↑ (6/6)	299	failed		
Relative organ weight							
Ovary	↔	↔	↓59%	85	59	140	93
Uterus, wet	↔	↔	↓60%	66	55	128	96
Uterus, blotted	↔	↔	↓21%	273	128	318	168
Uterine fluid weight	↔	↓42%	↓97%	42	34	139	104
No. with polycystic ovaries <sup>b</sup>	No data	↑ (4/8)	↑ (5/5)	81	24		
No. with corpora lutea <sup>a</sup>	No data	↔ (8/8)	↓ (0/5)	238	90		
No. of corpora lutea	No data	↔	↓ (none)	65	38	137	83
Corpora lutea area	No data	↓ 30%	↓ (none)	42	37	84	66

↑, ↓ Statistically significant increase or decrease compared to controls; ↔ no statistically significant effect.

<sup>a</sup>Control rate 8/8.

<sup>b</sup>Control rate 0/8.

From Kato et al. (380).

### 3.0 Developmental Toxicity Data

1 **Strengths/Weaknesses:** The strengths are the carefully performed and documented experiments. The  
2 major limitation is that the subcutaneous route of administration and the doses of bisphenol A were  
3 relatively high. The changes in the female reproductive organs seen are well documented, but given the  
4 extremely high dose of agent used, broadly unsurprising.

5  
6 **Utility (Adequacy) for CERHR Evaluation Process:** The results of this study reflect a careful  
7 documentation of the experiments performed. The study is adequate for the evaluation process but has  
8 limited utility due to concerns about the route of administration.

9  
10 **Toyama and Yuasa (381)**, supported in part by the Japanese Ministry of Environment and Ministry of  
11 Education, Science, Sports and Culture, examined the effects of neonatal bisphenol A [**purity not**  
12 **reported**] exposure on spermatogenesis during puberty and adulthood in rats and mice. [**No information**  
13 **was provided about chow or bedding and caging materials. The mouse data are reported in Section**  
14 **3.2.8.**] Wistar rats were sc injected on a  $\mu\text{g}/\text{pup}$  basis with bisphenol A in a DMSO and olive oil vehicle on  
15 PND 1, 3, 5, 7, 9, and 11 (PND 0 = day of birth). Bisphenol A doses were 1.0, 10.0, 100.0, and 600.0  
16  $\mu\text{g}/\text{pup}$ . Additional animals were treated with  $17\beta$ -estradiol and estradiol benzoate. Animals were killed  
17 weekly at 2–10 weeks of age, and other pups were killed at 24 and 31 days of age. There were 5  
18 animals/dose/time point in bisphenol A groups and apparently 5 vehicle control rats/time period. Testes  
19 were examined by light and electron microscopy. Males from each experimental group (a total of 11 rats)  
20 were mated with 2 females [**number tested in each dose group not reported**]. A total of 11 rat dams were  
21 allowed to complete pregnancy. [**It does not appear that statistical analyses were conducted.**]

22  
23 All rats given 0.600  $\mu\text{g}/\text{pup}$  bisphenol A died before 20 days of age and were excluded from analysis. In  
24 mature spermatids of 8-week-old rats in the vehicle control group, the incidences of deformed acrosomes,  
25 deformed nuclei, and abnormal ectoplasmic specialization were below 0.3%. In 8-week-old rats treated  
26 with  $\geq 0.010 \mu\text{g}/\text{pup}$  bisphenol A, the incidence of deformed acrosomes was >50–60%, the incidence of  
27 deformed nuclei was >40%, and the incidence of abnormal ectoplasmic specialization was >60–70%. [**Data**  
28 **were not shown for individual dose levels.**] Similar effects were observed in the groups treated with  $17\beta$ -  
29 estradiol and estradiol benzoate. No effects were reported at other ages. [**Data were not shown by study**  
30 **authors.**] The blood-testis barrier remained intact based on histologic observations. All tested males from  
31 the bisphenol A group were fertile, and sex ratio, litter sizes, and pup weights were reported to be normal.  
32 [**No results were shown for individual dose levels. Fertility data presented in Table 4 and 5 of the**  
33 **study, were not clearly identified by dose level.**] The study authors concluded that bisphenol A acts as an  
34 estrogen and induces transient changes in the male reproductive system of rodents that resolve in  
35 adulthood.

36  
37 **Strengths/Weaknesses:** The strengths include the use of multiple doses of bisphenol A and the use of both  
38 rats and mice, allowing interspecies comparisons. Weaknesses include selective and unclear data  
39 presentation, absence of statistical analyses, subcutaneous injection on a per pup basis, and failure to  
40 examine sperm morphology in the fertile 15 week old animals to determine whether the changes in sperm  
41 maturation seen at earlier time points had resolved or whether the animals were fertile in the face of such  
42 abnormalities.

43  
44 **Utility (adequacy) for CERHR Evaluation Process:** This study is inadequate and not useful for the  
45 evaluation process due to lack of clarity of design and analyses, route of administration and dosing  
46 procedures.

47  
48 **Kato et al. (382)**, supported by the Japanese Ministry of Education, Culture, Sports, Science and  
49 Technology and Ministry of Health, Labor and Welfare, examined the effects of neonatal exposure to  
50 bisphenol A on reproductive function of male rats. Sprague Dawley rats were fed CRF-1 diet, which was

### 3.0 Developmental Toxicity Data

1 described as having relatively low estrogenic activity compared to other Japanese rodent feeds. [No  
2 **information was provided on caging or bedding materials.**] Male rats used in this study were born to 12  
3 dams, assigned to 8 foster dams in groups of 7 based upon body weights, and distributed to dose groups.  
4 From PND 0 to 9 (PND 0 = day of birth), 24 male pups/group were sc injected with bisphenol A [**purity**  
5 **not indicated**] at 0 (ethanol/corn oil vehicle), 0.000024, 0.000120, 0.000600, 0.003, or 1 mg/pup/day  
6 bisphenol A. Study authors calculated average exposures of 0.002, 0.011, 0.056, 0.277, or 97 mg/kg  
7 bw/day. An additional group was treated with 10 µg/day 17β-estradiol (0.9 mg/kg bw/day) during the same  
8 time period. Eight rats/group were killed and necropsied at PND 10, 35, and 150. At the PND 10 necropsy,  
9 serum testosterone levels were measured by RIA, the testis was weighed and examined histologically, and  
10 expression changes in genes for hormone receptors and steroidogenic enzymes were determined by RT-  
11 PCR. The same endpoints were examined at the PND 35 necropsy in addition to measuring seminal vesicle,  
12 ventral prostate, and epididymis weights. The remaining rats were assessed for day of preputial separation.  
13 From PND 105 to 130, they were mated for 1 day a maximum of 4 times with an untreated female in  
14 proestrus. Females were killed on GD 13 (day of sperm = GD 0) and examined for corpora lutea,  
15 embryonic mortality, and implantation sites. Male rats were killed on PND 150. In addition to endpoints  
16 examined at earlier time periods, sperm endpoints and histopathology of ventral prostate were assessed.  
17 Statistical analyses included Bartlett method for homogeneity of variance followed by Dunnett method for  
18 homogeneous variances or Dunnett-type method with rank order for heterogeneous variances.  
19 Reproductive data were analyzed by Fisher exact probability test. Data obtained from the 17β-estradiol  
20 group were analyzed by Student *t*-test.

21  
22 There were no deaths or decreases in body weight in animals of the bisphenol A group. There were no  
23 effects on age of preputial separation, copulation rate, or fertility. In dams impregnated by bisphenol A-  
24 treated males, there were no effects on numbers of implantation sites, implantation losses, or live fetuses.  
25 Bisphenol A treatment had no adverse effects on sperm count, motility, or morphology. There were no  
26 effects on serum testosterone levels, histopathology of testis or prostate, or weights of testis, epididymis,  
27 seminal vesicle, ventral prostate, or penis. No significant changes were observed in mRNA for estrogen,  
28 androgen, or progesterone receptor or steroidogenic enzymes. In contrast to the bisphenol A groups, rats  
29 treated with 17β-estradiol experienced decreases in reproductive organ weights, altered gene expression,  
30 delayed and incomplete preputial separation, decreased copulatory rate, and decreased sperm numbers. The  
31 study authors concluded that neonatal bisphenol A exposure caused no adverse effects on reproductive  
32 function or gene expression of steroidogenic enzymes in the rat testis.

33  
34 There were no deaths or decreases in body weight in animals of the bisphenol A group. There were no  
35 effects on age of preputial separation, copulation rate, or fertility. In dams impregnated by bisphenol A-  
36 treated males, there were no effects on numbers of implantation sites, implantation losses, or live fetuses.  
37 Bisphenol A treatment had no adverse effects on sperm count, motility, or morphology. There were no  
38 effects on serum testosterone levels, histopathology of testis or prostate, or weights of testis, epididymis,  
39 seminal vesicle, ventral prostate, or penis. No significant changes were observed in mRNA for estrogen,  
40 androgen, or progesterone receptor or steroidogenic enzymes. In contrast to the bisphenol A groups, rats  
41 treated with 17β-estradiol experienced decreases in reproductive organ weights, altered gene expression,  
42 delayed and incomplete preputial separation, decreased copulatory rate, and decreased sperm numbers. The  
43 study authors concluded that neonatal bisphenol A exposure caused no adverse effects on reproductive  
44 function or gene expression of steroidogenic enzymes in the rat testis.

45  
46 **Strengths/Weaknesses:** This paper has a number of major strengths, notably the wide range of doses,  
47 appropriate use of statistics, inclusion of a positive control, and use of relevant endpoints. Weaknesses  
48 include route of administration and dosing on a per pup basis, thus not adjusting for bodyweight.

49  
50 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate but of limited utility due to  
51 route of administration and dosing on a per pup basis.

### 3.0 Developmental Toxicity Data

1 **Noda et al. (383)**, support not indicated, examined the effect of neonatal bisphenol A exposure on  
2 reproductive organs of Sprague Dawley rats. For five days beginning on PND 1 (day of birth = PND 0), 6–  
3 10 pups/sex/group (drawn from 2 litters) were sc injected with olive oil vehicle or bisphenol A [**purity not**  
4 **reported**] at 0.0001, 0.001, or 0.010 mg/rat/day. According to the study authors, the doses were equivalent  
5 to ~0.010, 0.100, or 1 µg/kg bw/day. A positive control group received diethylstilbestrol at the same doses  
6 as bisphenol A. Nonylphenol and genistein were also examined but will not be discussed here. Dose  
7 selection was based on diethylstilbestrol doses reported to have an effect. Stability, homogeneity, and  
8 concentration of dosing solutions were verified. Pups in each group were obtained from 2 dams. On PND 7,  
9 litters were adjusted to 4 males and females/dam when possible. Dams and pups were housed in  
10 polycarbonate cages until weaning at PND 21. At that time, pups were housed in wire mesh cages. Animals  
11 were fed MF feed (Oriental Yeast Co.). [**No information was provided on bedding used in**  
12 **polycarbonate cages.**] During the study, animals were examined for clinical signs, body weight, anogenital  
13 distance on PND 7, and day of vaginal opening or preputial separation. Estrous cycles were assessed from  
14 the time of vaginal opening until animals were killed on PND 47–50 (females in diestrus). Rats in  
15 persistent estrus were killed on PND 70. Reproductive organs were weighed. Testis was fixed in Bouin  
16 solution and all other reproductive organs were fixed in 10% neutral buffered formalin for  
17 histopathological examination. [**It was not indicated, but it is assumed that all pups were examined in**  
18 **each analysis.**] Data were analyzed by Bartlett test for homogeneity of variance, ANOVA, Dunnett test, or  
19 Kruskal-Wallis test.  
20

21 In the bisphenol A groups, there were no abnormal clinical signs or effects on body weight. Absolute  
22 anogenital distance was not affected, but anogenital distance adjusted by the square root of body weight  
23 cubed was decreased in females treated with the mid and high bisphenol A dose. There were no effects on  
24 day of vaginal opening or preputial separation or on estrous cycles. [**Data were not shown.**] No gross or  
25 histopathological abnormalities were reported in male or female reproductive organs. The study authors  
26 only reported organ weight effects relative to body weight, because the rats were killed at different ages.  
27 The only dose-related effect on reproductive organ weight was increased relative ventral prostate weight in  
28 the high dose group. Relative pituitary weight was increased in males of the low-dose group and females of  
29 the high-dose group. There were no effects on weights of testis, epididymis, seminal vesicle, uterus, or  
30 ovary in bisphenol A-treated animals. Effects observed in animals treated with 1 or more dose of  
31 diethylstilbestrol included delayed or incomplete preputial separation, estrous cycle disruption,  
32 underdeveloped reproductive organs (including ventral prostate), malformations in male and female  
33 reproductive organs, ovarian cysts, and uterine squamous metaplasia in glandular epithelium. The study  
34 authors noted that the shortened anogenital distance in females appeared to be biologically significant.  
35 However it was stated that the effect is of unknown relevance in female rats and was not observed in the  
36 rats treated with diethylstilbestrol. The study authors concluded that findings observed with bisphenol A  
37 were not toxicologically relevant.  
38

39 **Strengths/Weaknesses:** Strengths of this report include the use of 3 dose levels, the use of a positive  
40 control (diethylstilbestrol), the use of multiple endpoints to evaluate estrogenic effects. Weaknesses include  
41 the use of only 2 litters to constitute exposure groups, exposure by the subcutaneous route to bisphenol A  
42 (not the anticipated route of exposure in humans), and dosing on a per pup basis.  
43

44 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate due to the combination of  
45 small sample size (i.e., 2 litters) and sc route of administration.  
46

47 **Ho et al. (384)**, supported by NIH and Department of Defense, examined the effect of developmental  
48 exposure to bisphenol A on susceptibility of Sprague Dawley rats to prostate cancer. The dams and  
49 offspring used in this study were fed a soybean-free phytoestrogen-reduced diet (Zeigler Reduced Rodent  
50 Diet 2, Ziegler Brothers, Inc), housed in polysulfone cages [**with unspecified bedding**], and provided  
51 drinking water in glass bottles. On PND 1, 3, and 5 (day of birth = PND 0), 20–30 male pups/group were sc

### 3.0 Developmental Toxicity Data

1 injected with tocopherol-stripped corn oil vehicle, bisphenol A [**purity not indicated**] at 0.1 µg/pup (0.010  
2 mg/kg bw), or estradiol benzoate at 0.001 µg/pup (0.1 µg/kg bw) or 25 µg/pup (2500 µg/kg bw). Male rats  
3 from each litter were randomly assigned to treatment groups, but the total number of litters from which the  
4 pups were selected was not reported. Likewise, it is unclear, but assumed, that all doses were represented  
5 within litter rearing units. Pups were weaned on PND 21. At PND 90, half the rats from each treatment  
6 group were implanted with Silastic capsules containing 17β- estradiol and testosterone and the other half  
7 were implanted with empty capsules; the capsules were left in place for 16 weeks. The treatment was  
8 designed to result in a serum 17β-estradiol level of ~75 ng/L and testosterone level of ~3 µg/L, levels  
9 reported to induce prostatic intraepithelial neoplasia in 33% of Sprague Dawley rats. Rats were killed at 28  
10 weeks of age. Prostates were removed, and histopathological evaluations were conducted on each lobe.  
11 Immunohistochemistry techniques were used to measure proliferation. Apoptosis was measured using the  
12 terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique. PCR  
13 techniques were used to study methylation pattern and expression changes in prostate cell signaling  
14 proteins on PND 10, 90, and 200. Statistical analyses included chi-squared test, ANOVA, Fisher exact test,  
15 and Bonferroni test. The study authors stated that similar responses were observed in each of the 3 prostate  
16 lobes; and thus results were presented only for dorsal prostate. In bisphenol A-exposed compared to vehicle  
17 controls rats that did not receive 17β-estradiol/testosterone exposure in adulthood, there were no effects on  
18 dorsal prostate weight, histopathology alterations, proliferation index, or apoptotic index. In bisphenol A-  
19 treated compared to vehicle control rats that received 17β-estradiol/testosterone exposure in adulthood,  
20 there was increased incidence and severity of prostatic intraepithelial neoplasia (100 vs. 40% incidence). In  
21 the bisphenol A/17β-estradiol/testosterone group, proliferation and apoptosis indices were increased in  
22 regions where prostatic intraepithelial neoplasia (PIN) was observed. In humans PIN is an accepted  
23 precursor lesion to prostate cancer. In rodents the significance of PIN is less clear. Some transgenic mouse  
24 models will form PIN lesions which progress to adenocarcinoma in a manner broadly similar to that seen in  
25 humans. However, there are many examples in which mice form PIN lesions which do not progress to  
26 invasive disease. In rats, testosterone plus estradiol classically induces PIN lesions which progress to  
27 adenocarcinoma. The increase in incidence of PIN lesions seen following testosterone and estradiol  
28 treatment in BPA exposed rats in this study are certainly a cause for concern. The data presented do not  
29 address whether these lesions progress to cancer in a manner similar to PIN lesions seen in the classic  
30 testosterone plus estradiol model, or whether such progression occurs at a higher or lower rate. Changes  
31 observed in rats exposed to the high estradiol benzoate dose in the neonatal period but not 17β-  
32 estradiol/testosterone during adulthood included increased incidence and severity of prostatic intraepithelial  
33 neoplasia and elevated apoptosis and proliferation indices. The same effects, in addition to decreased  
34 prostate weight, were observed in rats receiving neonatal exposure to the high estradiol benzoate dose and  
35 adult exposure to 17β-estradiol/testosterone.

36  
37 In the investigation of a molecular basis for increased susceptibility to PIN, exposure to estrogenic  
38 compounds altered methylation pattern in several cell signaling genes. Phosphodiesterase type 4 variant, an  
39 enzyme involved in cyclic AMP breakdown, was selected for further study. Neonatal bisphenol A exposure  
40 resulted in hypomethylation of the phosphodiesterase type 4 variant gene and increased expression of that  
41 gene at 90 and 200 days of age, with or without 17β-estradiol/testosterone exposure in adulthood. Similar  
42 responses in phosphodiesterase type 4 variant gene methylation and expression were observed with  
43 exposure to the low and high 17 estradiol benzoate doses. The study authors concluded that developmental  
44 exposures of rats to bisphenol A increased susceptibility to precancerous prostate lesions resulting from  
45 prostate epigenomic alteration.

46  
47 **Strengths/Weaknesses:** This is a carefully performed study by a group with significant expertise in this  
48 area of work. The paper has many strengths, from the use of a relatively low dose level of bisphenol A to  
49 the search to identify molecular mechanisms, possibly including site-specific promoter methylation,  
50 underlying the observations made. Weaknesses include the use of a single dose level with subcutaneous

### 3.0 Developmental Toxicity Data

1 dosing. It could be suggested that carrying the study further in terms of animal age might have produced  
2 more dramatic phenotypes and clarified the relevance of PIN resulting from BPA exposure to prostate  
3 cancer (potentially enhancing cancer incidence) in this model. Failure to do this could be considered a  
4 weakness of the work.

5  
6 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is adequate and of limited utility for the  
7 evaluation process due to use of subcutaneous route of administration.

#### 8 9 *3.2.4.2 Neurobehavioral endpoints*

10 **Ishido et al. (385)**, supported by the National Institute for Environmental Studies and the Ministry of  
11 Economy, Trade, and Industry, examined the effects of postnatal intracisternal bisphenol A exposure on  
12 behavior of rats. Dams in this study were fed Standard laboratory chow (MF Diet; Oriental Yeast Corp.).  
13 **[No information was provided about caging or bedding materials.]** At 5 days of age, 5–7 male Wistar  
14 rat pups/group were injected intracisternally with a bisphenol A dose **[purity not indicated]** of 0  
15 (ethanol/olive oil vehicle), 0.00002, 0.0002, 0.002, or 0.020 mg. Pups were weaned at 3 weeks of age.  
16 Spontaneous motor activity was measured over a 12–24-hour period at 4–5 weeks of age. Rats were killed  
17 at 4 and 8 weeks of age, and brains were removed. RNA was isolated from midbrain and striatum for DNA  
18 microarray analysis. Expression of the gene for dopamine transporter in midbrain was studied by RT-PCR.  
19 Tyrosine hydroxylase expression in brain was measured at 8 weeks of age using an immunostaining  
20 method. Statistical analyses included ANOVA and Student *t*-test.

21  
22 In 4–5-week-old rats from the 0.020 mg bisphenol A group, motor activity was significantly increased and  
23 was 1.6 times higher than in control rats during the nocturnal period. In a dose response experiment, it was  
24 noted that hyperactivity was significantly increased at doses  $\geq 0.0002$  mg. Microarray analysis revealed that  
25 bisphenol A **[at an unspecified dose]** downregulated expression of dopamine D4 receptor gene 2-fold at 4  
26 weeks of age and dopamine transporter gene 2.8-fold at 8 weeks of age. Numerous other gene expression  
27 changes were observed but not discussed in detail by study authors. Analysis by RT-PCR confirmed that  
28 expression of the dopamine transporter gene was downregulated 3-fold in the midbrain of 8-week-old rats  
29 treated with bisphenol A in the neonatal period. In rats from the 0.020 mg bisphenol A group, tyrosine  
30 hydroxylase immunoreactivity was reduced in the substantia nigra at 8 weeks of age. The study authors  
31 interpreted the decrease in tyrosine hydroxylase immunoreactivity as degeneration of dopaminergic  
32 neurons. They concluded that bisphenol A affected the central dopaminergic system, resulting in  
33 hyperactivity that most likely occurred as a result of decreased tyrosine hydroxylase activity in midbrain.

34  
35 **Strengths/Weaknesses:** A significant weakness is the inability to correlate the internal exposure to  
36 bisphenol A provided by the intracisternal route with that seen by the oral route. Strengths of this paper  
37 include the use of a range of concentrations of bisphenol A. The correlation of changes in behavior patterns  
38 induced by bisphenol A with expression of specific dopamine receptor sets is also a strength. A significant  
39 weakness is the inability to correlate the doses of bisphenol A provided by this dosing mechanism with  
40 those seen by more common sc or oral routes, as well as uncertainty about the disposition of the bisphenol  
41 A that is injected into the cerebrospinal fluid.

42  
43 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
44 due to uncertainties around the intracisternal route of administration.

45  
46 **Masuo et al. (386)**, of the Japanese National Institute of Advanced Industrial Science and Technology and  
47 National Institute for Environmental Studies, investigated the effects in rats of an acute neonatal exposure  
48 to 6-hydroxydopamine, bisphenol A, nonylphenol, *p*-octylphenol, or diethylhexyl phthalate upon  
49 spontaneous motor activity, as well as catecholamine levels, dopaminergic neuron integrity by  
50 immunohistochemistry, and gene expression profiles. In the 6-hydroxydopamine group, 5-day-old male  
51 Wistar pups weighing about 10 g were first pretreated with 25 mg/kg desipramine ip on PND 5 in order to

### 3.0 Developmental Toxicity Data

1 protect noradrenergic neurons from the effects of 6-hydroxydopamine. These pups were then injected  
2 intracisternally 30 minutes later with 6-hydroxydopamine [**not discussed here**]. Other groups of pups were  
3 treated intracisternally with 0 (olive oil vehicle) or 87 nmol bisphenol A [**purity not provided**],  
4 nonylphenol, *p*-octylphenol, or diethylhexyl phthalate in olive oil (n = 6 or 7/group). In additional  
5 experiments, intracisternal bisphenol A treatments were used over a 0.087–87 nmol [**19.8 ng to 19.8 µg**]  
6 dose range. Following treatment, pups were randomly assigned to lactating dams and weaned at 3 weeks of  
7 age. Animals were housed in acrylic cages at 22° under 12 hour light/dark conditions and given free access  
8 to water and chow from Oriental Yeast Company.

9  
10 Spontaneous motor activity was assessed at 4–5 weeks of age using an automated activity-monitoring  
11 system over a 12 hour light/12 hour dark cycle, apparently for a single 24 hour period. [**Total number of**  
12 **cycles not indicated.**] Brain sections from 8–10 week old rats were snap frozen in liquid nitrogen. The  
13 striatum and whole mid-brains were used for cDNA microarray analyses. The frontal cortex, striatum,  
14 limbic regions including nucleus accumbens, septum, and olfactory tubercles were used to measure  
15 catecholamine levels by HPLC. Immunohistochemistry from whole brain sections was used to evaluate  
16 dopamine neuron integrity using tyrosine-hydroxylase monoclonal antibody reactivity [**number of rats not**  
17 **indicated**]. Most statistical analyses were performed using ANOVA techniques. Activity data were  
18 analyzed using repeated measures ANOVA to examine activity in 2 hour intervals, as well as across the  
19 dark, light, or full 24 hour period. Student *t*-tests were used to compare catecholamine levels.

20  
21 Spontaneous motor activity in rats treated with bisphenol A increased in a dose-dependent manner over the  
22 0.087 to 87 nmol range, with significance on pairwise comparison with controls at dose levels  $\geq 0.87$  nmol  
23 [**198 ng**]. Activity was increased in both the dark and light periods. Tyrosine hydroxylase activity was  
24 reduced in bisphenol A-treated rats, compared to controls. [**Quantification of immunohistochemical**  
25 **sections was not provided.**] Gene expression patterns in the midbrain differed in bisphenol A and 6-  
26 hydroxydopamine-treated animals.

27  
28 The authors concluded that neonatal exposure to bisphenol A was associated with an increase in  
29 spontaneous motor activity and reduced tyrosine hydroxylase activity. They hypothesized that bisphenol A  
30 may cause a deficit in the development of mesostriatal dopaminergic neurons, and that this increase either  
31 is greater than that produced by 6-hydroxydopamine lesions or involves additional neurochemical systems.  
32 A follow-up study (387) addressed these issues. The authors also proposed that bisphenol A-exposed rats  
33 can serve as animal models of attention deficit-hyperactivity disorder.

34  
35 **Strengths/Weaknesses:** A significant weakness is the inability to correlate the internal exposure to  
36 bisphenol A provided by the intracisternal route with that seen by the oral route.

37  
38 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
39 due to uncertainties around the intracisternal route of administration.

40  
41 **Masuo et al. (387)**, funded by the New Energy and Industrial Technology Development Organization, the  
42 Ministry of the Environment, and the Ministry of Economy, Trade, and Industry, Japan, followed up their  
43 previous study (386) with additional gene expression microarrays to elucidate potential molecular pathways  
44 associated with the effects of an acute, neonatal exposure to 6-hydroxydopamine, bisphenol A,  
45 nonylphenol, diethylhexyl phthalate, or dibutyl phthalate on spontaneous motor activity levels at 4-5 weeks  
46 of age. Pregnant Wistar rats were housed in acrylic cages with free access to tap water and laboratory chow  
47 (Oriental Yeast, Tokyo) and maintained on a 12 hour light/12 hour dark cycle. In the 6-hydroxydopamine  
48 group, 5 day old male pups, each about 10 g, were first pretreated with 25 mg/kg desipramine by ip  
49 injection (to protect noradrenergic neurons from the effects of 6-hydroxydopamine) and then given 6-  
50 hydroxydopamine intracisternally 30 minutes later. PND 5 male pups in other groups were intracisternally  
51 injected with olive oil vehicle, 87 nM bisphenol A [**19.8 µg**] [**purity not provided**], nonylphenol,

### 3.0 Developmental Toxicity Data

1 diethylhexyl phthalate, or dibutyl phthalate. **[Only the bisphenol A experiments will be discussed here.]**  
2 Following treatments, pups were randomly fostered to lactating dams (5–7 pups/per dam) and weaned at 3  
3 weeks of age. At 4–5 weeks of age, the spontaneous motor activity of bisphenol A treated rats was  
4 compared to vehicle treated rats (n = 6 or 7/group) using an automated activity-monitoring system over a  
5 12 hour light/12 hour dark cycle. Bisphenol A and vehicle-treated rats were killed at 8–10 weeks of age and  
6 the striatum and midbrain were harvested. RNA was extracted from 2 pooled striata/rat (n = 3/group) or 1  
7 midbrain/rat (n = 3/group) for cDNA microarray analyses. Gene expression values were evaluated relative  
8 to those of control treated rats. Repeated measures ANOVA was used for statistical analyses of  
9 spontaneous motor activity during 2 hour time intervals. Statistics were not described for microarray  
10 results.

11  
12 Neonatal exposure to bisphenol A in male rats significantly increased spontaneous motor activity at 4–5  
13 weeks during both the dark and light periods of the cycle when compared to controls. Gene expression  
14 profiles examined at 8–10 weeks of age for select genes potentially impinging on dopamine function and/or  
15 other pathways were altered in the adult striatum and midbrain of bisphenol A treated mice. The authors  
16 concluded that neonatal exposure to bisphenol A resulted in elevated spontaneous motor activity during  
17 both the light and dark phases. 6-Hydroxydopamine lesions increased motor activity only during the dark  
18 period. Comparisons of genetic expression in 6-hydroxydopamine and bisphenol A-treated rats suggested  
19 that the effects of bisphenol A may be mediated by alterations in dopamine as well as other systems. This  
20 profile of adverse effects was suggested to potentially serve as a model for human hyperactivity disorders.

21  
22 **Strengths/Weaknesses:** A significant weakness is the inability to correlate the internal exposure to  
23 bisphenol A provided by the intracisternal route with that seen by the oral route.

24  
25 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
26 due to uncertainties around the intracisternal route of administration.

27  
28 **Ishido et al. (388)**, support not indicated, examined the effects of neonatal bisphenol A exposure of rats on  
29 motor activity and gene expression in brain. Wistar rat dams were fed MF diet (Oriental Yeast, Tokyo,  
30 Japan). Pups were born from 10 pregnant dams and 5–7 male pups were assigned to each dam. At 5 days of  
31 age, male pups were injected intracisternally with vehicle (50% ethanol in olive oil) or 87 nmol **[19.8 µg]**  
32 bisphenol A. **[No information was provided on number of pups treated, purity of bisphenol A, or**  
33 **caging and bedding materials.]** Pups were also treated with 2 nonylphenol compounds and 3 phthalate  
34 compounds, but results for those compounds will not be discussed. Pups were weaned at 3 weeks of age.  
35 Spontaneous motor activity was measured in pups at 4–5 weeks of age. Rats were killed at 8 weeks of age,  
36 and RNA was isolated from midbrain for macroarray analyses of gene expression. **[The number of rats**  
37 **examined was not reported for any endpoint.]** Data for spontaneous motor activity were analyzed by  
38 ANOVA or Student *t*-test. **[There were no statistical analyses for gene expression data.]**

39  
40 Rats exposed to bisphenol A were significantly more active during the nocturnal phase than control rats (by  
41 ~1.4–1.6-fold). In midbrains of 8-week-old rats, expression levels were altered for 46 G protein-coupled  
42 receptor genes, which are involved in dopaminergic neurotransduction and many peptidergic  
43 neurotransduction processes. The study authors noted altered dopamine transporter gene expression, which  
44 was impaired by all chemicals tested. Bisphenol A also lowered galanin receptor 2 expression. The study  
45 authors concluded that intracisternal exposure to bisphenol A induced hyperactivity in rats, possibly by  
46 regulating gene or protein expression of G protein-coupled receptor and dopaminergic neurotransduction  
47 systems.

48  
49 **Strengths/Weaknesses:** Despite certain strengths, a significant weakness is the inability to correlate the  
50 internal exposure to bisphenol A provided by the intracisternal route with that seen by the oral route.

51

### 3.0 Developmental Toxicity Data

1 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process.

2  
3 **Patisaul et al. (389)**, supported by the American Chemistry Council, evaluated the effect of neonatal  
4 bisphenol A on the anteroventral periventricular nucleus of the Sprague Dawley rat. Pregnant rats (n = 5)  
5 were fed a phytoestrogen-free diet (Purina 5K96) during the last week of gestation. **[No information was  
6 provided about caging or bedding.]** Dams were permitted to litter. Pups were cross-fostered among all  
7 dams so that 4 dams reared 6 females and 6 males and 1 dam reared 5 males. Pups (n = 5–8/group) were  
8 randomly assigned to receive sc injections of 17 $\beta$ -estradiol 50  $\mu$ g/pup, genistein 250  $\mu$ g/pup, bisphenol A  
9 **[purity not indicated]** 250  $\mu$ g/pup, or sesame oil vehicle every 12 hours for 48 hours. The authors  
10 estimated that the twice daily dosing with 250  $\mu$ g/pup was approximately equivalent to 100 mg/kg bw/day.  
11 Injections began the morning of PND 1 (delivery = PND 0). On PND 19, the pups were transcardially  
12 perfused with ice-cold saline followed by paraformaldehyde. Brains were post-fixed in 20% sucrose in  
13 paraformaldehyde, sectioned coronally, and processed for immunohistochemistry for ER $\alpha$  and tyrosine  
14 hydroxylase. Sections were counterstained with Nissl stain. Cells of the anteroventral periventricular  
15 nucleus positive for ER $\alpha$ , tyrosine hydroxylase, or both were counted. Statistical analysis used 2-way  
16 ANOVA with sex and treatment as factors, followed by 1-way ANOVA and post hoc Fisher least  
17 significant difference test.

18  
19 There was a significant, sex-related effect on tyrosine hydroxylase-positive cells in the anteroventral  
20 periventricular nucleus with the number in males about 29% that of females **[estimated from a graph]**.  
21 The authors concluded that neonatal treatment with bisphenol A interfered with the normal testosterone-  
22 associated masculinization of the anteroventral periventricular nucleus. Because 17 $\beta$ -estradiol is  
23 aromatized to testosterone in the brain, the authors interpreted this effect of bisphenol A as anti-estrogenic.  
24 Cells staining for both ER $\alpha$  and tyrosine hydroxylase are not present in rodents after puberty, and the  
25 authors stated that these cells may play a role in the organization of the LH-surge. They postulated that the  
26 decrease in these cells with neonatal exposure to bisphenol A may result in cycle disruption in adulthood.

27  
28 **Strengths/Weaknesses:** Strengths of this study are the use of 17 $\beta$ -estradiol as a positive control and the  
29 measurement of ER $\alpha$  receptors. Weaknesses are the relatively high dose level of bisphenol A and the use of  
30 the subcutaneous route of exposure on a per pup basis without adjustment for body weight. Critical  
31 weakness include small sample size (5 treated dams) and lack of adequate experimental and statistical  
32 control for litter effects.

33  
34 **Utility (Adequacy) for CERHR Evaluation Process:** Despite certain strengths, this study is inadequate  
35 for the evaluation process for the reasons cited above.

36  
37 **Patisaul et al. (389)**, supported by the American Chemistry Council, investigated the effects of an acute  
38 neonatal exposure to bisphenol A or genistein (**not discussed here**) on the SDN-POA and the anteroventral  
39 periventricular nucleus in the adult male rat. Five pregnant Sprague-Dawley rats were obtained and  
40 maintained on a 12 hour/12 hour light/dark cycle, with free access to water and a soy-free, phytoestrogen-  
41 free diet that was maintained throughout the duration of the experiment. **[Details on housing (individual  
42 or group), type of caging, and bedding material were not provided.]** Most of the dams were cross-  
43 fostered with 6 male and 6 female pups. Starting on PND 1, all male pups were given sc injections every 12  
44 hours over 48 hours with 250  $\mu$ g bisphenol A **[purity not provided]** or oil vehicle. **[Assuming a Sprague  
45 Dawley pup weighs ~7.5 g, this dose would be equivalent to ~66 mg/kg bw/day.]** On PND 85, males  
46 were gonadectomized. Six ovariectomized female rats served as controls. After a recovery period, the rats  
47 were given sc injections of 10  $\mu$ g estradiol benzoate, and 48 hours later, a sc injection of 500  $\mu$ g  
48 progesterone. The authors note that this protocol has consistently induced *fos* expression in GnRH neurons,  
49 leading to LH release in females. About 8 hours later, the animals were killed, formalin-perfused, and  
50 brains were harvested. Regions containing the SDN-POA and anteroventral periventricular nucleus were

### 3.0 Developmental Toxicity Data

1 cryopreserved. SDN-POA sections were serially stained with Nissl or labeled for calbindin-d28K. The  
2 vascular organ of the lamina terminalis was double-immunostained for Fos and GnRH. An automated  
3 stereomicroscope was used to gauge the volume areas of the anteroventral periventricular nucleus, the  
4 SDN-POA, the calbindin-immunoreactive regions of the SDN-POA, and number of calbindin-positive  
5 nuclei. Calbindin-positive nuclei were also counted by independent evaluators blinded to the treatments.  
6 Quantification analyses of GnRH and Fos staining were evaluated visually. Statistical analysis was  
7 performed using ANOVA, and Fisher's least significant difference test.

8  
9 Acute neonatal treatment of bisphenol A did not affect the volume of the SDN-POA. Similarly, the  
10 volumes of the calbindin-immunoreactive regions of the SDN-POA were roughly equivalent to SDN  
11 volumes [**estimated from a graph**] with no apparent bisphenol A treatment effect. Bisphenol A treatment  
12 induced a significant increase [**~50-60% estimated from a graph**] in calbindin-positive nuclei. Bisphenol  
13 A had no effect on the volume of the anteroventral periventricular nucleus or the total number of GNRH-  
14 positive nuclei, and no induction of Fos protein was identified.

15  
16 The authors noted that the long-term effect of neonatal exposure to bisphenol A on male brain development  
17 and reproductive behavior cannot be predicted solely on anatomical changes in sexually dimorphic brain  
18 regions. They concluded that the development of more precise and predictive biomarkers is needed.

19  
20 **Strengths/Weaknesses:** Strengths of this study are the use of 17 $\beta$ -estradiol as a positive control.  
21 Weaknesses are the relatively high dose level of bisphenol A and the use of the subcutaneous route of  
22 exposure on a per pup basis without adjustment for body weight. Critical weakness include small sample  
23 size (5 treated dams) and lack of adequate experimental and statistical control for litter effects.

24  
25 **Utility (Adequacy) for CERHR Evaluation Process:** Despite certain strengths, this study is inadequate  
26 for the evaluation process for the reasons cited above.

27  
28 **Shikimi et al. (390)**, supported by the Japan Society for the Promotion of Science for Young Scientists,  
29 examined the effects of bisphenol A exposure on Purkinje cell development in rats. [**No information was  
30 provided about feed or composition of caging and bedding materials.**] At 6–9 days of age, 4 male or  
31 female Fisher rats/group received bisphenol A [**purity not provided**] at 0 (sesame oil vehicle), 0.050, or  
32 0.500 mg/day by injection into the cerebrospinal fluid near the region of the cerebellum. During the same  
33 time period, additional groups of 4 rats received 0.500 mg/day tamoxifen, 0.500 mg/day bisphenol A +  
34 0.500 mg/day tamoxifen, or 5  $\mu$ g/day estradiol benzoate through the same exposure route. [**Both male and  
35 female rats were treated, but it was not indicated if there were equal numbers in each group; both  
36 sexes were apparently evaluated together.**] At 10 days of age, pups were killed and vermal cerebella  
37 were removed and sectioned. Purkinje cells were examined morphologically following identification by  
38 calbindin-D<sub>28K</sub> immunostaining. Data were analyzed by ANOVA, followed by Duncan multiple range test.

39  
40 Treatment with the high dose of bisphenol A increased Purkinje fiber length. There was no effect on cross-  
41 sectional soma area or Purkinje cell number as a result of bisphenol A treatment. Co-treatment with  
42 tamoxifen inhibited the increase in dendritic length that was observed following treatment with bisphenol A  
43 alone. Estradiol benzoate also induced an increase in dendritic length of Purkinje fibers that was blocked by  
44 tamoxifen. Treatment with tamoxifen alone also reduced dendritic fiber length. The effects of octylphenol  
45 were also examined and an increase in dendrite length was observed. The study authors concluded that  
46 bisphenol A induced Purkinje dendritic growth, possibly through the ER.

47  
48 **Strengths/Weaknesses:** The use of estradiol benzoate as a positive control is a strength of this study.  
49 Weaknesses are the injection into cerebrospinal fluid.

50

### 3.0 Developmental Toxicity Data

1 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
2 due to uncertainties surrounding the route of administration (i.e., difficulty of relating a cerebrospinal  
3 injection to human exposures).  
4

5 **Zsarnovszky et al. (391)**, supported by NIH, NIEHS, and the American Heart Association, evaluated the  
6 effect of intracerebellar injection of bisphenol A on the development of activated extracellular signal-  
7 regulated kinase (ERK)-positive cells in cerebellar sections in Sprague Dawley rats. Neonatal rats on PND  
8 4–19 underwent a single direct injection under anesthesia of bisphenol A or 17 $\beta$ -estradiol under stereotactic  
9 guidance into cerebellar folia 6 and 7. **[For bisphenol A, only PND 10 results were given. The number  
10 of animals at each age was not specified, but a figure legend indicated at least 6/dose group. The  
11 purity of the chemicals was not specified. The day of birth was not defined.]** Concentrations of the  
12 chemicals were 10<sup>-12</sup> to 10<sup>-6</sup> M **[bisphenol A concentrations of 0.23 ng/L to 0.23 mg/L]**. Uninjected,  
13 mock-injected, and vehicle-injected controls were used. Brains were removed and fixed 6 minutes after the  
14 onset of the injection. Sections were processed for immunohistochemistry using an antibody that  
15 recognized activated ERK. Quantitative analysis was performed on images of folium 9. Statistical analysis  
16 was performed using ANOVA with post hoc Tukey-Kramer multiple comparison test. Response to  
17 different chemicals and different concentrations on PND 10 were compared using 2-factor ANOVA with  
18 post hoc Bonferroni test. Adult rats were also treated but were not included in the quantitative analysis.  
19

20 The qualitative appearance of the immunostained sections was similar after bisphenol A and 17 $\beta$ -estradiol.  
21 In the 10<sup>-12</sup> to 10<sup>-9</sup> M dose range, the quantitative responses to the 2 chemicals were similar. Activated  
22 ERK-positive cells increased with a median effect concentration of 7.46 pM for 17 $\beta$ -estradiol and 3.25 pM  
23 **[0.74 ng/L]** for bisphenol A. Both chemicals were described as having an inhibitory effect at higher doses.  
24 **[The data graph shows drop-offs to control densities at 10<sup>-9</sup> and 10<sup>-10</sup> M, with a second increase in  
25 density at 10<sup>-7</sup> and 10<sup>-5</sup> M.]** Co-administration of 10<sup>-10</sup> M 17 $\beta$ -estradiol with bisphenol A 10<sup>-12</sup>–10<sup>-10</sup> M  
26 **[0.23–23 ng/L]** resulted in a concentration-dependent decrease in activated ERK-positive cells compared to  
27 the administration of 17 $\beta$ -estradiol alone. The authors concluded that 17 $\beta$ -estradiol regulates ERK  
28 signaling in the developing cerebellum and that bisphenol A can mimic and also inhibit this estrogenic  
29 effect, with potentially adverse affects on brain development and function.  
30

31 **Strengths/Weaknesses:** The use of 17 $\beta$ -estradiol as a positive control is a strength of this study.  
32 Weaknesses are the intracerebellar injection and the administration on a per pup basis.  
33

34 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
35 due to uncertainties surrounding the route of administration (i.e., difficulty of relating a cerebrospinal  
36 injection to human exposures).  
37

#### 38 3.2.5 Mouse—oral exposure only during pregnancy

##### 39 3.2.5.1 Studies without neurobehavioral endpoints

40 **Morrissey et al. (316)**, supported by NTP/NCTR, examined the effects of prenatal bisphenol A exposure in  
41 rats and mice in studies conducted according to GLP. The studies are also available as NTP publications for  
42 rats (317) and mice (318). The study was conducted in two sets of rats and mice and data were pooled for  
43 each species. **[The data for rats were discussed in Section 3.2.1.]** Animals were fed Purina 5002 diet,  
44 housed in polypropylene or polycarbonate cages with stainless steel wire lids with Ab-Sorb-Dri cage  
45 bedding. Pregnant CD-1 mice were randomly assigned to groups of  $\geq 10$  animals in each set of the study,  
46 for a total of  $\geq 20$  animals/dose. On GD 6–15 (GD 0 = sperm or plug), mice were gavaged with bisphenol A  
47 at 0 (food-grade corn oil), 500, 750, 1000, or 1250 mg/kg bw/day. Doses were based on results of  
48 preliminary studies and were expected to result in 10% maternal mortality at the high dose and no toxicity  
49 at the low dose. The purity of bisphenol A was >95%, and 2,4'-bisphenol A was reported as an impurity.  
50

### 3.0 Developmental Toxicity Data

Concentrations of dosing solutions were verified. Pregnant animals were weighed during the study. Mice were killed on GD 17. Liver and uteri were weighed, and corpora lutea and implantation sites were examined. Fetuses were sexed, weighed, and examined for viability and external, visceral, and skeletal malformations. Data were analyzed by Bartlett test for homogeneity of variance, ANOVA and/or William multiple comparison, Dunnett, and/or Fisher exact probability tests. **[Data were presented and analyzed on a per litter basis.]**

Clinical signs reported in mice treated with bisphenol A included arched back, lethargy, piloerection, rough coat, vaginal bleeding, vocalization, alopecia, weight loss, and wheezing. One or 2 of 29–34 dams died in each of the 3 lowest dose groups and 6 of 33 dams died in the 1250 mg/kg bw/day group. Statistically significant effects are summarized in Table 75. Absolute liver weight was increased in the 500, 750, and 1000 mg/kg bw/day dose groups, and relative liver weights were increased in all bisphenol A dose groups. Decreased gravid uterine weight and dam body weight gain during the gestation and treatment periods attained statistical significance at the 1250 mg/kg bw/day dose. The number of litters available for evaluation in the control and each dose group was 26, 23, 21, 23, and 21. Increased resorptions/litter and decreased fetal body weights/litter attained statistical significance in the high-dose group. There was no effect on the number of live fetuses/litter at birth or on fetal malformations/litter. The study authors concluded that bisphenol A is not teratogenic in mice at doses that result in maternal toxicity.

**Table 75. Maternal and Developmental Toxicity in Mice Gavaged with Bisphenol A**

Endpoint	Dose in mg/kg bw/day							
	500	750	1000	1250	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
Dam weight in treatment period	↔	↔	↔	↓43%	881	661	1159	1039
Gravid uterine weight	↔	↔	↔	↓32%	983	690	1243	1123
Relative dam liver weight	↑9%	↑13%	↑17%	↑26%	618	411	755	541
Resorptions/litter	↔	↔	↔	↑2.8-fold	817	377	1245	1162
Fetal body weight/litter	↔	↔	↔	↓15%	1079	785	1249	1024

↑,↓ Statistically significant increase, decrease; ↔ no statistically significant change. Morrissey et al. (316).

**Strengths/Weaknesses: Strengths include** the oral route of exposure as well as the design and sample sizes used. The use of very high doses is a weakness.

**Utility (Adequacy) for CERHR Evaluation Process:** This paper is adequate and of high utility in the evaluation in providing information on conventional teratogenic endpoints.

**vom Saal et al. (392)**, supported by NIH, examined the effects of bisphenol A exposure on male reproductive organs and sperm production in mice. The CF-1 mice used in this study were purchased in 1979 and maintained as an outbred stock in a closed colony. Dams were fed Purina breeder chow (#5008) during pregnancy and lactation, and male offspring were fed Purina #5001 standard lab chow after weaning. Housing consisted of polypropylene cages with corn cob bedding. Bisphenol A **[purity not reported]** in tocopherol-stripped corn oil vehicle was fed to 7 mice/group by electronic micropipette at 0.002 or 0.020 mg/kg bw/day on GD 11–17 (day of vaginal plug = GD 0). One group of 6 mice was given the vehicle control, and a group of 5 mice was not handled. Based on results of in vitro assays conducted by the study authors, the 0.02 mg/kg bw/day bisphenol A dose was predicted to be bioactive in mice. Additional mice were treated with the same doses of octylphenol. Females delivered pups naturally on GD 19, and pups were weaned on PND 23 (day of birth not defined). Male siblings were housed 3/cage until 5 months of age. Randomly selected males were housed individually at 5 months of age and killed 1 month later. Body, testes, epididymides, preputial glands, and seminal vesicles were weighed in 11 control mice

### 3.0 Developmental Toxicity Data

1 and 7 treated mice/group. Data from the two control groups did not differ significantly and were combined  
 2 for analyses of organ and body weight. Data for prostate weight were reported by Nagel et al. (275). Daily  
 3 sperm production was determined in 8 control males/group and 5 treated males/group. **[It was not stated  
 4 how data from the 2 control groups were handled for sperm analyses.]** Sperm data were analyzed by  
 5 ANOVA. Organ weight data were analyzed by ANCOVA, Pearson's correlation analysis, ANOVA, and  
 6 least significant means test. **[It was not clear if the offspring or litter were considered the statistical  
 7 unit; only one randomly selected male per litter was used per F. vom Saal, personal communication,  
 8 June 20, 2007.]**  
 9

10 Statistically significant findings are summarized in Table 76. Exposure to bisphenol A resulted in dose-  
 11 related reductions in daily sperm production efficiency (i.e., per g testis) that attained statistical significance  
 12 at the highest dose level. Some significant but non-dose related effects were observed for body and organ  
 13 weights. Epididymal weights were reduced at both doses. At the low dose, body and seminal vesicle  
 14 weights were reduced and preputial weight was increased. In mice treated with octylphenol, daily sperm  
 15 production was reduced at the low dose but there was no effect on reproductive organ weights. The study  
 16 authors concluded that exposure of the fetus to low doses of endocrine-disrupting chemicals can affect the  
 17 size and function of reproductive organs.  
 18

19 **Table 76. Sperm Production and Male Reproductive Organ Weights in Mice Exposed to Bisphenol A**  
 20 **During Gestation**

Endpoint	Dose in mg/kg bw/day <sup>a</sup>					
	0.002	0.020	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
Sperm production efficiency	↔	↓19%	0.011	0.007	0.010	0.007
Body weight	↓9%	↔				
Preputial weight	↑36%	↔				
Seminal vesicle weight	↓12%	↔				
Epididymal weight	↓12%	↓8%				

<sup>a</sup>Benchmark doses were not estimated for values obtained from graphs and non-dose-related effects; errors were assumed to be SEM, as reported earlier in the paper. From vom Saal et al. (392).

21  
 22 **[The NTP Statistics Subpanel (340) noted that vom Saal et al. (392) did not apparently require**  
 23 **overall differences by ANOVA to be significant before applying the least significant difference test,**  
 24 **which is prone to false positive findings without the overall protection of ANOVA. The NTP**  
 25 **Subpanel was not able to confirm any of the significant findings reported for bisphenol A. The NTP**  
 26 **Subpanel noted that in theory, their reanalysis of organ weights was not necessarily in conflict with**  
 27 **the findings of the study authors because of the use of different statistical methods (Dunnett test**  
 28 **versus Fisher least significant difference test).]**  
 29

30 **Strengths/Weaknesses:** Strengths are the use of oral delivery and low dose levels. Weakness are the  
 31 inability to assume the genetic comparability and responsiveness of CF-1 mice maintained in a closed  
 32 colony for almost 20 years is comparable to other sources of CF-1 mice), failure to weight-adjust the  
 33 maternal dose daily, the lack of information on testis weight (which is needed for consideration of daily  
 34 sperm production), small sample size for sperm production measurement, and the questions about the  
 35 statistical analysis. An additional weakness is the unusual/unexplained findings of low dose only effect on  
 36 weights.  
 37

38 **Utility (Adequacy) for CERHR Evaluation Process:** The body weight data contained in this paper are  
 39 adequate for the evaluation process, however overall utility is limited because of sample size and statistical

### 3.0 Developmental Toxicity Data

1 concerns. Data on reproductive organ weights and sperm production are considered inadequate for the  
2 evaluation.

3  
4 **Nagel et al. (275)**, supported by NIH and the University of Missouri-Columbia, examined the effect of  
5 prenatal bisphenol A exposure on mouse prostate weight. The mice used in this study were the same ones  
6 used in the study by vom Saal et al. (392), and experimental details are provided in the above summary of  
7 that study. CF-1 mice were fed Purina Laboratory Chow 5001 and housed in polypropylene cages with corn  
8 cob bedding. The mice (7/group) were dosed with bisphenol A [**purity not reported**] at 0.002 and 0.020  
9 mg/kg bw/day on GD 11–17. A control group of 6 mice was given the tocopherol-stripped corn oil vehicle  
10 during the same time period. Vehicle and dosing solutions were fed to the mice using a micropipette. A  
11 second control group of 5 dams was unhandled. Because there were no significant differences between the  
12 2 control groups, data from the 2 groups were pooled. Females were allowed to litter. Pups were weaned at  
13 23 days of age and housed 3/cage. One male/litter was selected and housed individually for 1 month. Body  
14 weights of males were measured throughout the study. Selected males were killed at 6 months of age for  
15 measurement of prostate weight. Data for prostate weight were analyzed by ANCOVA using body weight  
16 as the covariate. If it was determined that body weight did not account for differences in prostate weight,  
17 data were reanalyzed by ANOVA without adjustment for body weight. Selection of 1 male/litter controlled  
18 for litter effects. Body weights were lower in males from the 0.002 mg/kg bw/day group than in controls.  
19 Statistical analyses revealed that prostate weight was not related to body weight. Compared to control  
20 values, prostate weights were 30% higher in the 0.002 mg/kg bw/day group and 35% higher in the 0.020  
21 mg/kg bw/day group. The study authors concluded that bisphenol A alters the reproductive system of mice  
22 at doses near reported ranges of human exposure.

23  
24 **[The NTP Statistics Subpanel (340) concluded that Nagel et al. (275) used appropriate statistical**  
25 **methods, and the Subpanel reached essentially the same conclusions as the study authors regarding**  
26 **elevated prostate weight.]**

27  
28 **Strengths/Weaknesses:** Strengths are the use of the same methods as vom Saal et al. (392) and the use of  
29 dose levels in the range of human exposure. The independent confirmation of the data analysis by the NTP  
30 Statistics Subpanel is another strength. The use of a small sample size, closed mouse colony and the failure  
31 to present any histopathological analyses are weaknesses. The Purina 5001 chow has high and variable  
32 levels of soy phytoestrogens, and the corn cob bedding is known to be problematic due to antiestrogenic  
33 constituents. This study did not use a positive control, although there are earlier reports from this laboratory  
34 using diethylstilbestrol.

35  
36 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is adequate and useful for the evaluation  
37 process.

38  
39 **Cagen et al. (393)**, support not indicated [**authors noted to work in industry**], examined the effects of  
40 prenatal bisphenol A exposure on the developing reproductive system of male mice. The study attempted to  
41 duplicate the findings by vom Saal et al. (392) and Nagel et al. (275) by repeating their procedures.  
42 Exceptions were (1) use of larger group sizes to increase statistical power; (2) use of 4 dose levels instead  
43 of 2; (3) use of 2 methods to determine sperm counts; (4) killing of male offspring at 90 instead of 180  
44 days; (5) conducting the study according to GLP (6) obtaining mice from a commercial source instead of an  
45 in-house bred colony; and (7) housing males individually after weaning. In the study by Cagen et al., CF-1  
46 mice gaining more than 4.5 g weight from GD 0 to 10 were randomly assigned to groups of 28 animals and  
47 administered bisphenol A (>99% pure) 0.0002, 0.002, 0.020, or 0.2 mg/kg bw/day on GD 11–17. Two  
48 negative control groups with 28 dams each were given the tocopherol-stripped corn oil vehicle. Because  
49 results from the two vehicle control groups were statistically equivalent, data from the two groups were  
50 pooled. A positive control group of 28 mice was given 0.2 µg/kg bw/day diethylstilbestrol. Dosing  
51 solutions were dripped into the animals' mouths using a micropipette. Concentrations of dosing solutions

### 3.0 Developmental Toxicity Data

1 were verified prior to dosing. Animals were fed certified rodent chow #5002. Water was provided in glass  
2 bottles with Teflon seals. Cages were made of polypropylene with steel lids. Corn cob bedding was used.  
3 Music was played at low volume to provide background noise. Dams were monitored for clinical signs,  
4 food intake, body weight gain, and fertility endpoints. Pups were counted and sexed at birth (PND 0) and  
5 monitored for survival and weight gain until weaning on PND 22. Litters were culled to 8 pups on PND 4,  
6 leaving as many males as possible. At weaning, no more than 4 males/litter (65–95 males/group) were  
7 randomly selected to continue in the study and housed individually. The males were monitored for body  
8 weight gain and feed intake until they were killed on PND 90. Brain, liver, kidneys, and reproductive  
9 organs were weighed. Daily sperm production and epididymal sperm counts were determined and a  
10 histopathological examination of testes was conducted. The litter was considered the experimental unit in  
11 statistical analyses. Data were analyzed by Levene test, ANOVA, Dunnett test, rank transformation,  
12 Wilcoxon rank sum test with Bonferroni correction, Fisher exact probability test, and binomial distribution  
13 test.

14  
15 There were no clinical signs or significant differences in body weight gain or feed intake in dams. The  
16 numbers of dams that died of unknown causes during the study were: 2 receiving vehicle controls; 1 dosed  
17 with diethylstilbestrol; 3 dosed with 0.0002 mg/kg bw/day bisphenol A; and 1 each in the 0.002 and 0.020  
18 mg/kg bw/day bisphenol A groups. The number of total pups/litter was significantly lower than controls in  
19 the 0.2 mg/kg bw/day bisphenol group (mean  $\pm$  SD = 9.60  $\pm$  3.85 compared to 12.37  $\pm$  3.02 in the control  
20 group). In communications with the animal vendor, it was determined that litter size in the control group  
21 exceeded typical litter sizes (9–10 pups), and the study authors therefore concluded that the effect was not  
22 treatment related. Bisphenol A had no significant effects on gestation index or duration, percentage of male  
23 pups at birth, or pup survival and body weight during the lactation period. The same endpoints were  
24 unaffected in the diethylstilbestrol group.

25  
26 Terminal body weights were increased [**by 7%**] in the 0.020 mg/kg bw/day group and [**by 5%**] in the 2  
27 mg/kg bw/day group. Bisphenol A did not affect absolute or relative (to body or brain) weights of  
28 reproductive organs including prostate, preputial gland, seminal vesicle, or epididymis. Non-dose-related  
29 effects were observed for brain and kidney weights, and the study authors concluded that the effects were  
30 not treatment-related. There were no significant effects on cauda epididymal sperm concentration, daily  
31 sperm production, or efficiency of sperm production. Testicular histopathology was not affected by  
32 bisphenol A treatment. [**Data were not shown by authors.**] Reproductive development of male offspring  
33 was also unaffected by diethylstilbestrol. The study authors noted that the diethylstilbestrol dose was  
34 considered the “maximum effect” oral dose by vom Saal but was lower than doses affecting male offspring  
35 in other studies. The study authors also noted that the effects of bisphenol A on prostate weight and sperm  
36 production reported by vom Saal et al. (392) and Nagel et al. (275) were not repeated in this study. They  
37 concluded that bisphenol A should not be considered a selective reproductive or developmental toxicant.

38  
39 [**The NTP Statistics Subpanel (340) concluded that the statistical methods used by Cagen et al. (393)**  
40 **were appropriate. Although the Subpanel agreed with the study author conclusions, they noted that**  
41 **(1) a significant ANOVA is not a requirement for Dunnett test and (2) a Bonferroni correction of the**  
42 **Wilcoxon-rank sum test was not needed because the study authors already required significance by**  
43 **ANOVA, which was sufficient.]**

44  
45 **Strengths/Weaknesses:** The attempt to replicate the studies of vom Saal et al. (392) and Nagel et al. (275),  
46 the use of litter analysis, the large sample sizes, and the agreement of the NTP Subpanel with the author  
47 conclusions are strengths. With respect to this study as a replication, weaknesses include design differences  
48 relating to strain, dietary differences, age at evaluation, and the use of solo housing rather than small group  
49 housing. The lack of response of the positive control DES group is problematic

50

### 3.0 Developmental Toxicity Data

1 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
2 due to absence of response of the positive control group.

3  
4 **Ashby et al. (394)**, support not indicated [2 authors from industry], examined the effects of prenatal  
5 bisphenol A exposure on the mouse reproductive system. The study attempted to duplicate the findings  
6 reported by vom Saal et al. (392) and Nagel et al. (275). Both generations of CF-1 mice were fed RM1 diet  
7 containing 6.5% soy during periods when they were not pregnant or lactating, and dams were fed RM3 diet  
8 containing 18.5% soy during pregnancy and lactation. On postconception days 11–17, 8 dams/group were  
9 dosed with bisphenol A (99% pure) at 0, 0.002, or 0.020 mg/kg bw/day. The negative control group was  
10 administered the tocopherol stripped corn oil vehicle. A positive control group of 7 dams received  
11 diethylstilbestrol at 0.2 µg/kg bw/day. A naïve group of 7 dams was not weighed or dosed. The dosing  
12 solution was slowly expelled from a pipette placed in the animals' mouths. Day of vaginal plug detection  
13 was designated postconception day 1, however, females that had no vaginal plugs but gained >3.5 g were  
14 arbitrarily considered to be 10 days pregnant. Females with vaginal plugs and those that gained >3.5 g were  
15 distributed evenly among treatment and control groups. Females that gained >1 but <3.5 g were considered  
16 to be pregnant, but because the day of pregnancy could not be determined, they were assigned to the naïve  
17 control group. Dams were allowed to litter. All female offspring were weighed and monitored for vaginal  
18 opening. Females were killed at ~44 weeks of age, and liver, kidney, and reproductive organs were  
19 weighed. Male pups were housed as littermates until PND 112 (day of birth designated as PND 1). To  
20 determine the effects of housing, ~3 males from 4–7 litters/group (11–21 males/group) were randomly  
21 selected and housed separately from PND 112 until study termination, which occurred ~71 days later. The  
22 remaining male pups from 4–5 litters/group from each litter (11–17/group) were housed together. Singly  
23 housed males were weighed and killed on PND 183–185, and group-housed males were weighed and killed  
24 on PND 186–187. Equal numbers of males from each group were killed each day. Liver, kidney, and  
25 reproductive organs were weighed, and testicular sperm count and efficiency were determined. Technicians  
26 were blinded to experimental conditions. Measures taken to reduce stress to animals included administering  
27 test agents by drip feeding, minimal handling of pups, and minimal environmental noise. Selection of 3  
28 males from each litter increased statistical power compared to previous studies (275, 395). Statistical  
29 analyses were dually conducted using the individual offspring and the litter as the statistical unit. Data were  
30 evaluated by ANOVA and Dunnett test. Results from vehicle-treated and naïve controls were pooled when  
31 there was no evidence of a vehicle effect. Data from individually housed and group housed-males were  
32 pooled when they did not differ significantly.

33  
34 There were no significant differences in litter sizes or percentage of males/litter. In female offspring from  
35 the bisphenol A groups, there were no significant effects on body weight or organ weights, including  
36 cervix, uterus, vagina, and ovary. Age and weight at vaginal opening were also unaffected in groups  
37 exposed to bisphenol A. Vaginal opening was delayed in the diethylstilbestrol-treated group and in the  
38 naïve control group.

39  
40 Significant effects included increased terminal body weights in the low-dose group, increased testis weight  
41 in both dose groups, and increased epididymis weight in the high-dose group. Because testis and  
42 epididymis weights relative to body weights were nearly identical to controls [**data not shown by study**  
43 **authors**], the authors considered the finding equivocal. Although prostate weights were slightly higher in  
44 the bisphenol A groups, there were no statistically significant effects on prostate weight when adjusted for  
45 body weight and litter effects. Daily sperm production was increased in both dose groups, but the study  
46 authors considered the finding equivocal due to low biological significance. The study authors noted that  
47 the study failed to confirm the increase in prostate weight and decrease in sperm production reported in the  
48 studies by vom Saal et al. (395) and Nagel et al. (275), but results were consistent with those reported by  
49 Cagen et al. (393). Possible reasons for variability between studies were stated as differences in background  
50 sound level, diet, and animal body weights. The study authors also mentioned the possibility of genetic drift  
51 occurring in mice bred in-house in the vom Saal laboratory.

1  
2 **[The NTP Statistics Subpanel (340) essentially reproduced the findings reported by Ashby et al.**  
3 **(394).]**  
4

5 **Strengths/Weaknesses:** Strengths are the rather close replication of the designs of the studies by vom Saal  
6 et al. (392) and Nagel et al. (275) with diet as the only major difference, the use of both solo and group  
7 housed mice, and the support of the conclusions by the NTP Statistics Subpanel. The use of small samples  
8 is an understandable weakness given that this study was designed to be a replicate study. The lack of  
9 response of the positive control DES group is problematic

10  
11 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
12 due to absence of response of the positive control group and small sample sizes.  
13

14 **Howdeshell et al. (396)**, support not indicated, examined the effect of prenatal bisphenol A exposure on  
15 age of puberty in female mice. **[No information was provided about chow or composition of bedding**  
16 **and cage materials.]** CF-1 mice (n = 21/group) were fed oil vehicle **[type of oil not specified]** or  
17 bisphenol A **[purity not reported]** at 0.0024 mg/kg bw/day on GD 11–17 **[day of vaginal plug not**  
18 **defined]**. On GD 19, pups were obtained by cesarean section. Intrauterine position of pups (i.e., located  
19 next to male or female pups) was noted at that time. Pups were fostered by untreated mothers and weaned  
20 on PND 22. Body weights were measured, and pups were monitored for vaginal opening and time to estrus.  
21 Results were analyzed according to all pups from each dose group or in relation to intrauterine position.  
22 The study authors stated that fetuses positioned between 2 male mice were exposed to the lowest levels of  
23 17 $\beta$ -estradiol, while exposures to 17 $\beta$ -estradiol were highest in fetuses positioned next to female fetuses.  
24 Data were analyzed on a litter basis to control for maternal effects. Age of vaginal opening was covaried  
25 with weight at weaning. Numbers of female offspring evaluated were 75–111/group for body weight and  
26 51–58/group for vaginal opening. The study authors attempted to evaluate females from each intrauterine  
27 position in each litter. **[No additional information was provided for statistical analysis in this brief**  
28 **communication.]**  
29

30 Body weight at weaning was significantly increased in females in the bisphenol A group. When analyzed  
31 according to intrauterine position, body weights were 22% higher than controls in females who were not  
32 positioned next to a male fetus and 9% higher in females who had been positioned next to 1 male in utero.  
33 There were no significant effects on age of vaginal opening. **[It was not clear if the data presented were**  
34 **covaried with body weight.]** Bisphenol A treatment significantly reduced the period between vaginal  
35 opening and first estrus by ~2.5 days. When evaluated according to intrauterine position, a significant  
36 decrease in time to first estrus was observed in females who were not positioned next to a male pup  
37 (accelerated by ~5 days) and in females positioned next to 1 male **[~2 days]**. No statistically significant  
38 findings were observed in females who had been positioned next to 2 males in utero. The study authors  
39 concluded that prenatal exposure to bisphenol A at environmentally relevant levels altered postnatal growth  
40 and reproductive function in female mice but that natural variations in individual endogenous 17 $\beta$ -estradiol  
41 levels influenced the response to bisphenol A.  
42

43 The results of this study were also discussed in a publication by Howdeshell and vom Saal (397), which  
44 indicated that the work was supported by NIH and reported additional findings. There was a bisphenol A-  
45 associated reduction in pup survival between birth and weaning. Complete litter death occurred in 6 of 21  
46 litters in the bisphenol A group compared to 1 of 21 litters in the control group. Significantly increased  
47 body weight of male pups at weaning was also reported for the bisphenol A group. Body weights were  
48 highest in males who were positioned next to 2 female pups in utero and were 10% higher than body  
49 weights of control males positioned next to 2 female fetuses in utero. No increase in body weight occurred  
50 in males that were positioned between two male fetuses in utero. Although the authors identified a litter-

### 3.0 Developmental Toxicity Data

1 based analysis, it was not always clear that this applied to all analyses (in Study Figure 1, the n values  
2 exceed the number of dams, suggesting that some of the data were analyzed on a per pup basis.

3  
4 **[The NTP Statistics Subpanel (340) requested the Howdeshell et al. (396) dataset for reanalysis, but it  
5 was not provided by study authors.]**

6  
7 **Strengths/Weaknesses:** Strengths are the oral route of exposure and the use of a low dose level of  
8 bisphenol A. The omission of a description of husbandry conditions and lack of clarity of statistical  
9 procedures are weaknesses. Use of only a single dose is a weakness. Further, the use of time from vaginal  
10 opening to first estrus is not a standard endpoint for assessing puberty in mice and is of questionable  
11 biological significance.

12  
13 **Utility (adequacy) for CERHR evaluation process:** This paper is adequate for the evaluation process but  
14 utility is limited due to uncertainties in data analyses

15  
16 **Gupta (398)**, supported by NIH, examined the effects of bisphenol A exposure on the reproductive system  
17 of male mice. CD-1 mice were received on GD 12 (GD 0 = day of breeding). The mice were fed Purina  
18 Chow-5 L9 at the Charles Rivers Laboratory and Purina Chow 5012 at the study author's laboratory. **[No  
19 information was provided on bedding or caging materials.]** On GD 16–18, 15 mice/group were fed the  
20 corn oil/12% ethanol vehicle or 0.050 mg/kg bw/day bisphenol A **[purity not reported]**. Additional groups  
21 of mice were administered diethylstilbestrol at 0.1 and 200 µg/kg bw/day and Aroclor at 0.050 mg/kg  
22 bw/day during the same time period. The bisphenol A dose level was based on a level reportedly  
23 considered safe by the FDA. Following delivery, litters were culled to 8 pups, with at least 3 males. Body  
24 weight and anogenital distance were examined in 3 pups/litter (45 pups) on PND 3, 2 pups/litter (30 pups)  
25 on PND 21, and 1 offspring/litter (15 offspring) on PND 60. **[Although Table 1 of the study lists the n  
26 value as 15–45/group, a statement in the methods section indicated that an equal number of pups  
27 (n=1–3) were pooled from each litter.]** Prostate and epididymis were weighed in 15 offspring/group on  
28 PND 3, 21, and 60. Whole-tissue mounts of prostate were examined for growth in 15-day-old offspring (n  
29 = 4/group). Androgen binding was measured in prostates isolated at 3, 21, and 60 days of age, with 2–6  
30 prostates pooled, depending upon age; an n of 5 was reported in Figure 2 of the study. Data were analyzed  
31 by ANOVA. **[It was not clear if the offspring or litter was considered the statistical unit.]**

32  
33 Body weights of male offspring were not affected by bisphenol A treatment. In male pups of the bisphenol  
34 A group compared to the control group, anogenital distance adjusted for body weight was significantly  
35 increased **[by 22%]** on PND 3, **[by 25%]** on PND 21, and **[by 33%]** on PND 60. Prostate weights in males  
36 of the bisphenol A group were significantly increased **[by 56%]** on day 3, **[by 39%]** on day 21, and **[by  
37 101%]** on day 60. Relative (to body weight) epididymis weight in the bisphenol A group was significantly  
38 reduced **[by 35%]** on PND 60. Prostate growth was reported to be qualitatively increased by bisphenol A  
39 exposure. Androgen receptor binding was increased on PND 21 and 60 **[by ~344% on PND 21 and 358%  
40 on PND 60, estimated from a graph]**. Similar effects were reported following treatment with the low dose  
41 of diethylstilbestrol and Aroclor. In contrast, the high dose of diethylstilbestrol reduced body weights,  
42 anogenital distance, prostate weight, and androgen receptor binding. Presentation of pathology data are  
43 superficial, thus questioning interpretation.

44  
45 The report also included an in vitro study to examine the effects of bisphenol A on prostate growth. The  
46 urogenital sinus was dissected from GD 17 fetuses and cultured for 7 days in media containing 0, 5, or 50  
47 ng/L bisphenol A with and without the addition of testosterone. The urogenital sinus was also incubated in  
48 0.1 or 0.5 ng/L diethylstilbestrol and 5 or 30 ng/L Aroclor. Prostates obtained from cultures were then fixed  
49 in Bouin solution and examined histologically. A similar protocol was used to examine androgen binding in  
50 cultured prostates, except that only the high doses of each compound were examined, and cells were  
51 cultured for 6 days. Bisphenol A at 50 ng/L increased prostate size **[by 140%]** in the absence of

### 3.0 Developmental Toxicity Data

1 testosterone and **[by 150%]** in the presence of testosterone. Androgen binding in prostate was increased  
2 **[by 200%]** following treatment with bisphenol A. Similar effects were reported with diethylstilbestrol and  
3 the high Aroclor dose. The study authors concluded that the effects of in vivo studies were reproduced in in  
4 vitro studies, which suggests a direct effect on reproductive organs of fetal mice.

5  
6 In a subsequent commentary, Elswick et al. (399) noted several concerns and requested clarification of the  
7 data analysis performed by Gupta. It was noted that statistical analyses were insufficiently described to  
8 determine if analyses in addition to ANOVA were conducted. It was not indicated if post hoc tests were  
9 used or if corrections were made for multiple comparisons. Table 1 of the study was noted to contain a  
10 footnote indicating  $P < 0.05$  (larger) or  $P < 0.05$  (smaller). It was stated that determining a mean and  
11 conducting a one-tailed post hoc test based upon whether the mean is larger or smaller is a source of  
12 potential bias in the statistical analyses. Analyses conducted by Elswick et al. indicated that the assumption  
13 of homogeneity of variance, a requirement for ANOVA, was not met for some data such as anogenital  
14 distance on PND 3 (Table 1 of the study) and prostate size (Table 3 of the study). Therefore, questions were  
15 raised about whether homogeneity testing was done or if data were transformed to account for lack of  
16 homogenous variances prior to ANOVA. Failure to consider the litter as the experimental unit was noted in  
17 cases where the sample size was listed as 30 and 45, while only 15 dams/group were treated. It was noted  
18 that if anogenital distance was measured in the same animal at different time points, a repeated-measures  
19 ANOVA would have been the appropriate statistical test. It was stated that correction of anogenital distance  
20 by the cube root of body weight instead of body weight would have been preferred to avoid overcorrection;  
21 ANCOVA with body weight as a covariate would have been a better method for correcting anogenital  
22 distance, and the best method would have been a nested ANCOVA (dam within treatment). Questions were  
23 raised about whether sampling 1 pup/litter on PND 60 provided a reliable estimate, especially for highly  
24 variable endpoints such as anogenital distance, which can be affected by sex of the adjacent fetuses in the  
25 uterus. Organ weights were also stated to be variable, and it was questioned whether sampling 1  
26 offspring/litter on PND 60 resulted in a reliable estimate.

27  
28 Gupta (400) responded to the questions raised by Elswick et al. Regarding the question of post hoc tests for  
29 data analyzed by ANOVA, Gupta stated that comparisons using the least significant difference test support  
30 the effect reported in the original paper. Gupta stated that the use of 1-tailed tests was never mentioned and  
31 that the criticism was unfounded. The numbers of offspring examined at each age was reiterated **[with no**  
32 **mention of considering the litter the statistical unit]**. It was stated that individual animals were not  
33 identified because it would have required using a toe clip or tattoo, which is stressful to the animals.  
34 Therefore, it was not known if the same animals were examined for anogenital distance at the different time  
35 points and use of the repeated-measures ANOVA would not have been appropriate. Regarding use of 1  
36 animal/litter, it was stated that it is the standard procedure accepted by NIEHS to control for litter effects.  
37 Correction of anogenital distance by body weight was stated to be appropriate because of a significant  
38 correlation between body weight and anogenital distance ( $r = 0.47$ ,  $P < 0.001$ ). Adjustment for litter effects  
39 was stated to occur because litter was nested within treatment in the ANOVA. Gupta noted a typographical  
40 error in Table 3 of the original paper. Standard deviations for the 50 ng/L bisphenol A and Aroclor groups  
41 were mistakenly indicated to be 10-fold higher than the actual values (i.e., the actual values were 0.024 for  
42 bisphenol A and 0.032 for Aroclor). The errors made it appear that there were differences in variances  
43 between groups, when actually there were not. Gupta stood by his original conclusion that low levels of  
44 bisphenol A alter the development of the male reproductive tract.

45  
46 **Strengths/Weaknesses:** Strengths are the oral route of administration, the use of a low dose level of  
47 bisphenol A, the use of diethylstilbestrol as a positive control, the prostate measurements at 3 postnatal  
48 time points, and the use of an in vitro study to support the in vivo results. The use of a single dose level,  
49 and questionable histopathological presentation and evaluation are weaknesses. An additional weakness is  
50 that more than one male per litter was used for some endpoints without adequate statistical control for litter  
51 effects.

### 3.0 Developmental Toxicity Data

1 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for  
2 evaluation of prostate weight, biochemical endpoints, and body weight and AGD at PND 60 but not other  
3 endpoints where litter effects were not adequately controlled for (i.e., those where 30 or 45 pups were  
4 examined from 15 litters).

5  
6 **Iida et al. (401)**, supported by the Japan Society for Promotion of Science, examined the effect of prenatal  
7 bisphenol A exposure on spermatogenesis in adult mice. **[No information was provided about**  
8 **composition of feed, caging, or bedding.]** On GD 10–17 **[day of vaginal plug not defined]**,  $\geq 3$  ddY  
9 mice/group were orally administered bisphenol A **[purity not reported]** at 0 (corn oil vehicle), 1, 10, or  
10 100 mg/kg bw/day. **[The specific method of oral dosing was not stated.]** At 60 days of age, 4–5 male  
11 mice/dose group (obtained from 3 litters/dose group) were weighed and killed. Testes were removed and  
12 fixed in paraformaldehyde for histopathological evaluation by light microscopy. At 120 days of age,  
13 testicular histopathology was examined by light and electron microscopy in 3 mice/group from the control  
14 and 10 mg/kg bw/day groups. Data were analyzed by ANOVA. **[It was not clear if the litter or offspring**  
15 **were considered the statistical unit.]**

16  
17 No effects on body weight were observed in 60-day-old mice. Significant and dose-related increases in the  
18 incidence of abnormal seminiferous tubules were observed in mice exposed to bisphenol A. The incidence  
19 of abnormal seminiferous tubules in the control and each respective treatment group was 3.7, 15.2, 17.7,  
20 and 31.5%. **[Benchmark dose analysis using a probit model and n = 3 litters gave a BMD<sub>10</sub> = 44 and a**  
21 **BMDL<sub>10</sub> = 17 mg/kg bw/day.]** Examples of seminiferous tubule lesions included luminal space loss in  
22 tubules, reduced numbers of maturing elongate spermatids, decreased tubular diameter, aberrant  
23 distribution of spermatogenic cells in epithelium, and accumulation of material within tubules. In the 120-  
24 day-old mice exposed to 10 mg/kg bw/day, the same types of lesions were observed at a higher incidence  
25 than controls (28.3 compared to 5.14%). Electron microscopic examinations of 2 abnormal seminiferous  
26 tubules from exposed 120-day-old mice revealed the presence of round but not elongated spermatids,  
27 leading study authors to suggest disrupted spermatogenesis. Disorganized arrangement of Sertoli cells was  
28 also observed in the 120-day-old mice of the 10 mg/kg bw/day group. The study authors noted that  
29 degeneration of Sertoli cells may be the cause of aberrant distribution of spermatogenic cells.

30  
31 **Strengths/Weaknesses:** The oral route of delivery is a strength of this study. The lack of information on  
32 details of husbandry, the small sample size (4-5 male mice from 3 litters per dose group) and the lack of  
33 adjustment for litter effects, inadequate methods for histopathological preservation and evaluation (i.e., use  
34 of paraformaldehyde for paraffin embedding) are weaknesses.

35  
36 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
37 based on methodology.

38  
39 **Timms et al. (402)**, supported by NIEHS and US EPA, examined the effects of bisphenol A exposure on  
40 development of the prostate in mice. CD-1 mice were fed soy-based Purina 5008 chow, provided drinking  
41 water in glass bottles, and housed in polypropylene cages. **[The type of bedding material was not**  
42 **indicated.]** On GD 14–18 (day of mating = GD 0), pregnant mice were fed by micropipette with 0.010  
43 mg/kg bw/day bisphenol A **[purity not indicated]** (n = 6), the tocopherol-stripped corn oil vehicle (n = 5),  
44 0.1  $\mu\text{g/kg}$  bw/day ethinyl estradiol (n = 5), or 0.1  $\mu\text{g/kg}$  bw/day diethylstilbestrol (n = 5), the positive  
45 control. The dose of bisphenol A was based on previous findings that suggested bisphenol A was 100-fold  
46 less potent than diethylstilbestrol in permanently increasing prostate size in mice. On GD 19, fetuses were  
47 removed by cesarean section, and during the removal process, intrauterine position of male fetus relative to  
48 sex of adjacent fetuses was recorded. To reduce effects associated with sex hormone exposure from the  
49 adjacent fetus, 1 male/litter that developed between a male and female fetus was examined. Prostate  
50 morphology was determined by a 3D computer reconstruction technique. Immunohistochemistry  
51 techniques were used to measure levels of proliferating cell nuclear antigen and mouse keratin 5. Statistical

### 3.0 Developmental Toxicity Data

1 analyses included ANOVA, followed by Fisher least-squares mean test when statistical significance was  
 2 obtained. In a separate study, prostate morphology was examined in 4 pregnant mice/group that were dosed  
 3 with vehicle or 200 µg/kg bw/day diethylstilbestrol according to the procedures described above.  
 4

5 Bisphenol A increased numbers of ducts, volume, and proliferation in one or more prostate regions, as  
 6 outlined in Table 77. The pattern of proliferating cell nuclear antigen staining was similar to that observed  
 7 with mouse keratin 5, a basal epithelial cell maker. The study authors also reported a 56% increase in the  
 8 volume of the coagulating glands. **[Data were not shown by study authors.]** An abnormal narrowing was  
 9 observed in the portion of the urethra near the neck of the bladder. **[The volume of the cranial urethra**  
 10 **was reduced by 35% compared to controls. Malformation of prostatic sulci was reported, but no**  
 11 **information was provided on incidence or severity.]** Similar effects on the prostate were reported in  
 12 mice exposed to ethinyl estradiol and the low dose of diethylstilbestrol. Narrowing of the cranial urethra  
 13 was observed in mice exposed to ethinyl estradiol. In contrast, exposure to the high diethylstilbestrol dose  
 14 resulted in inhibited morphogenesis of the prostate. The study authors concluded that the differentiating  
 15 urogenital system of male mice is very sensitive to a low dose of bisphenol A.  
 16

17 **Table 77. Effects on Prostate Development in Mice Following Prenatal Exposure to 0.010 mg/kg**  
 18 **bw/day Bisphenol A**

Endpoint <sup>a</sup>	Prostate region		
	Dorsolateral	Ventral	Dorsolateral and ventral
No. of prostate ducts	↑41%	↔	↑40%
Prostate duct volume	↑99%	↑78%	↑91%
Proliferating cell nuclear antigen staining	↑44%	↔	No data

↑,↓ Statistically significant increase, decrease; ↔ no statistically significant effect.

<sup>a</sup>Percent changes calculated by CERHR differed slightly from values presented by authors; it was not clear which part of the prostate the authors' values represented.

From Timms et al. (402).

19  
 20 **Strengths/Weaknesses:** Strengths are the oral route of administration, the low dose level of bisphenol A,  
 21 the use of diethylstilbestrol and ethinyl estradiol as positive controls, and the sophisticated measures  
 22 applied to the prostate. Weaknesses are the use of a single dose level and small sample size, although the  
 23 Panel judged it to be adequate for the methodology.  
 24

25 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is adequate and of high utility for the  
 26 evaluation.  
 27

28 **Palanza et al. (403)**, supported by NIEHS, NIH, MURST, the University of Parma, and the National  
 29 Council for Research, examined the effects of bisphenol A treatment on maternal behavior following  
 30 exposure of mice during prenatal development and/or adulthood. The CD-1 mice used in this study were  
 31 maintained as an outbred colony. Mice were housed in polypropylene cages with corn cob bedding. During  
 32 pregnancy and lactation, mice were fed Purina 5008 (soy-based) chow. After weaning, mice were fed  
 33 Purina 5001 (soy-based) chow. Water was provided in glass bottles. On GD 14–18 (GD 0 = day of vaginal  
 34 plug), 14 mice were fed the tocopherol-stripped corn oil vehicle and 9 mice were fed 0.010 mg/kg bw/day  
 35 bisphenol A **[purity not reported]** using an electronic micropipette. Dams were housed 3/cage after  
 36 mating and individually housed on GD 17. Body weights of dams were measured during gestation. The day  
 37 of birth was considered PND 1, and offspring were weaned on PND 20. At 2–2.5 months of age, F<sub>1</sub> female  
 38 offspring from vehicle- and bisphenol A-treated dams were mated and exposed to vehicle or 0.010 mg/kg  
 39 bw/day bisphenol A on GD 14–18. There were 4 groups of F<sub>1</sub> females that were exposed during gestation-  
 40 adulthood to vehicle-vehicle (n = 20), vehicle-bisphenol A (n = 15), bisphenol A-vehicle (n=15), and  
 41 bisphenol A–bisphenol A (n=15). Maternal behavior was observed in F<sub>1</sub> dams every 4 minutes during a

### 3.0 Developmental Toxicity Data

1 120-minute period on PND 2–15. On PND 1, F<sub>2</sub> pups were weighed, sexed, and counted. Litters were then  
 2 culled to 10 pups, with equal numbers of male and female pups when possible. Pups were weighed during  
 3 the lactation period and cliff-drop aversion and righting reflex were evaluated in all pups of a subset of 8  
 4 litters/group on PND 3, 5, 7, and 9. For statistical analyses, all pup data were adjusted for litter. Data were  
 5 analyzed by ANOVA, Holms *t*-test, and/or Fisher protected least-squared difference test.

6  
 7 Bisphenol A treatment did not affect gestational body weight gain in F<sub>0</sub> or F<sub>1</sub> dams. Statistically significant  
 8 effects for F<sub>1</sub> maternal behavior collapsed across 14 observation days are presented in Table 78. Exposure  
 9 to bisphenol A either in gestation or in adulthood resulted in decreases in the percentage of time the dams  
 10 spent nursing and in the nest and increases in the percentage of time the dams spent nest building, resting  
 11 alone, grooming, and out of the nest. Increased activity was also observed in the group exposed to  
 12 bisphenol A in adulthood. The only significant effect observed in mice exposed to bisphenol A during  
 13 gestation and adulthood was increased time resting. When data were presented for individual evaluation  
 14 days, time resting was significantly increased on PNDs 9, 10, 11, 12, and 14 in the group exposed to  
 15 bisphenol A during gestation. Time spent resting was significantly increased on PND 9 and 14 in the group  
 16 exposed to bisphenol A during gestation and adulthood. No other significant effects were observed on  
 17 specific evaluation days. There were no significant differences in the number of live F<sub>2</sub> pups/litter, sex  
 18 ratio, or body weight at birth or in weight gain during the lactation period. **[Data were not shown]**. No  
 19 significant effects were observed for cliff aversion or righting reflexes. The study authors concluded that  
 20 reduced levels of nursing behavior were observed in mice exposed to bisphenol A only as fetuses or only as  
 21 adults. **[Because this study involves effects of adult exposure on maternal behaviors, it is also**  
 22 **discussed in Section 4.2]**

23  
 24 **Table 78. Maternal Behavior Effects in Mice Exposed to Bisphenol A During Gestation and/or**  
 25 **Adulthood**

Percent time <sup>a</sup>	Bisphenol A exposure during gestation/adulthood		
	Bisphenol A/vehicle	Vehicle/bisphenol A	Bisphenol A/bisphenol A
Nursing	↓15%	↓14%	↔
Nest building	↑73%	↑146%	↔
Resting alone	↑67%	↑29%	↑46%
Grooming	↑25%	↑18%	↔
Active	↔	↑18%	↔
In nest	↓12%	↓10%	↔
Out of nest	↑17%	↑12%	↔

<sup>a</sup>Data were presented graphically. Values were provided by the study author (personal communication, P. Palanza, February 26, 2007).

↑,↓ Statistically significant increase/decrease compared to vehicle-vehicle group, ↔ no statistically significant effect.

From Palanza et al. (403).

26  
 27 **Strengths/Weaknesses:** Strengths are the oral route of administration, the low dose level of bisphenol A,  
 28 and the exploration of effects on complex maternal behaviors. It is unusual that pre- and postnatal exposure  
 29 had effects but not the combination of pre- and postnatal exposure, and failure to explain this finding is a  
 30 weakness. The use of a diet high in soy isoflavones is an additional weakness.

31  
 32 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is adequate and of high utility for the  
 33 evaluation process.

34  
 35 **Nishizawa et al. (404),** supported by the Japanese Ministry of Education, Culture, Sports, Science, and  
 36 Technology, examined the effects of prenatal bisphenol A exposure on expression of retinoic acid receptor

### 3.0 Developmental Toxicity Data

1  $\alpha$  and retinoid X receptor  $\alpha$  in mouse embryos. ICR mice were fed standard feed (CM, Oriental Yeast,  
2 Tokyo). [No information was provided about caging and bedding materials.] Mice were orally dosed  
3 with bisphenol A [purity not indicated] at 0 (olive oil vehicle) or 0.002 mg/kg bw/day on 6.5–11.5, 6.5–  
4 13.5, 6.5–15.5, and 6.5–17.5 days post coitum. Day of vaginal plug was considered 0.5 days post coitum.  
5 [No information was provided about the specific method of oral dosing.] Twelve dams/group were  
6 killed at 12.5, 14.5, 16.5, and 18.5 days post coitum, 24 hours after receiving the last dose. Expression of  
7 mRNA for retinoic acid receptor  $\alpha$  and retinoid X receptor  $\alpha$  was measured by RT-PCR in fetal cerebrum,  
8 cerebellum, and gonads. Data were analyzed by ANOVA. [It was not clear if the litter or offspring was  
9 considered the measurement unit.] Numerous changes in mRNA expression were observed following in  
10 utero exposure to bisphenol A, and they varied according to sex, tissue, and dosing period. The study  
11 authors concluded that these findings suggest a novel mechanism of bisphenol A toxicity mediation by  
12 disruption of the expression of retinoic acid receptor  $\alpha$  and retinoid X receptor  $\alpha$ .

13  
14 **Strengths/Weaknesses:** Strengths are the oral route of delivery, the use of a low dose level of bisphenol A,  
15 and the exposure at different time periods. The study has value for understanding mechanisms of action  
16 although these changes were not tied to any adverse findings that might be related to these changes.  
17 Weaknesses include the use of a single dose level and lack of clarity on number of embryos per litter  
18 sampled. This is not considered a critical weakness because it is known that standard procedures for these  
19 methods require pooling of embryos within litter.

20  
21 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is adequate but of limited utility for the  
22 evaluation because of the mechanistic nature of the endpoints.

23  
24 **Nishizawa et al. (405)**, supported by the Japanese Ministry of Education, Culture, Sports, Science, and  
25 Technology and by the Japan Society for the Promotion of Science, examined the effects of bisphenol A  
26 exposure on expression of mRNA for arylhydrocarbon and retinoid receptors in mouse embryos. ICR mice  
27 were fed standard diet (CM; Oriental Yeast, Tokyo). [No information was provided about caging or  
28 bedding materials.] Pregnant mice were orally dosed with bisphenol A [purity not indicated] at 0 (olive  
29 oil vehicle), 0.00002, 0.002, 0.20, or 20 mg/kg bw/day from 6.5 to 13.5 days post coitum or 6.5 to 17.5  
30 days post coitum. Day of vaginal plug detection was considered 0.5 days post coitum. [No information  
31 was provided about the specific method of oral dosing.] Twelve pregnant mice/group were killed on 14  
32 and 18.5 days post coitum, 24 hours after the last bisphenol A dose was administered. RT-PCR analyses  
33 were conducted to determine expression of mRNA for retinoic acid, retinoid X, and arylhydrocarbon  
34 receptors in fetal cerebrum, cerebellum, ovary, and testis. Data were analyzed by ANOVA. [It was not  
35 clear if the litter or offspring was considered the measurement unit.] Numerous changes in mRNA  
36 expression were observed following bisphenol A exposure and they varied according to dose, sex, tissue,  
37 and exposure period. The study authors concluded the this study demonstrates a novel mechanism by which  
38 bisphenol can induce endocrine disruption through upregulation of arylhydrocarbon receptor (a key factor  
39 in the metabolism of some xenobiotics compounds) and retinoid receptors (key factors in nuclear receptor  
40 signal transduction).

41  
42 **Strengths/Weaknesses:** The wide dose range from 0.00002 to 20 mg/kg bw/day and the oral route are  
43 strengths. The study has value for understanding mechanisms of action although these changes were not  
44 tied to any adverse findings that might be related to these changes.. Weaknesses include the lack of  
45 specification of the method of oral dosing and lack of clarity on sample origins and sizes for each assay.  
46 Again, this is not considered a critical weakness because it is known that standard procedures for these  
47 methods require pooling of embryos within litter.

48  
49 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is adequate but of limited utility for the  
50 evaluation because of the mechanistic nature of the endpoints.

51

### 3.0 Developmental Toxicity Data

1 **Nishizawa et al. (406)**, supported by the Japan Society for the Promotion of Science, examined the effects  
2 of bisphenol A exposure on expression of aryl hydrocarbon receptors, related factors, and metabolizing  
3 enzymes in mouse embryos. ICR mice were fed standard diet (CM, Oriental Yeast, Tokyo). **[No**  
4 **information was provided about caging and bedding materials.]** Mice were orally dosed with bisphenol  
5 A **[purity not indicated]** at 0 (olive oil vehicle), 0.00002, 0.002, 0.2, or 20 mg/kg bw/day from 6.5–13.5  
6 days post coitum and 6.5 to 17.5 days post coitum. Day of vaginal plug was considered 0.5 days post  
7 partum. **[No information was provided about the method of oral dosing.]** Another group of mice was  
8 dosed with 5 µg/kg bw/day 17β-estradiol during the same time periods. Twelve mice/group were killed at  
9 14.5 and 18.5 days post coitum, 24 hours after receiving the final dose. Embryos were dissected to obtain  
10 cerebrum, cerebellum, ovary, testis, and liver. RT-PCR analysis was used to measure mRNA levels of  
11 genes. Western immunoblotting was used to measure protein levels of CYP1A1 and glutathione-S-  
12 transferase in liver. Data were analyzed by ANOVA. **[It was not clear if the litter or offspring was**  
13 **considered the measurement or statistical unit.]**

14  
15 Numerous changes in mRNA expression were observed following bisphenol A exposure, and they varied  
16 according to dose, sex, tissue, and exposure period. In at least one sex and time period, exposure to 17β-  
17 estradiol increased expression of mRNA arylhydrocarbon receptor in all tissues, arylhydrocarbon receptor  
18 repressor in testes and ovaries, arylhydrocarbon receptor nuclear translocator in brain or testes, *CYP1A1* in  
19 brain, and glutathione S-transferase in brain. Changes in protein levels of CYP1A1 and glutathione S-  
20 transferase in liver were also examined in embryos at 18.5 days post coitum and levels of both proteins  
21 were increased with exposure to bisphenol A at doses ≥0.2 mg/kg bw/day and with exposure to 17β-  
22 estradiol. The study authors proposed a novel mechanism of toxicity involving up-regulation of mRNA for  
23 arylhydrocarbon receptor and other factors by bisphenol A.

24  
25 **Strengths/Weaknesses:** The wide dose range and the oral route are strengths. The study has value for  
26 understanding mechanisms of action although these changes were not tied to any adverse findings that  
27 might be related to these changes. Weaknesses include the lack of specification of the method of oral  
28 dosing and lack of clarity on sample origins and sizes for each assay. This is not considered a critical  
29 weakness because it is known that standard procedures for these methods require pooling of embryos  
30 within litter.

31  
32 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is adequate but of limited utility for the  
33 evaluation because of the mechanistic nature of the endpoints.

34  
35 **Imanishi et al. (407)**, supported by the Ministry of Education, Culture, Sports, Science, and Technology of  
36 Japan, used DNA microarrays to investigate potential mode of action of bisphenol A on alterations in  
37 expression of 20 nuclear hormone receptors and a few other genes in the mouse placenta. ICR male and  
38 female mice were housed in polycarbonate cages, given ad libitum access to tap water and CM rodent feed  
39 (Oriental Yeast, Tokyo, Japan), and maintained under standard 12h/12h light/dark cycle. Between 6.5 and  
40 17 days post-coitum, pregnant dams were orally administered 0 or 0.002 mg/kg bw/day bisphenol A  
41 **[purity not provided]** in olive oil **[method of oral administration not given]**. The dams were killed 18.5  
42 days post-coitum, and placentas and fetuses were frozen at –80 C. Placental RNA from male and female  
43 embryos was separately extracted, reverse transcribed, and hybridized to a microarray chip for 18 hours at  
44 42 C. Images were analyzed using Atlas navigator software, and statistical analyses were performed using  
45 the Pearson correlation coefficient, normalized to the Fisher *z* transformation. Differentially expressed  
46 genes were identified using paired *t*-test, and significant changes were noted in percent values increased or  
47 decreased relative to control mRNA expression values. **[The number of dams used and arrays run was**  
48 **not given. It was not clear if the litter or offspring were considered the statistical unit.]**

49  
50 Nuclear receptor genes that showed differential expression in male and/or female fetuses were: neuron-  
51 derived orphan receptor 1, retinoic acid related orphan receptor γ, estrogen receptor β, liver X receptor α,

### 3.0 Developmental Toxicity Data

1 progesterone receptor, chicken ovalbumin upstream promoter transcription factor  $\alpha$ , germ cell nuclear  
2 factor, steroidogenic factor 1, and photoreceptor-specific nuclear receptor. Nuclear receptor genes that did  
3 not show differential expression included thyroid hormone receptor  $\beta$ , peroxisome proliferators activated  
4 receptor  $\alpha$  and  $\gamma$ , constitutive androstane receptor, farnesoid X receptor, chicken ovalbumin upstream  
5 promoter transcription factor  $\beta$ , testis receptor  $\beta$ , estrogen related receptor  $\gamma$ , aryl hydrocarbon receptor,  
6 small heterodimer partner, and dosage-sensitive sex reversal receptor. Other genes the expression of which  
7 was both significantly altered in pair-wise comparison with control treatment and exhibited opposing up- or  
8 downregulation in a sex-dependent manner included fast skeletal troponin C, probasin, RNA-specific  
9 adenosine deaminase, and ADAM25/testase 2,  $\alpha$ -fetoprotein and kinesin light chain 1. These genes were  
10 downregulated in placentas of male fetuses and upregulated in placentas of female fetuses. Placentas of  
11 male and female fetuses exhibited downregulation if  $\alpha$ -fetoprotein ( $\downarrow$ 60%, male and  $\downarrow$ 24%, female) and  
12 kinesin light chain 1 ( $\downarrow$ 70%, male and  $\downarrow$ 10%, female).

13  
14 The authors conclude that fetal sex-based differences in placental physiology resulting from bisphenol A  
15 exposure may lead to subsequent sex-specific developmental perturbation. They also indicated that  
16 important but largely unknown effects of bisphenol A may occur with respect to a cluster of orphan nuclear  
17 receptors, which exhibited significant changes in gene expression.

18  
19 **Strengths/Weaknesses:** Strengths: The evaluation of several molecular endpoints including gene activity  
20 for several receptors that are not commonly examined, oral dosing, and use of a low dose represent  
21 strengths. Weaknesses are the use of only one dose level of BPA and absence of many critical experimental  
22 details such as the number of litters used

23  
24 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for inclusion due to lack of  
25 reporting key experimental details

26  
27 **Yoshino et al. (408)**, supported by the Japanese Ministry of Education, Science, Sports, and Culture and  
28 the Japan Private School Promotion Foundation, examined the effect of prenatal bisphenol A exposure on  
29 immune response in mice. **[No information was provided about feed or caging and bedding materials.]**  
30 DBA/1 J mice were fed bisphenol A **[purity not indicated]** at doses of 0 (ethanol/corn oil vehicle), 0.003,  
31 0.030, 0.300, or 3 mg/kg bw/day for 18 days **[stated to be 17 days in the Methods section but 18 days in**  
32 **other parts of the report]**, beginning on the day of a 24-hour mating period (day 0). Twelve mice/group  
33 were treated and 7–9/group became pregnant. **[The specific method of oral dosing was not described.]** At  
34 8 weeks of age (day 77) 5 mice/group/sex were randomly selected and immunized by ip injection with hen  
35 egg lysozyme. Representation of litter was not specified. Blood was collected and spleens were removed 3  
36 weeks following immunization (day 98). Serum levels of hen egg lysozyme-specific immunoglobulin G  
37 (IgG), IgG1, and IgG2A were measured by ELISA. Spleen cell suspensions were prepared, and  
38 proliferation was assessed by incorporation of  $^3\text{H}$ -thymidine following a 72-hour incubation with hen egg  
39 lysozyme. Spleen cell suspensions were also prepared for measurement of interferon- $\gamma$  and interleukin-4  
40 secretion by ELISA. An additional 6 mice/group/sex were killed at 8 weeks of age (day 77). Spleens were  
41 removed and expression of  $\text{CD3}^+\text{CD8}^+$  and  $\text{CD3}^+\text{CD4}^+$  molecules on splenic lymphocytes was examined  
42 using monoclonal antibodies and flow cytometry. Thymus and spleen were fixed in 4% formaldehyde and  
43 examined histologically. Data were analyzed by Mann-Whitney  $U$  test. **It was not clear if the litter of**  
44 **origin was accounted for in statistical analyses.**

45  
46 Bisphenol A treatment had no significant effect on pregnancy rate, sex ratio, or body weight of offspring.  
47 There were several significant immune responses for male mice. **[Results in female mice were said to be**  
48 **similar to those observed in male mice but the data were not show by study authors.]** At bisphenol A  
49 doses  $\geq 0.03$  mg/kg bw/day, production of anti-hen egg lysozyme IgG2a following immunization was  
50 increased. Effects observed at  $\geq 0.3$  mg/kg bw/day included increases in production of anti-hen egg  
51 lysozyme IgG and secretion of interferon- $\gamma$  and interleukin-4. Additional findings at the high dose (3 mg/kg

### 3.0 Developmental Toxicity Data

1 bw/day) were increases in spleen cell proliferation and production of anti-hen egg lysozyme IgG1  
2 following immunization. Augmentation of interferon- $\gamma$  and interleukin-4 secretion following incubation of  
3 spleen cells with hen egg lysozyme was examined in the high-dose group only and found to be increased.  
4 **[Increases in CD3<sup>+</sup>CD8<sup>+</sup> and CD3<sup>+</sup>CD4<sup>+</sup> expression on lymphocytes were reported in males and**  
5 **females exposed to bisphenol A, but the doses at which the effects occurred were not specified.]** No  
6 histopathological alterations were reported for the spleen or thymus. The study authors explained that  
7 effects on IgG2a and interferon- $\gamma$  were indicators of T helper 1 immune responses and effects on IgG1 and  
8 interleukin-4 were indicators of T helper 2 responses. They concluded that the findings suggest that  
9 prenatal exposure to bisphenol A may up-regulate immune responses in mice.

10  
11 **Strengths/Weaknesses:** The oral route of administration and the wide range of doses are strengths.  
12 Weaknesses include small sample size (n=5), lack of clarity regarding statistical handling of factors such as  
13 litter and sex effects.

14  
15 **Utility (Adequacy) of CERHR Evaluation:** This study is inadequate for the evaluation process due to the  
16 reasons stated above.

17  
18 **Berger et al. (409)**, supported by The Natural Sciences and Engineering Research Council of Canada,  
19 examined the effect of bisphenol A exposure on ovum implantation and pup survival in mice. CF-1 mice  
20 were housed in polypropylene cages and were fed Harlan Teklad 22/5 rodent feed, which was stated to  
21 contain soy. **[No information was provided about bedding materials.]** On GD 1–4 or 5 **[described as**  
22 **GD 1–5 in methods section and GD 1–4 in study figures and tables]** (GD 0 = day of vaginal plug), 31  
23 mice in the control group were sc injected with peanut oil vehicle and 5–15 mice/group were sc injected  
24 with bisphenol A (97% purity) at 0.0005, 0.0015, 0.0046, 0.0143, 0.0416, 0.125, 0.375, 1.125, 3.375, or  
25 10.125 mg/animal/day. In a second experimental group, BPA was administered through a diet containing  
26 3% or 6% BPA added to peanut butter and chow. In a 3<sup>rd</sup> experimental group maintained on chow, BPA  
27 was administered at 0.11, 1.0, 3.0, or 9.0% in separate offerings of peanut butter alone. Pregnancy  
28 disruptions in orally exposed mice are discussed in Section 3.2.5.1. **[In the first experimental group, if it**  
29 **is assumed that the mice weighed 0.02 kg at the start of gestation (115), CERHR estimated bisphenol**  
30 **A intakes of 0.025, 0.075, 0.23, 0.72, 2.1, 6.3, 19, 56, 170, and 500 mg/kg bw/day.]** Mice were allowed to  
31 litter. Pups were counted on the day of parturition and observed for survival for 5 days. Pups were weaned  
32 at 28 days after birth and at that time, body weight and sex ratio were determined. Data were analyzed by  
33 ANOVA, chi-squared test, and Newman-Keuls multiple comparisons. **[It was not clear if all offspring**  
34 **data were analyzed on a pup or litter basis.]** A study examining implantations in sc treated females is  
35 discussed in Section 4.2.1.1 Percent of females giving birth was significantly decreased in the 10.125  
36 mg/day group (~28% vs 97% in control group). Numbers of pups born were significantly decreased in the  
37 3.375 and 10.125 mg/day group (~8 and 2 pups in each of the dose groups and 13 pups in the control  
38 group). There were no treatment-related effects on pup weight or sex ratio at weaning. [As discussed in  
39 Section 3.2.5.1, **it appears that with oral exposure, pregnancy disruption occurred at higher bisphenol**  
40 **A levels (68.8 mg/day, 3440 mg/kg bw/day) than with sc exposure (10.125 mg/day, ~500 mg/kg**  
41 **bw/day)].** The study authors concluded that the amount of bisphenol A required for pregnancy disruption  
42 was higher than typical environmental levels but that it is not known if bisphenol A could have additive or  
43 synergistic effects with other environmental estrogens.

44  
45 **Strengths/Weaknesses:** A strength of the subcutaneous study is that it examined a wide range of bisphenol  
46 A dose levels. The comparison of the differential effects of sc and oral routes of bisphenol A administration  
47 is also a strength. Weaknesses include the limited/unequal number of mated mice in each dose group,  
48 absence of maternal data to ascertain the potential impact of maternal toxicity on pregnancy,  
49 methodological deficiencies regarding fertility assessment, and the use of a diet that contains  
50 phytoestrogens.

51

### 3.0 Developmental Toxicity Data

1 **Utility (Adequacy) for CERHR Evaluation Process:** Due to the limited number of mated mice per dose  
2 level (n=5-15), methodological concerns, absence of key statistical information as well as maternal  
3 information, this study is inadequate for the CERHR evaluation process.  
4

#### 5 *3.2.5.2 Studies with neurobehavioral endpoints*

6 **Narita et al. (410)**, supported by the Japanese Ministry of Health, Labor, and Welfare, and Ministry of  
7 Education, Culture, Sports, Science, and Technology, conducted a series of studies to examine the effects  
8 of bisphenol A on the dopaminergic system of mice exposed during development. Only brief details were  
9 provided about the studies. In each study, ddY mice received feed containing bisphenol A from mating to  
10 weaning of their offspring. **[No information was provided on purity of bisphenol A, type of feed, caging  
11 and bedding materials, the number of dams treated, or the ages or sexes of offspring that were  
12 tested.]** Statistical analyses included ANOVA with Bonferroni/Dunnett test. **[It was not clear if the litter  
13 or offspring was considered the statistical unit.]** In a place conditioning-study, testing was conducted in  
14 6–14 mice/group born to dams exposed to bisphenol A at 0, 0.03, 0.3, 3, 500, or 2000 mg/kg food.  
15 **[Assuming a female mouse eats ~0.2 kg feed/kg bw/day (115), bisphenol A intake would have been  
16 0.006, 0.06, 0.6, 100, or 400 mg/kg bw/day.]** During the preconditioning period, mice were placed in one  
17 section of a cage following injection with saline **[specific route not reported]** and in another section of the  
18 cage following sc injection with 1 mg/kg bw morphine. On the day of testing, the amount of time spent in  
19 each section of the cage was recorded. Mice from the lowest dose group (0.03 mg/kg food) and 2 highest  
20 dose groups (500 and 2000 mg/kg) food spent more time in the section of the cage associated with  
21 morphine injection. **[Compared to controls, the time spent in the morphine-associated section of the  
22 cage was~ 9.5-, 7-, and 9-fold longer in each of the respective dose groups.]** Total locomotor activity  
23 was measured for 3 hours in 5–15 mice/group born to dams exposed to 0, 0.03, 3, or 2000 mg/kg food.  
24 Following sc injection with 10 mg/kg bw morphine, activity was increased in mice from the low- (0.03  
25 mg/kg food ) and high- (2000 mg/kg food) dose groups compared to the control group **[increased by ~9-  
26 fold in the low dose group and 12-fold in the high-dose group]**. Binding of <sup>35</sup>S-guanosine-5' [γ-thio]-  
27 triphosphate in the limbic system was measured in 3 samples/group obtained from offspring of dams  
28 exposed to 0.03, 3, or 2000 mg/kg food. Dopamine-induced binding of <sup>35</sup>S-guanosine-5' [γ-thio]-  
29 triphosphate in the limbic system was increased at each dose level compared to controls **[by ~32, 18, and  
30 56%]**. Based on their findings, the study authors concluded that prenatal and neonatal exposures to low  
31 bisphenol A doses can potentiate central dopamine receptor-dependent neurotransmission in the mouse.  
32

33 **Strengths/Weaknesses:** This paper is so poorly written that it is extremely difficult to understand many  
34 sentences (let alone paragraphs) and to determine precisely what was done, why, and what happened. The  
35 main weakness of the paper is therefore its inability to pass its message to the reader. Given this limitation,  
36 it is difficult to determine whether the paper has any strengths, and if so what they might be.  
37

38 **Utility (Adequacy) for CERHR Evaluation:** This paper is inadequate for the evaluation process because  
39 of the lack of methodological details and the poor communication of the study results.  
40

41 **Kawai et al. (411)**, supported by Core Research for Evolutional Science and Technology and Japan  
42 Science and Technology, examined the effects of prenatal bisphenol A exposure on aggressive behavior in  
43 male mice. **[No information was provided about feed or bedding and caging materials.]** Pregnant CD-1  
44 mice were randomly assigned to groups of 7 and orally dosed by micropipette with 0.002 or 0.020 mg/kg  
45 bw/day bisphenol A **[purity not reported]** on GD 11–17. A control group of 9 mice received the corn oil  
46 vehicle by micropipette during the same time period. Doses were said to be within the range of human  
47 exposures. Pups were weaned on PND 21 (day of birth = PND 0), and randomly selected males from the  
48 same litter were housed in groups of 4 or 5. Aggression testing was conducted at 8, 12, and 16 weeks of  
49 age. For the testing, 15 control male mice from the 9 litters were randomly selected to be opponents and  
50 housed 5/cage. Opponents were used only once/day for testing. During testing of mice from the control and  
51 treated groups, the subject was housed alone for 5 minutes prior to placing the opponent mouse into the

### 3.0 Developmental Toxicity Data

1 cage. Behavior with the opponent mouse was observed for 7 minutes. The numbers of mice evaluated were  
2 26–32/group at 8 weeks of age, 18–24/group at 12 weeks of age, and 10–16/group at 16 weeks of age.  
3 Randomly selected mice were killed at 9, 13, and 17 weeks of age, one week following behavior testing,  
4 for measurement of testis weight and serum testosterone level. **[The results section states that testis**  
5 **weights and serum testosterone levels were obtained at 8, 12, and 16 weeks of age.]** Eight mice/group  
6 were killed after the first 2 test periods and 10–16 mice/group were killed after the last test period. Mice  
7 that were not killed were tested at the next evaluation period, so that mice killed after 16 weeks of age were  
8 tested a total of 3 times. Statistical analyses included ANOVA and Spearman rank correlation test. **[It does**  
9 **not appear that the litter was considered the statistical unit.]**

10  
11 Aggression scores, as determined by contact time, were significantly increased compared to the control  
12 group at 8 weeks of age in both the low- (124% increase) and high- (146% increase) dose bisphenol A  
13 groups. No treatment-related effects on aggression score were observed at 12 and 16 weeks of age. In the  
14 low-dose group, relative (to body weight) testis weight was 10% lower than controls at 8 weeks of age and  
15 18% lower than controls at 12 weeks of age. Relative testis weight was 11% lower than control values in  
16 the high-dose group at 12 weeks of age. No significant effects were observed for serum testosterone levels.  
17 There were no correlations between serum testosterone levels and contact time in aggression testing. The  
18 study authors concluded that prenatal bisphenol A exposure of mice resulted in behavioral changes and  
19 decreased relative testis weight that was more pronounced at the lower dose.

20  
21 **Strengths/Weaknesses:** Strengths are the use of 2 low dose levels and the oral route of administration. The  
22 lack of husbandry information, inappropriate presentation of testis weight data, variable degrees of repeated  
23 behavioral testing, and the apparent lack of consideration of possible litter effects are weaknesses.

24  
25 **Utility (adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation process  
26 due to the reasons stated above.

27  
28 **Kawai et al. (412)**, supported by Japan Sciences Technology and Core Research for Evolutional Science  
29 and Technology, evaluated the brain expression of *ER $\alpha$*  and *ER $\beta$*  in male mice exposed in utero to  
30 bisphenol A. Pregnant ICR mice were fed bisphenol A in corn oil by micropipette on GD 11–17 at 0 or  
31 0.002 mg/kg bw/day (n = 18/group). Mice were housed singly in polypropylene cages. **[The first day of**  
32 **gestation was likely designated as GD 0, according to a figure. Type of feed and bedding material**  
33 **were not given.]** Litters were reared by their dams until weaning on PND 21 **[birth = PND 0]**. Males from  
34 the same litters were housed 4 or 5/cage. Randomly selected males **[8–12/group, without mention of litter**  
35 **of origin]** were killed at 4–5, 8–9, or 12–13 weeks of age. Testosterone was measured by RIA in trunk  
36 blood serum. Brains were perfusion fixed and processed for immunostaining with antibody to *ER $\alpha$* , *ER $\beta$* ,  
37 serotonin, and serotonin transporter. Fields were selected within the dorsal raphe nucleus and *ER $\alpha$* - or  
38 *ER $\beta$* -positive neurons were counted in every fourth section (n = 8 or 9 animals/group). Staining for  
39 serotonin and serotonin transporter involved overlapping dendrites, making it difficult to count positive  
40 neurons, and densitometric methods were used to quantify staining for serotonin and serotonin transporter  
41 (n = 8–12 animals/group). Data were analyzed using 2-way ANOVA and post-hoc Student *t*-test.

42  
43 The number of neurons in the dorsal raphe nucleus expressing *ER $\alpha$*  and *ER $\beta$*  was increased by bisphenol A  
44 at 5 and 13 weeks but not at 9 weeks. There were no significant differences at any time point in serum  
45 testosterone concentrations. The authors identified a “tendency” for serotonin and serotonin transporter  
46 immunoreactivity to be increased by bisphenol A in the dorsal raphe nucleus, but there were no statistical  
47 differences between bisphenol A-treated and control brains at any time point. The authors concluded that it  
48 is possible that alterations in ER in the brain may be responsible for emotional and behavioral alterations in  
49 mice.

### 3.0 Developmental Toxicity Data

1 **Strengths/Weaknesses:** This was a reasonable attempt to detect effects and explore a connection between  
2 bisphenol A, brain receptors, and aggressive behavior. This study is weakened by the use of only one dose,  
3 lack of experimental details, and uncertain accounting for litter and repeated measures/sections effects in  
4 analyses.

5  
6 **Utility (Adequacy) for CERHR Evaluation Process:** This study is deemed inadequate for inclusion due  
7 to unclear statistical procedures regarding litter and nested factors associated with repeated measurements.

8  
9 **Laviola et al. (413)**, supported by Italian Ministry of Health, Ministry of Universities and Research, and  
10 the University of Parma, examined the effect of prenatal bisphenol A exposure on *d*-amphetamine-  
11 reinforcing effects in mice. [No information was provided about feed, housing, or bedding  
12 composition.] CD-1 mice were trained to drink the tocopherol-purified corn oil vehicle through a syringe.  
13 The mice were randomly assigned to groups, and 10–12/group were exposed to bisphenol A [purity not  
14 reported] at 0 (vehicle) or 0.010 mg/kg bw by feeding from a syringe on GD 11–18 [day of vaginal plug  
15 not defined]. Another group of mice was exposed to methoxychlor; those findings will not be discussed.  
16 Litters were culled to 10 pups ( $5 \pm 1$  of each sex) within 12 hours of parturition. Offspring were weaned  
17 and group housed with littermates of the same sex on PND 25. At 60 days of age, 3 offspring/sex/litter (1  
18 sex/litter at each *d*-amphetamine dose) were subjected to conditioned place-preference testing. For the test,  
19 animals were acclimated to the apparatus on the first day of testing. On alternate days over a 4-day period,  
20 animals were ip injected with 0, 1, or 2 mg/kg bw *d*-amphetamine and confined to one compartment of the  
21 apparatus for 20 minutes. On the other days of the 4-day period, animals were injected with saline and  
22 confined in another section of the apparatus for 20 minutes. On the fifth day of testing, animals were not  
23 treated and were given free access to the entire apparatus for 10 minutes. The amount of time spent in the  
24 compartment associated with *d*-amphetamine treatment was measured. Data were analyzed by a split-plot  
25 ANOVA, in which the litter was considered the block variable, and Tukey HSD test. Prenatal treatment  
26 was described as a between litters factor and all other variables were described as within litter factors.

27  
28 No differences were reported for birth weight and sex ratio at birth. [Data were not shown by authors.]  
29 There were no significant effects of bisphenol A treatment on locomotor activity. Conditioned place-  
30 preference occurred in control females following injection with either *d*-amphetamine dose, but was not  
31 observed in females treated with bisphenol A. In males, both the vehicle control and the bisphenol A group  
32 displayed a preference for the *d*-amphetamine-associated compartment following treatment with the high *d*-  
33 amphetamine dose. Therefore, there was no change in preference following bisphenol A treatment of  
34 males. The study authors concluded that prenatal bisphenol A exposure affected organization of the brain  
35 dopaminergic system in female mice leading to long-term alterations in neurobehavioral function.

36  
37 **Strengths/Weaknesses:** Strengths of this study include robust and appropriate design and analysis,  
38 adequate sample size, and oral dosing. The use of only 1 dose level is a weakness.

39  
40 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility in the  
41 evaluation.

#### 42 3.2.6 Mouse—parenteral exposure only during pregnancy

43 **Markey et al. (414)**, supported by NIH, the Massachusetts Department of Health, the International Union  
44 Against Cancer, and the World Bank, examined the effect of prenatal bisphenol A exposure on mammary  
45 gland development in mice. CD-1 mice were fed RMH 3000 rodent diet, which showed negligible activity  
46 in estrogenicity testing. Caging and bedding were also reported to test negative in estrogenicity assays.  
47 Dams (6–10/group) were estimated to have received the DMSO vehicle or bisphenol A [purity not  
48 reported in the manuscript;  $97 \pm 2\%$  per A. Soto, personal communication, March 2, 2007] at 0.000025  
49 or 0.000250 mg/kg bw/day through a sc pump from GD 9–20 (GD 1 = day of vaginal plug). [The original  
50 publication stated that bisphenol A doses were 25 and 250  $\mu\text{g}/\text{kg}$  bw/day, but units were corrected to  
51

### 3.0 Developmental Toxicity Data

1 **ng/kg bw/day in an addendum released for the study].** Doses were not adjusted for increasing body  
2 weight as dams gained weight during pregnancy. Dams were allowed to litter and offspring were weaned at  
3 19 days of age. At 10 days, 1 month, and 6 months of age, 6–10 female offspring/group were killed during  
4 each time period. **[Number of litters represented was not stated but there may have been 1**  
5 **offspring/litter based on the numbers examined.]** Vaginal smears were assessed in mice following  
6 puberty, and post-pubertal mice were killed during proestrus. Prior to being killed, females were injected  
7 with bromodeoxyuridine, and incorporation of bromodeoxyuridine in mammary glands was determined by  
8 an immunohistochemistry method. Histological and morphometric analyses of mammary glands were also  
9 conducted. Data were analyzed by ANOVA, least significant difference test, and *t*-test. [The statistical  
10 analyses considered litter differences, method unstated.]

11  
12 At 1 month of age, the rate of ductal migration into the stroma was increased in the low-dose group and  
13 decreased in the high-dose group; values in the 2 treatment groups were significantly different from one  
14 another but neither dose group was significantly different from the control group. Bisphenol A treatment  
15 increased percentages of ducts and buds at 6 months of age. Bromodeoxyuridine incorporation was  
16 decreased in epithelial cells at both doses at 10 days of age, decreased in stromal cells at the high dose at 1  
17 month of age, and increased in stromal cells at both dose levels at 6 months of age. At 1 month of age, the  
18 ratio of bromodeoxyuridine-positive epithelial to stromal cells was 4:1 in the control group, 2:1 in the  
19 0.000025 mg/kg bw/day group, and 6:1 in the 0.000250 mg/kg/bw/day group. The percentage of alveoli  
20 containing secretory products was increased at the low dose at 6 months of age. The study authors  
21 concluded gestational exposure to low doses of bisphenol A alters timing of DNA synthesis in mammary  
22 epithelium and stroma, resulting in a histoarchitecture that is not typical for a virgin mouse.

23  
24 **Strengths/Weaknesses:** The examination of the mammary gland, a system not often studied, is a strength.  
25 A critical weakness is the uncertainty of the DMSO concentration as a vehicle and therefore pump  
26 performance. An additional weakness is that the proliferative changes reported in mammary tissues in  
27 virgin mice have not been satisfactorily established as precursors of breast cancer.

28  
29 **Utility (adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
30 given exposure uncertainties.

31  
32 **Markey et al. (415)**, supported by NIH and the Massachusetts Department of Public Health, examined the  
33 effects of prenatal bisphenol A exposure on development of the female reproductive system and mammary  
34 gland in mice. CD-1 mice were fed Purina Rodent Chow that tested as having negligible estrogenicity.  
35 Cages and bedding tested negative for estrogenicity in the E-SCREEN assay. Water was provided in glass  
36 bottles. Mice ( $n = 6-10$ /group) were administered bisphenol A [**purity not indicated in the manuscript;**  
37  **$97 \pm 2\%$  per A. Soto, personal communication, March 2, 2007] at 0 (DMSO vehicle), 0.000025, or  
38 0.000250 mg/kg bw/day by sc pump from GD 9 through the remainder of pregnancy (GD 1 = day of  
39 vaginal plug). [**The dose levels were incorrect in the original and were corrected by an erratum (416).]**  
40 Number of offspring, sex ratio, body weight, and age at vaginal opening were assessed. Beginning at 3  
41 months of age and continuing for 2 weeks, estrous cyclicity was assessed by visual examination of the  
42 external vagina and confirmation by vaginal smears. Female offspring (6–10/group) were killed at 1, 3, 4,  
43 6, 9, and 12 months of age on the afternoon of proestrus. Reproductive organs were grossly assessed, and  
44 morphometric measurements were obtained for ovary and mammary gland. [**Although the methods**  
45 **section suggests that morphometric measurements were obtained at each time period of sacrifice, it**  
46 **does not appear that the measurements were taken at 12 months of age. The 1-month data were**  
47 **reported in a previous publication (414).]** A histopathological evaluation of the ovary was conducted at 3  
48 months of age. Reproductive organ weights were obtained at 1, 3, and 6 months of age. [**As in other**  
49 **studies reported from this laboratory, different litters were represented at each time period (A. Soto,**  
50 **personal communication March 2, 2007).]** Statistical analyses included ANOVA, Kruskal-Wallis, and  
51 Mann-Whitney tests. [**It was not clear if the litter or offspring was considered the statistical unit.]****

### 3.0 Developmental Toxicity Data

1 Bisphenol A exposure had no significant effect on litter size or sex ratio. A significant interaction between  
2 age for body weight and treatment was reported from 2 to 12 months of age but the effect on body weight  
3 was not explained. No significant effects were observed for vaginal opening in treated mice. Significant  
4 increases were observed in percentages of 3-month-old mice with estrus/metestrus for  $\geq 4$  or 8 days. At 6  
5 months of age, the incidence of fluid-filled ovarian bursae was increased in both treatment groups.  
6 Reproductive organ weights were not affected at 1 or 6 months of age, but at 3 months of age, absolute and  
7 relative (to body weight) weights of vagina were decreased in the high-dose group. The percentage of ovary  
8 tissue consisting of antral follicles was increased in the high-dose group at 3 months of age. No significant  
9 differences were observed for mammary structures at 4 months of age. At 6 months of age, the percentage  
10 of alveolar buds/lobulo-alveoli was increased in both dose groups compared to the control group. The  
11 percentage of alveolar buds/lobulo-alveoli was decreased in the low-dose group compared to control group  
12 at 9 months of age. The study authors concluded that exposure of mice to environmentally relevant doses of  
13 bisphenol A during the development of estrogen-sensitive tissues results in effects that are manifested in  
14 adulthood.

15  
16 **Strengths/Weaknesses:** The examination of the mammary gland, a system not often studied, is a strength.  
17 A critical weakness is the uncertainty of the DMSO concentration as a vehicle and therefore pump  
18 performance.

19  
20 **Utility (adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
21 given exposure uncertainties.

22  
23 **Vandenberg et al. (417)**, supported by NIEHS and Tufts, examined the effects of prenatal bisphenol A  
24 exposure on mouse mammary gland development. CD-1 mice were fed Harlan Teklad 2008, which was  
25 reported to contain 20 fmol/g estrogen equivalents. The type of caging and bedding used was not reported  
26 but they were stated to test negative for estrogenicity in the E-SCREEN. Water was supplied in glass  
27 bottles. On GD 8 (GD 1 = day of vaginal plug) mice were implanted [**sc (A. Soto, personal**  
28 **communication, March 2, 2007)**] with osmotic pumps that delivered the 50% DMSO vehicle or bisphenol  
29 A [**purity not reported in manuscript;  $97 \pm 2\%$  per A. Soto, personal communication, March 2, 2007**]  
30 at 0.000250 mg kg bw/day. The bisphenol A dose was selected because it was predicted (or estimated) to  
31 be environmentally relevant and shown to alter mammary endpoints (414, 418). Pumps were left in place  
32 until dams were killed on GD 18. [**The number of dams treated was not reported in the paper. The**  
33 **Expert Panel has been informed that there were 20–30/group (A. Soto, personal communication,**  
34 **March 2, 2007).**] Fetal mammary glands were mounted whole or sectioned to examine mammary gland  
35 development in 36–40 offspring/group. Immunohistochemistry techniques were used to measure expression  
36 of *Ki67* and *Bax* in mammary structures from 4–8 offspring/group. Mammary collagen localization was  
37 assessed using Masson Trichrome stain in 6–17 mice/group. Expression of mRNA for *ER $\alpha$* , *ER $\beta$* , adipocyte  
38 lipid binding protein, *Col-1*, and *PPAR $\gamma$*  were measured by RT-PCR in mammary glands from 4–6  
39 offspring/group. Litter was accounted for in design and analyses by assigning 1 individual/litter to each  
40 group or endpoint. Statistical analyses included *t*-tests, ANOVA, Mann-Whitney *U* non-parametric tests,  
41 and chi-squared tests.

42  
43 Morphometric analysis revealed significantly higher ductal area and extension in the bisphenol A group  
44 than in controls. In the control group, females positioned next to 2 females in utero had significantly fewer  
45 branching points than females positioned next to 1 or 2 males; this difference was not observed in the  
46 bisphenol A group. In fetuses that were not positioned next to a male, significantly more branching points  
47 were observed in the bisphenol A than in the control group. Control females positioned next to 2 males had  
48 significantly larger epithelial duct area than control females not positioned next to a male; this difference  
49 was not observed in the bisphenol A group. In bisphenol A-treated females positioned next to 1 male,  
50 ductal extension was significantly greater than in control females positioned next to 1 male.

### 3.0 Developmental Toxicity Data

1 In the bisphenol A group, epithelial cells were less rounded, more evenly spaced, and more dense than in  
2 controls. Bisphenol A did not significantly affect Ki67 (a proliferation marker) expression in mammary  
3 epithelium. Lumen formation was observed in 6 of 16 control mice and 0 of 10 bisphenol A-exposed mice.  
4 Significantly decreased numbers of Bax-positive (apoptotic) cells were observed in the inner epithelial cord  
5 (not in contact with basement membrane) of bisphenol A-exposed than control mice. Optical density of  
6 histological staining was significantly lower in the fat pad of the bisphenol A-exposed than control group.  
7 Fat pads of the bisphenol A group compared to control group were found to be significantly less cellular,  
8 contain more Bax-positive cells, and have more vacuoles at a distance <1 mm from the epithelial  
9 compartment. Study authors interpreted the effect as increased epithelial penetration and advanced  
10 maturation of fat pads. No significant differences were observed for PPAR $\gamma$  or adipocyte lipid binding  
11 protein mRNA expression. Density of collagen deposits was lower in the entire mammary gland but higher  
12 in the periductal stroma (within 10  $\mu$ M of the epithelium) of the bisphenol A than the control group.  
13 Bisphenol A exposure did not affect collagen type I, *ER $\alpha$* , or *ER $\beta$*  mRNA expression. *ER $\alpha$*  protein  
14 expression in the stroma was also unaffected by bisphenol A exposure. Study authors concluded that  
15 advanced maturation of fat pad and changes in extracellular matrix may be the cause of altered growth, cell  
16 size, and lumen formation in mammary epithelium of mouse fetuses exposed to bisphenol A.

17  
18 **Strengths/Weaknesses:** Strengths of this paper are the rigor with which the measurements were made, and  
19 the fact that the authors were trying to quantify endpoints that are difficult to measure (e.g., the relationship  
20 of the stroma to the epithelium). The relevance of the endpoints is a strength as is the low dose used. The  
21 single dose and subcutaneous route of administration are weaknesses. A critical weakness is inappropriate  
22 statistical analysis of a complex study design that may have produced too many positive findings and a lack  
23 of statistical accounting for litter effects (i.e., 36-40 pups presented in Table 1 of paper and only 20-30  
24 litters treated).

25  
26 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation process  
27 because of insufficient control for litter effects.

28  
29 **Honma et al. (419)**, supported by the Japanese Ministry of Education, Culture, Sports, Sciences, and  
30 Technology, examined the effect of prenatal bisphenol A exposure on the reproductive system of female  
31 mice. Mice were fed commercial diet (CE-2, CLEA, Tokyo, Japan). **[No information was provided about  
32 bedding or caging materials.]** Ten ICR/Jcl mice/group were sc injected with bisphenol A **[purity not  
33 reported]** in sesame oil at 0, 0.002, or 0.020 mg/kg bw/day on GD 11–17 (GD 0 = vaginal plug).  
34 Additional mice were injected with diethylstilbestrol at 0.02–2  $\mu$ g/kg bw/day. Pups were sexed, counted,  
35 and weighed at birth. At 22 days of age, offspring were weaned and litter sizes were adjusted to 8 pups.  
36 Male and female offspring were weighed during the postnatal period. Anogenital distance was measured in  
37 males and females at 22 and 60 days of age. Females were monitored for vaginal opening. Vaginal smears  
38 were obtained for 30 days following vaginal opening. Female offspring were mated with untreated males  
39 from 90 to 120 days of age. F<sub>2</sub> pups were counted and sexed at birth. The litter was considered the  
40 experimental until in statistical analyses. Data were analyzed by ANOVA and Student or Welch *t*-test.

41  
42 Statistically significant findings are summarized in [Table 79](#). There were no effects on gestation duration,  
43 number of pups/litter, or sex ratio. Body weights were slightly lower in high-dose males at birth, both dose  
44 groups of females at weaning, and high-dose males and females at 60 days of age. Anogenital distance was  
45 increased in low-dose females at weaning and both dose groups of males at 60 days of age. Age of vaginal  
46 opening and 1<sup>st</sup> estrus was accelerated in the high-dose group, and body weight at vaginal opening was  
47 lower in both dose groups. Estrous cycle length was increased in both dose groups. Total days that  
48 cornified cells were present in vaginal smears was increased and total days that lymphocytes were detected  
49 was decreased in the low-dose group. In F<sub>1</sub> offspring there were no significant effects on mating, number of  
50 F<sub>2</sub> pups/litter, or sex ratio of F<sub>2</sub> pups. Results in mice dosed with diethylstilbestrol were similar to those

### 3.0 Developmental Toxicity Data

1 observed in mice dosed with bisphenol A. The study authors concluded that prenatal exposure to low doses  
2 of bisphenol A results in early vaginal opening in mice but did not affect female reproductive function.

3

4 **Table 79. Effects in Mice Exposed to Bisphenol A During Prenatal Development**

Endpoint	Dose (mg/kg bw/day)					
	0.002	0.020	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
Female body weight						
Weaning	↓10%	↓7%	0.065	0.017	0.088	0.021
PND 60	↔	↓4%	0.054	0.021	0.11	0.021
Male body weight						
Birth	↔	↓5%	0.054	0.020	0.031	0.015
PND 60	↔	↓6%	0.048	0.020	0.044	0.020
Anogenital distance						
Females at weaning	↑6%	↔				
Males on PND 60	↑6%	↑8%	0.035	0.020	0.035	0.020
Age at vaginal opening <sup>a</sup>	↔	↓1.3 days				
Body weight at vaginal opening <sup>a</sup>	↓10%	↓11%				
Age at 1 <sup>st</sup> estrus <sup>a</sup>	↔	↓1 day				
Estrous cycle length	↑1.3 day	↑1 day	0.021	0.007	0.12	0.021
Cornified cells in vaginal smear	↑3.1 days	↔	0.17	0.020	0.44	0.021
Lymphocytes in vaginal smear	↓2.2 days	↔	0.26	0.020	0.26	0.020

↑,↓ Statistically significant increase, decrease; ↔ no significant effect.

<sup>a</sup>Value estimated from a graph by CERHR; data from graphs were not modeled.

From Honma et al. (419).

5

6 **Strengths/Weaknesses:** Strengths are that this study represents one of the few studies that appropriately  
7 examines the onset of puberty in the mouse as an endpoint, it uses low dose levels of bisphenol A,  
8 relatively large sample sizes, and effectively uses a positive control at 3 dose levels. The lack of AGD  
9 measurement at birth and difficulty of measurement at PND 60 are weaknesses. The Expert Panel was unable  
10 to confirm the statistical significance of the effects shown in Table II of the manuscript.

11

12 **Utility (Adequacy) for CERHR Evaluation Process:** The study is adequate for inclusion but of limited  
13 utility due to statistical questions about body weight and AGD and subcutaneous route of exposure.

14

15 **Iwasaki and Totsukawa (420)**, support not indicated, examined the effect of prenatal bisphenol A  
16 exposure on reproductive development of female mice. ICR mice were fed F1 diet (Funabashi, Chiba,  
17 Japan) and housed in polycarbonate cages containing an unspecified chip bedding. On GD 7–18 (GD 0 =  
18 day of copulatory plug), 6 dams/group received bisphenol A [**purity not reported**] at 0 (DMSO vehicle)  
19 0.00025, 0.025, or 2.5 mg/kg bw/day by sc injection. A positive control group of mice received 100 µg/kg  
20 bw/day 17β-estradiol [**route not specified**]. Dams were weighed during the study. Pups were counted and  
21 sexed on PND 0, and pup viability was determined on PND 4. Pups were weaned on PND 21, and male  
22 pups were killed and discarded. Female pups (24–41/group) were observed for vaginal opening. On PND  
23 21, 1 pup/litter(4/group) from the low- and mid-dose group was injected with 3 µg/kg bw/day 17β-estradiol  
24 for two days and then killed. Uterine weights were assessed and expression of the *ERα* gene in uterus was  
25 determined using a colorimetric method. Statistical analyses included ANOVA, ANOVA on ranks  
26 (Kruskall-Wallis test), and Dunnett test. [**It was not clear if the litter or offspring was considered the**  
27 **statistical unit.**]

28

29 Weight gain was described as increased in all treated dams compared to control dams, but there was no  
30 evidence of a dose-response relationship and statistical significance was not achieved. Pup birth weight was

### 3.0 Developmental Toxicity Data

1 significantly lower [6%] in the low-dose group compared to the control group. There were no differences  
2 in litter size at birth. Pup viability on PND 4 was significantly reduced [by 26%] in the low-dose group.  
3 Age of vaginal opening was significantly delayed by 3 days in the low-dose group, but significantly  
4 accelerated by 2.2 days in the high-dose group. Following 17 $\beta$ -estradiol exposure, uterine weight was  
5 significantly decreased [by ~85%] in the low-dose bisphenol A group and significantly increased [by  
6 ~29%] in the mid-dose bisphenol A group. Although expression of *ER $\alpha$*  mRNA was observed at 132% of  
7 control levels in the mid-dose bisphenol A group following exposure to 17 $\beta$ -estradiol, the effect did not  
8 attain statistical significance. Expression of *ER $\alpha$*  gene was not detectable in the low-dose bisphenol A  
9 group following 17 $\beta$ -estradiol exposure. No significant effects were reported in mice treated with 17 $\beta$ -  
10 estradiol. The study authors concluded that “The levels tested in this study appear to be dangerous.”  
11

12 **Strengths/Weaknesses:** The use of 3 dose levels, including low doses, and the use of 17 $\beta$ -estradiol as a  
13 positive control are strengths of this study. Weaknesses include the use of DMSO as a vehicle, the  
14 subcutaneous route of administration, the small sample size, lack of significant effects detected in the 17 $\beta$ -  
15 estradiol positive control group, and the failure to account for litter in statistical analyses.  
16

17 **Utility (Adequacy) for CERHR Evaluation Process:** The study is inadequate for the evaluation process.  
18

19 **Nakamura et al. (421)**, supported by grants from the Japanese government, examined the effects of  
20 prenatal exposure to bisphenol A on the morphology and expression of certain genes related to brain  
21 development in the mouse neocortex. In the first experiment ICR/Jc1 mouse dams were injected  
22 subcutaneously with either 0 (sesame oil vehicle) or 20  $\mu$ /kg bw/day bisphenol A [**purity not indicated**]  
23 daily from GD 0 (defined as the day that a vaginal plug was detected) until GD10.5, GD12.5, GD14.5 or  
24 GD16.5. [**No information was provided on feed, caging materials, bedding.**] Dams were then given a  
25 single ip injection of 5-bromo-2'-deoxyuridine (BrdU). Fetuses were collected either one hour following  
26 BrdU treatment (to assess precursor cell proliferation) or 2 or 3 days following BrdU treatment (to assess  
27 neuronal migration and differentiation). Brains were fixed in 4% buffered paraformaldehyde for  
28 morphometry and immunohistochemical evaluation. The sections of the neocortex were sectioned into  
29 three zones: ventricular zone, intermediate zone, and cortical plate (the neocortex at GD12.5 was divided  
30 into the ventricular zone and the primordial plexiform layer). Ten fetuses from two or more dams were  
31 collected at each time point. In the second study, ICR/Jc1 dams were treated as described above and fetal  
32 telencephalons were collected on GD12.5, GD14.5, or GD16.5 and frozen in liquid nitrogen and stored at –  
33 80° for mRNA expression analyses (n = 10-15 fetuses in each group).  
34

35 There were no significant differences in the pattern of immunoreactivity for K1-67 (a marker for cell  
36 proliferation), nestin (a marker for neural progenitors), Musashi (another marker for neural progenitors),  
37 and histone H<sub>3</sub>. However, a marker for young neurons, Tuj1, was more prominent in the intermediate zone  
38 at GD14.5 and GD16.5 in the bisphenol A group. The authors also looked at the immunoreactivity pattern  
39 for PDI, a microsomal enzyme that contains binding sites for T<sub>3</sub> and estradiol. PDI is believed to act as a  
40 buffer for these hormones in cells. PDI is of interest because bisphenol A has been reported to bind to the  
41 T<sub>3</sub> binding sites of PDI with 10-100 fold lower affinity than T<sub>3</sub> (Hiroi, 2006) and inhibit the binding of T<sub>3</sub> to  
42 PDI when bound. PDI immunoreactivity was increased in the neocortex of bisphenol A treated fetuses from  
43 GD12.5 until GD16.5 and in subplate cells at GD14.5.  
44

45 There were no differences in BrdU labeled cells in any neocortical zone from brains collected 1 hour  
46 following BrdU treatment. However, the BrdU-labeled cells analyzed two days following BrdU injection  
47 were decreased in the ventricular zone of BPA-treated mice at GD14.5 (labeled at GD12.5) and GD16.5  
48 (labeled at 14.5) and increased in the cortical plate at GD14.5 (labeled at GD12.5). The authors used  
49 quantitative RT-PCR to examine the expression of several genes involved in brain development including  
50 those that help regulate the maintenance of neural stem cells and promote gliogenesis (*Hes1* and *Hes5*),

### 3.0 Developmental Toxicity Data

1 promote neurogenesis (*Mash1*, *Math3*, and *Ngn2*), and relate to thyroid hormone action (*LICAM*, *THR-alpha*, and *THR-beta*). The gene expression of *Math3*, *Ngn2*, *Hes1*, *LICAM*, and *THR-alpha* were  
2 significantly up-regulated in the bisphenol A treated group at GD14.5 (*Hes1* and *Hes5* were significantly  
3 down-regulated at GD12.5). Overall, the authors interpreted these findings as suggesting that bisphenol A  
4 might disrupt normal neocortical development by accelerating neuronal differentiation and migration.  
5

6  
7 **Strengths/Weaknesses:** The strengths of this study are that a reasonable sample size (10) for this type of  
8 study was used although the presumed dam effect was only partly controlled for by choosing 10 pups from  
9 two different dams. The study used a low dose (20ug/kg) delivered sc to a pregnant mouse. The results  
10 revealed an effect on neocortical development in developing fetuses. Neurogenesis and gene expression  
11 were affected by BPA  
12

13 **Utility:** This is an adequate study for evaluation purposes but of limited utility because dam effects were  
14 only partly controlled for and because of the subcutaneous route of administration.  
15

16 **Nikaido et al. (422)**, supported by the Japanese Ministry of Health, Labor, and Welfare examined the  
17 effects of bisphenol A exposure on mammary glands and reproductive systems of mice. Outbred CD-1  
18 (ICR) mice were fed NIH-07 (a low-phytoestrogen diet) and provided with water supplied in polycarbonate  
19 bottles with rubber stoppers. The mice were housed in polyisopentene cages with white pine chip bedding.  
20 Beginning on GD 15 (plug day not specified), mice were sc injected with 0 (DMSO vehicle), 0.5, or 10  
21 mg/kg bw/day bisphenol A ( $\geq 99\%$  purity) or 0.5 or 10  $\mu\text{g}/\text{kg}$  bw/day diethylstilbestrol for 4 days. **[The  
22 control group contained 6 dams/group, but the number of dams in treated groups was not clear.]**  
23 Additional groups of mice were treated with the same doses of genistein, resveratrol, or zearalenone.  
24 Female pups were weaned at 21 days of age. Onset of vaginal opening was monitored. Estrous cyclicity  
25 was monitored in 12 mice/group at 9–11 weeks of age. At 4, 8, 12, and 16 weeks of age, 6 randomly  
26 selected mice/group were weighed and killed. Ovaries, uterus, vagina, and mammary glands were  
27 preserved in 10% formalin for histopathological evaluation. Differentiation of mammary structures was  
28 evaluated in whole mounts. Statistical analyses included homogeneity of variance tests followed by  
29 ANOVA or Kruskal-Wallis test. When *P* values were below 0.05, Fisher protected least significant  
30 difference test was conducted. **[It appears that offspring were considered the statistical unit.]**  
31

32 Body weight gain of offspring was increased by bisphenol A treatment, and at 16 weeks of age, body  
33 weight compared to controls was higher **[by ~50%]** in the low-dose group and **[by ~23% ]** in the high-dose  
34 group. Vaginal opening was accelerated by 1.2 days at the high-dose group. Estrous cycle length was  
35 increased by 2.8 days in the low-dose group and 3 days in the high-dose group as a result of increased time  
36 spent in diestrus. Corpora lutea were observed in all control mice at each age. No corpora lutea were  
37 observed in 2 of 6 mice of the low-dose group and 3 of 6 mice of the high-dose group at 4 weeks of age,  
38 but all mice had corpora lutea at 4, 8, 12, and 16 weeks of age. With the exception of vaginal cornification  
39 observed in mice lacking corpora lutea, no histopathological abnormalities were observed in the uterus or  
40 vagina. Two of three mice with corpora lutea in the high-dose bisphenol group had greater mammary  
41 alveolar differentiation compared to control mice at 4 weeks of age. No differences in mammary  
42 differentiation were observed at later ages. The study authors concluded that both the high and low dose of  
43 bisphenol A produced transient changes in the mammary gland and reproductive tracts of mice. Transient  
44 effects on the reproductive tract and mammary gland were also observed with genistein and  
45 diethylstilbestrol, while prolonged effects were induced by zearalenone.  
46

47 **Strengths/Weaknesses:** The lack of clarity regarding sample size and the weak description of the  
48 histopathology findings are weaknesses, as are the use of DMSO as a vehicle, the subcutaneous route of  
49 administration, and statistical concerns.  
50

51 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process.

### 3.0 Developmental Toxicity Data

1 **Park et al. (423)**, support not indicated, treated ICR mice during pregnancy. Bisphenol A [**purity not**  
2 **indicated**] in corn oil was given ip at dose levels of 0, 0.05, 0.5, or 5 mg/kg bw on the day of mating and  
3 every 3 days for a total of 6 doses (n = 12/group). Dams were killed on GD 18 (plug = GD 0) for  
4 determination of litter size, fetal weight, and sex ratio. The uterus and right ovary were removed from each  
5 dam, fixed in Bouin fluid, and sections were stained with hematoxylin and eosin for light microscopy.  
6 Results were analyzed with least significant difference test [**apparently on a per fetus basis**].

7  
8 Maternal weight was not altered by treatment. Fetal body weight was decreased in the high-dose group by  
9 14% for males and 12% for females. There was no effect on litter size or sex ratio. There was no treatment  
10 effect on dam uterine or ovarian weight. Histopathology of the dam ovary was reportedly not affected by  
11 treatment. Histopathology of the dam uterus showed thickening of the endometrium in the 0.05 and 0.5  
12 mg/kg bw groups and uterine muscle damage in the 5 mg/kg bw group. [**The damage is not otherwise**  
13 **described. The photomicrographs available in the report were not interpretable due to poor**  
14 **reproduction quality.**] The authors concluded that bisphenol A at low doses does not produce  
15 reproductive toxicity in mice. [**This paper was written in Korean with an English abstract and tables.**  
16 **A translation was provided to CERHR by the American Plastics Council.**]

17  
18 **Strengths/Weaknesses:** The use of 3 dose levels is a strength. The lack of information on husbandry  
19 conditions, the ip dose route, failure to account for litter effects in statistical analyses, and the poor  
20 presentation of histopathology results are weaknesses.

21  
22 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process.

23  
24 **Park et al. (424)**, support not indicated, treated ICR mice during pregnancy. Bisphenol A [**purity not**  
25 **indicated**] in corn oil was given ip at dose levels of 0, 0.05, 0.5, or 5 mg/kg bw on the day of mating, and  
26 every 3 days for a total of 6 doses (n = 3–6/group). Offspring were evaluated on PND 45 for body weight,  
27 reproductive organ weight and histopathology, semen analysis, complete blood count, and serum  
28 chemistry. [**There were 24 female and male offspring evaluated per dose group (not indicated whether**  
29 **12 of each sex). Litter of origin appears not to have been considered. No information was provided on**  
30 **standardization of litters, diet, or cage/bedding materials.**] Statistical analysis was performed using the  
31 least significant difference test. [**It was not clear if the litter or offspring was considered the statistical**  
32 **unit.**]

33  
34 There was a statistically significant 6% decrease in male body weight in the high-dose group; a comparable  
35 body weight decrement in female offspring was not statistically significant. There were no statistically  
36 significant treatment effects on the weights of the testis, epididymis, seminal vesicles, coagulating glands,  
37 uterus, or ovary. Sperm concentration, viability, motility, and morphology were not affected by treatment.  
38 Blood endpoints were not affected by treatment except for a statistically significant 6% increase in  
39 erythrocyte count in male offspring and a 2% decrease in serum albumin in female offspring. An 11%  
40 increase in blood urea nitrogen in mid-dose female offspring was not dose related. Histopathology of the  
41 testis and ovaries was described as unaffected by treatment. Uterine intimal proliferation was described in  
42 the mid- and high-dose female offspring. [**The histological methods were not described. The**  
43 **photomicrographs available in the report were not interpretable due to poor reproduction quality.**]  
44 The authors concluded that bisphenol A at low doses does not produce reproductive toxicity in mice. [**This**  
45 **paper was written in Korean with an English abstract and tables. A translation was provided to**  
46 **CERHR by the American Plastics Council.**]

47  
48 **Strengths/Weaknesses:** The inadequate description of methods, unacceptable small sample size, the ip  
49 dosing, inappropriate statistical analyses, and the poor presentation of histology results are weaknesses of  
50 this study.

51

### 3.0 Developmental Toxicity Data

1 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
2 due to the reasons stated above.

3  
4 **Sato et al (425)**, support not indicated, investigated the effects in mice of in utero exposure to bisphenol A  
5 on fetal growth, offspring reproductive and brain development, and behavior. Pregnant Jcl-ICR mice (n =  
6 20) were given s.c. injections of bisphenol A [**purity not indicated**] 100 mg/kg bw/day, ethinyl estradiol  
7 0.2 or 0.02 mg/kg bw/day, or olive oil vehicle on GD 11–19 [**Plug day was not defined. Information**  
8 **regarding caging material, animals per cage, feed, culling, and weaning was not provided.**] Pups were  
9 evaluated for onset of pivoting, righting, straight line walking, and grasp reflex. Open field testing was  
10 conducted at 40 days of age. Offspring were killed at 40 or 60 days of age and organs were weighed and  
11 processed for histology using hematoxylin and eosin [**fixation method not given**]. Brain myelin was  
12 evaluated using Klüver-Barrera staining. Statistical analyses were performed using the Student *t*-test. [**The**  
13 **pup appears to have been used as the statistical unit.**]

14  
15 There were 11/93 stillborn fetuses after in utero exposure to bisphenol A, but no data were provided for the  
16 control group. There were no significant effects of bisphenol A treatment on litter size or offspring body  
17 weight at birth, 20, or 60 days of age. There were no significant effects of bisphenol A treatment on days at  
18 acquisition of pivoting, righting, straight-line walking, or grasp reflexes. In open field testing, mice in the  
19 bisphenol A-treated group showed significantly less defecation than controls [**39% less**]. There was no  
20 statistically significant difference between groups in grooming, rearing, line-crossing of inner and outer  
21 fields, or latency to first line crossing. At 60 days of age, seminiferous tubules from bisphenol A-exposed  
22 male offspring had a significant reduction in mean diameter [**↓16.6%**] and cell layer thickness [**↓25%**]  
23 compared to controls. There was no significant bisphenol A effect on brain myelination at 60 days of age or  
24 in mean diameter at 40 and 60 days of age of the tractus mamillothalamicus. The authors suggest that *in*  
25 *utero* exposure to 100 mg/kg bw/day bisphenol A induces alterations in behavior similar to that seen at  
26 reduced plasma corticosterone levels and that bisphenol A exposure induces gross and cellular changes in  
27 seminiferous tubules, suggesting potential perturbation in hormone pathways involved in development.

28  
29 **Strengths/Weaknesses:** The use of multiple doses of estrogen as a positive control is a strength.  
30 Weaknesses include the evaluation of a single dose of BPA, subcutaneous dosing and lack of details  
31 regarding husbandry. Behavioral methods were chosen from less sophisticated screening approaches and  
32 data were not appropriately analyzed using the litter as the statistical unit. Further, there is no description of  
33 sex ratios in groups given behavioral testing, despite established sex differences in endpoints measured in  
34 the open field evaluation. As a result, behavioral findings are unreliable.

35  
36 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for inclusion in the  
37 evaluation process due to the reasons stated above.

38  
39 **Rubin et al. (426)**, supported by NIEHS, examined sexual differentiation in mice perinatally exposed to  
40 bisphenol A. Animals were fed rodent diet 2018 (Harlan Teklad, St. Louis), which was reported to have  
41 negligible for estrogenicity (20 fmol 17 $\beta$ -estradiol equivalents/g). Caging and bedding materials were not  
42 indicated but were reported to have negligible estrogenic activity in the E-SCREEN assay. Water was  
43 supplied in glass bottles. On GD 8 (GD 1 = day of vaginal plug) through the 16<sup>th</sup> day of lactation, CD-1  
44 mice were sc dosed by osmotic pump with the 50% DMSO vehicle or bisphenol A [**purity not reported**]  
45 at 0.000025 or 0.000250 mg/kg bw/day. [**The numbers of dams exposed was not indicated.**] Litters were  
46 culled to 8 pups (4/sex) on the day following birth. Litters were weaned on PND 22–24 (day of birth not  
47 defined). Anatomical examination and assessment of tyrosine hydroxylase neurons in the anteroventral  
48 periventricular preoptic area by an immunohistochemistry technique were conducted before puberty (PND  
49 22–24) in 7 or 8 offspring/sex/group (2/sex/litter). Open-field testing was conducted in 14–17  
50 offspring/group (1 offspring/sex/litter) at 6–9 weeks of age. The study authors expressed concern about  
51 possible hormonal effects because their historical records indicated that regular estrous cycles are not

### 3.0 Developmental Toxicity Data

1 observed in group-housed females at 6–9 weeks of age. Therefore, open-field testing was repeated in 27–  
2 29-day-old offspring (n = 10–12/sex/group) exposed to 0 or 0.000250 mg/kg bw/day bisphenol A.  
3 Statistical analyses included 2-way ANOVA, *t*-test, and ANOVA with Bonferroni post hoc test.  
4

5 In control offspring, the total number of tissue sections through the anteroventral periventricular preoptic  
6 area was greater in females than males, but the sexually dimorphic difference was not observed in either  
7 treatment group. The number of sections through the anteroventral periventricular preoptic area was  
8 significantly lower in females from the high-dose bisphenol A than control group. In the control offspring,  
9 the number of tyrosine hydroxylase-positive neurons in the anteroventral periventricular preoptic area was  
10 higher in females and in males but this sexually dimorphic difference was not observed in the high-dose  
11 group. The number of tyrosine hydroxylase-positive neurons in the anteroventral periventricular preoptic  
12 area was lower in females in the high-dose bisphenol A than control group. The results for tyrosine  
13 hydroxylase-positive neurons were based on counting of all sections. When counting was limited to 7  
14 sections or 4 mid sections, the sexually-dimorphic difference observed for tyrosine hydroxylase-positive  
15 neurons in the control group was not observed in either treatment group. When limited to 3 caudal sections,  
16 the sexually dimorphic difference observed for tyrosine hydroxylase-positive neurons was maintained in  
17 the low-dose group and was borderline significant ( $P = 0.06$ ) in the high-dose group. Bisphenol A exposure  
18 had no significant effect on the number of tyrosine hydroxylase-positive neurons in the arcuate nucleus. In  
19 open-field testing of 6–9 week old animals, significant effects in control females compared to control males  
20 included more rearing and time spent in the center and less time stopped. Sexually dimorphic differences in  
21 rearing and time spent in center were not observed in either bisphenol A treatment group and the sexually  
22 dimorphic difference in time stopped was not observed in the low-dose group. In open-field testing  
23 conducted at 4 weeks of age, control females compared to males reared more times and spent less time  
24 stopped. The sexually dimorphic differences were not observed in animals exposed to 0.000250 mg/kg  
25 bw/day (the only dose tested in 4-week-old animals). The number of rearings was significantly lower in 4-  
26 week-old females in the 0.000250 mg/kg bw/day group than in controls. The study authors concluded that  
27 bisphenol A may alter important events during critical periods of brain development.  
28

29 **Strengths/Weaknesses:** The strengths of this paper are the care taken to control for extraneous estrogenic  
30 exposure, the delivery of BPA at 2 doses, both low, delivery from GD 1 to PND 16, the reasonable sample  
31 sizes, and the inclusion as outcome measurements of behavior, anatomy, and an index of neurochemical  
32 effects in the brain. Significant weaknesses include the use of sc osmotic pumps, uncertainty about sample  
33 size and whether litter effects were adequately controlled for.  
34

35 **Utility (Adequacy) for CERHR Evaluation Process:** This is inadequate for the evaluation process due to  
36 the combination of route of administration and statistical concerns.  
37

38 **Toyama (427)**, supported in part by the Japanese Ministry of Education, Culture, Sports Science, and  
39 Technology, examined the effects of prenatal Bisphenol A exposure in CL/P mice, a strain with a high  
40 background rate of cleft lip/palate. The study was published in Japanese and a translation was provided by  
41 the American Plastics Council. Mice were fed CA-1 (Japan CLEA, Inc.). **[No information was provided  
42 about caging or bedding materials.]** On GD 9.5 (GD 0 = day of vaginal plug), 25 dams/group were sc  
43 dosed with olive oil vehicle or bisphenol A **[purity not reported]** at 0.001, 0.01, 0.1, 1, or 10 mg/kg bw.  
44 Dams were killed on GD 18 and fetuses (169–184/group) were examined for cleft lip/palate or thymic  
45 anomaly (i.e., hypoplasia). Data were analyzed by Student *t*-test and chi-squared test. **[It appears that  
46 offspring were considered the statistical unit.]**  
47

48 There were no significant differences for numbers of implantations or fetal survival. The incidence of cleft  
49 lip/palate in fetuses from the control and each respective treatment group was 8.3, 8.0, 6.1, 1.8, 4.9, and  
50 6.2%. There were no differences in the types of cleft palate observed in each group. Incidence of thymic  
51 anomaly in the control and each respective dose group was 11.8, 10.8, 6.1, 1.8, 4.9, and 6.2%. Incidence of

### 3.0 Developmental Toxicity Data

1 cleft/lip palate or thymus anomalies was lower in bisphenol A-treated than control groups and was lowest  
2 in the 0.1 mg/kg bw bisphenol A group. **[Results of statistical analyses for cleft lip/palate and thymic  
3 anomaly were difficult to interpret.]** A higher tendency for complication of cleft lip/palate and thymus  
4 hypoplasia **[possibly fetuses with both types of defects]** was observed in the bisphenol A groups;  
5 respective incidences in the control and each treatment group was 36, 57.1, 61.8, 100, 77.8, and 72.7%. The  
6 study authors concluded that U-shaped dose response curves were observed for cleft lip/palate and thymus  
7 hypoplasia and that complication of cleft lip/palate and thymus hypoplasia tended to be lower in the  
8 bisphenol A groups.

9  
10 **Strengths/Weaknesses:** Strengths of this study include that the authors explored a wide range of BPA  
11 doses. Time of dosing was appropriate with respect to palate development. The hypothesis that BPA  
12 administration is protective is interesting. Weaknesses include the route of administration, absence of  
13 exposure assessment, confusion on statistical analyses, absence of historical control perspective, and strain  
14 of mouse used. This strain of mouse has a high incidence of cleft palate making interpretation of these data  
15 challenging.

16  
17 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the CERHR evaluation  
18 process because of the combination of strain selection, confusion on statistical analyses, use of sc route of  
19 exposure, and use of offspring as the unit of analysis.

20  
21 **Berger et al. (409)**, supported by The Natural Sciences and Engineering Research Council of Canada,  
22 examined the effect of bisphenol A exposure on blastocyst implantation and pup survival in mice. CF-1  
23 mice were housed in polypropylene cages mice and fed Harlan Teklad 22/5 rodent chow, a soy-containing  
24 feed. **[No information was provided about bedding materials.]** On GD 1–4 or 5 **[inconsistently  
25 described in report]**, 6–15 mice/group were administered bisphenol A through a peanut butter supplement,  
26 or a mixture of feed and peanut butter. Mice were allowed to litter. Pups were counted on the day of  
27 parturition and observed for survival for 5 days. Pups were weaned at 28 days after birth and at that time,  
28 body weight and sex ratio were determined. Data were analyzed by chi-squared test. **[It was not clear if  
29 offspring data were analyzed on a pup or litter basis.]**

30  
31 In the study in which the diet was supplemented with peanut butter, bisphenol A was added to the peanut  
32 butter at 0, 0.11, 1, 3, or 9%. Based on weights of unconsumed peanut butter, the study authors estimated  
33 mean bisphenol A intake at 0, 1.08, 8.33, 16.50, or 13.59 mg/day. **[Assuming that the mice weighed 0.02  
34 kg at the start of gestation (I15), CERHR estimated bisphenol A intakes of 54, 417, 825, and 680  
35 mg/kg bw/day].** Peanut butter consumption was significantly decreased in the 9% group. There were no  
36 treatment effects on number of females delivering litters. Survival of pups from birth to weaning was lower  
37 in the 9% group (76.1%) than in the control group (98.2%) and 2 complete litters were lost in the 9%  
38 group. There was no significant difference in sex ratio of pups at weaning. There also did not appear to be  
39 an effect on pup weight at weaning.

40  
41 In the study in which feed was dosed, mice were fed 1 part feed to 2 parts peanut butter. The feed/peanut  
42 butter mixture contained bisphenol A (97% purity) at 0, 3, or 6%. The study authors estimated bisphenol A  
43 intake at 0, 66.7, or 68.8 mg/day. **[Assuming that the mice weighed 0.02 kg at the start of gestation  
44 (I15), CERHR estimated bisphenol A intakes of 0, 3335, or 3440 mg/kg bw/day.]** Feed intake was  
45 significantly decreased in the 6% group. Controls were fed with the same quantity of food consumed by  
46 treated mice on the previous day. Delivery of litters in the 3% group was not affected but there were no  
47 litters delivered in the 6% group. Pup weight and sex ratio at weaning were not affected in the 3% group.  
48 Pregnancy disruption in the sc dosed mice is discussed in Section 3.2.6. **[It appears that with sc exposure,  
49 pregnancy disruption occurred at lower bisphenol A levels (10.125 mg/day, ~500 mg/kg bw/day) than  
50 with oral exposure (68.8 mg/day, 3440 mg/kg bw/day)]** The study authors concluded that the amount of

### 3.0 Developmental Toxicity Data

1 bisphenol A required for pregnancy disruption was higher than typical environmental levels but that it is  
2 not known if bisphenol A could have additive or synergistic effects with other environmental estrogens.

3  
4 **Strengths/Weaknesses:** Major weaknesses include absence of key statistical information on the  
5 appropriate control for possible litter effects, absence of similar effects at the same estimated dose level,  
6 inability to discriminate between potential maternal toxicity and the findings in the offspring, and the  
7 absence of exposure data (i.e., does the matrix affect exposure?).

8  
9 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the CERHR evaluation  
10 process

#### 11 3.2.7 Mouse—oral exposure postnatally with or without prenatal exposure

12 **Nagao et al. (428)**, support not indicated, examined the effects of bisphenol A in mice following exposure  
13 during different life stages. An initial study compared the sensitivity of male juvenile C57BL/6N and ICR  
14 mice to 17 $\beta$ -estradiol. Following sc dosing of 10 mice/strain/group with 10  $\mu$ g/kg bw/day 17 $\beta$ -estradiol on  
15 PND 27–48, there were no weight changes or histopathological alterations in reproductive organs of ICR  
16 mice. In contrast, C57BL/6N mice exposed to 17 $\beta$ -estradiol experienced significant decreases in absolute  
17 and relative weights of testes, epididymides, and seminal vesicles. In addition, epididymal sperm was  
18 reduced and there was increased severity of seminal vesicle and Leydig cell atrophy. The study authors  
19 concluded that C57BL/6N mice are sensitive to estrogen and this strain of mice was used in the remaining  
20 experiments.

21  
22  
23 Life stages examined in experiments with bisphenol A included prenatal development, adolescence, and  
24 adulthood. The studies conducted during prenatal development and adolescence are described here, and the  
25 study conducted during adulthood is described in Section 4.2. C57BL/6N mice were fed PLD  
26 (phytoestrogen-low diet, Oriental Japan). They were housed in polycarbonate cages with wood bedding.  
27 Daidzein and genistein levels were analyzed in the diet, tap water, and bedding and found to be below 0.5  
28 mg/100 g. Bisphenol A (stated to be 99% pure in the study with adult mice) was administered to juvenile or  
29 pregnant mice by gavage at doses of 0.002, 0.020, or 0.200 mg/kg bw/day. Control animals were gavaged  
30 with 0.5% carboxymethyl cellulose [assumed to be the vehicle]. Juvenile males (30 /group (obtained from  
31 10 litters) were treated on PND 21–43 (day of birth not defined). At six weeks of age, 25 mice/group were  
32 necropsied. Ten pregnant C57BL/6N mice/group were treated on GD 11–17 (GD 0 = day of vaginal plug).  
33 Fetuses were removed by cesarean section on GD 18 and that day was considered PND 0. Litters were  
34 fostered to untreated dams. On PND 4, females were disposed and litters were culled to 3 males. Males  
35 were weaned on PND 21 and housed individually in polycarbonate cages. At 12 weeks of age, males were  
36 weighed and 25 males/group were killed and necropsied. During necropsy of males that had been exposed  
37 during prenatal development or during adolescence, testes, epididymis, and seminal vesicles with  
38 coagulating glands were weighed. In the study conducted in adult mice, it was noted that ventral prostates  
39 were not weighed due to difficulties in obtaining only prostate and determining the precise weight of the  
40 organ. Epididymal sperm counts were obtained. Histopathological examinations were conducted for  
41 reproductive organs fixed in Bouin solution. For males exposed during gestation, the litter was considered a  
42 single sample. Data were analyzed by Bartlett's test to determine homogeneity of variance, followed by  
43 ANOVA when homogeneity of variance was obtained or Wallace-Wallace analysis of ranks when variance  
44 was not homogenous. Dunnett test was used for multiple comparisons.

45  
46 There were no significant effects on embryo mortality after birth, body weight gain, or terminal body  
47 weight. **[Data were not shown.]** The only reproductive organ weight effect was a significant, but non-dose  
48 related [6%] decrease in absolute seminal vesicle weight in the low-dose bisphenol A group. Organ  
49 weights were not affected in males exposed during adolescence. Sperm density was unaffected by  
50 bisphenol A exposure. No treatment-related lesions were observed in testes or other reproductive organs

### 3.0 Developmental Toxicity Data

1 including ventral prostate. **[Data were not shown.]** The study authors concluded that low-dose bisphenol A  
2 exposure of mice did not reduce sperm density or disrupt male reproductive system development.

3  
4 **Strengths/Weaknesses:** Strengths are the use of 3 low dose levels, the oral route of administration, the  
5 careful description of methods, the use of a low-phytoestrogen diet, and the confirmation that the strain of  
6 mice used was estrogen sensitive.

7  
8 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
9 evaluation process.

10  
11 **Kabuto et al. (134)**, supported by the Kagawa Prefectural College of Health Sciences, examined the role of  
12 oxidative stress in bisphenol A-induced toxicity in mice. ICR mice were fed standard laboratory chow  
13 containing 24% protein (MF Oriental Yeast Co., Tokyo, Japan). **[No information was provided about**  
14 **bedding or caging materials.]** From 1 week prior to mating through gestation and lactation, 6 mice/group  
15 were given drinking water containing the 1% ethanol vehicle or bisphenol A **[purity not reported]** at 5 or  
16 10 µg/L. **[Based on the reported water intake of 5 mL/day and an assumed body weight of 0.02 kg**  
17 **(115), it is estimated that bisphenol A intake in mice at the start of pregnancy was 0.0013 or 0.0025**  
18 **mg/kg bw/day.]** Mice gave birth about 3 weeks following mating and pups were housed with dams for 4  
19 weeks. **[Based on an assumed body weight of 0.0085 kg and assumed water intake rate of 0.003 L/day**  
20 **(115), it is estimated that intake of bisphenol A in weanling males was 0.0018 or 0.0035 mg/kg**  
21 **bw/day].** At 4 weeks of age, male pups were killed and brain, kidney, liver, and testis were weighed in 8–  
22 13 mice/group. Tissues were homogenized to determine activities of superoxide dismutase, catalase, and  
23 glutathione peroxidase and concentrations of glutathione and L-ascorbic acid in 6–8 mice/group. Tissue  
24 level of thiobarbituric acid-reactive substance, a biogenic macromolecular peroxidation indicator, was  
25 measured in 6 mice/group. Data were analyzed by ANOVA followed by Scheffe F test. **[It appears that**  
26 **offspring were considered the statistical unit in some analyses.]**

27  
28 Organ weight effects included decreased brain weight at the low dose, decreased kidney weight at the high  
29 dose, and decreased testis weight at both doses. **[Relative organ weights were not determined.]** In the  
30 high-dose group, thiobarbituric acid-reactive substance levels were increased in brain, kidney, and testis.  
31 Changes in antioxidant enzyme levels included decreased catalase activity in testis and increased  
32 glutathione oxidase activity in kidney. No significant effects were observed for superoxide dismutase  
33 activity or glutathione or ascorbic acid levels in any of the tissues examined. The study authors concluded  
34 that bisphenol A exposure during gestation and lactation results in oxidative stress and peroxidation in  
35 offspring that ultimately lead to underdevelopment of brain, kidney, and testis.

36  
37 **Strengths/Weaknesses:** The delivery of bisphenol A in drinking water at low dose levels is a strength.  
38 Weaknesses include small sample size of exposed dams (n=6), inappropriate use of the pup as the  
39 experimental unit in statistics, and mechanistic data without functional correlates.

40  
41 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation process  
42 due to inappropriate statistical procedures and small sample size.

43  
44 **Takao et al. (429)**, support not indicated, examined the effects of bisphenol A exposure on expression of  
45 *ERα* and *ERβ* in the testis of young mice. **[No information was provided about feed, caging, or bedding**  
46 **materials.]** Three-week-old male C57BL/6 mice (n = 7/group) were administered bisphenol A **[purity not**  
47 **indicated]** through drinking water at 0 (ethanol vehicle), 0.5, or 50 mg/L for 8 weeks. **[Assuming a**  
48 **weanling mouse drinks ~0.35 L/kg bw/day (115), bisphenol A intake would have been ~0, 0.175, or**  
49 **17.5 mg/kg bw/day.]** The stability of bisphenol A was not determined, but water bottles were changed 2  
50 times a week to maintain a stable concentration of bisphenol A in drinking water. Mice were killed at an  
51 unspecified period following exposure, and the testis and spleen were weighed. The testis was examined for

### 3.0 Developmental Toxicity Data

1 ER $\alpha$ - and ER $\beta$ -positive cells using an immunohistochemistry method and *ER $\alpha$*  and *ER $\beta$*  mRNA using a  
2 semi-quantitative RT-PCR technique. Data were analyzed by ANOVA followed by Fisher protected least  
3 significant difference test.

4  
5 Exposure to 50 mg/L bisphenol A resulted in a decreased number of ER $\beta$ -positive cells and increased  
6 number of ER $\alpha$ -positive cells. Expression of *ER $\beta$*  mRNA was decreased and expression of *ER $\alpha$*  mRNA was  
7 increased following exposure to 50 mg/L bisphenol A. There were no differences in body weight or  
8 absolute or relative weights of testis or spleen following bisphenol A treatment. The study authors  
9 concluded that differential modulation of ER $\alpha$  and ER $\beta$  could be involved in effects observed following  
10 bisphenol A exposure.

11  
12 **Strengths/Weaknesses:** The delivery of bisphenol A in drinking water and the measurement of ER in the  
13 testis are strengths. The lack of clarity on age at sacrifice, limited number of endpoints assessed, and  
14 marginal sample size (n=7) are significant weaknesses.

15  
16 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation process  
17 based on the limitations noted above.

18  
19 **Matsumoto et al. (430)**, support not indicated, examined the effect of maternal bisphenol A exposure on  
20 growth of offspring in mice. Mice were fed standard rodent chow (CE-2, Japan Clea). **[No information**  
21 **was provided on caging and bedding materials.]** Mice of the ddY strain were exposed to bisphenol A  
22 ( $\geq 97\%$  purity) through feed at 0 or 1% from GD 14 through PND 7. The study authors stated that the  
23 bisphenol A dose was equivalent to 1000 mg/kg bw/day. **[The number of dams treated was not**  
24 **indicated. Day of vaginal plug and day of birth were not defined].** Mice delivered pups on PND 21.  
25 During the postnatal period, body weight was monitored in 31 pups from the control group and 61–89 pups  
26 from the bisphenol A group. Serum prolactin levels were measured by RIA in 3 dams/group 4 days  
27 following delivery. Pups were killed on PND 7, and stomach weight was measured. Data were analyzed by  
28 Student *t*-test. **[It was not clear if the litter or offspring was considered the statistical unit.]**

29  
30 No differences were reported for live pups at birth. During the postnatal period, body weights of pups in the  
31 bisphenol A group were significantly lower **[by ~40%]** than control group pups. No deaths were reported  
32 for pups in the control group, but 30% of pups in the bisphenol A group died before PND 7. On PND 1,  
33 milk could be seen in stomachs of pups from the control group, but not the bisphenol A group. **[The**  
34 **number of pups evaluated for milk in stomach was not reported].** On PND 7, stomach weight was  
35 significantly lower **[by 40%]** in pups from the bisphenol A than control group. Serum prolactin level was  
36 significantly reduced **[by 46%]** in dams from the bisphenol A group. The authors concluded that  
37 administration of a high bisphenol A dose to mice resulted in suppressed postnatal growth of offspring  
38 which probably resulted from an insufficient supply of milk, which might have been due to decreased  
39 prolactin secretion. **[Because of the implications of this study for lactation competence, this paper will**  
40 **be discussed again in Section 4.2.]**

41  
42 **Strengths/Weaknesses:** Weaknesses of the study are the difficulty in calculating bisphenol A intake, the  
43 likely high exposure level, the lack of information on dam number and husbandry, and the high level of pup  
44 body weight decrement and mortality.

45  
46 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation process  
47 due to the reasons stated above.

48  
49 **Suzuki et al. (431)**, supported by the Japanese Ministry of Health, Labor, and Welfare and the Ministry of  
50 Education, Culture, Sports, Science, and Technology conducted a study to determine the effect of prenatal  
51 bisphenol A exposure on dopamine-receptor mediated actions in mice. Female ddY mice were fed chow

### 3.0 Developmental Toxicity Data

1 containing bisphenol A at 0.002, 0.5, or 2 mg/g feed from mating through weaning of offspring. **[No**  
2 **information was provided on the number of dams treated, purity of bisphenol A, or the type of chow,**  
3 **bedding, or caging materials. Assuming a female mouse eats ~0.2 kg feed/kg bw/day (115), bisphenol**  
4 **A intake would have been 0.4, 100, or 400 mg/kg bw/day.]** Male offspring were subjected to a series of  
5 tests **[age at testing not stated]**. In a conditioned place-preference test, groups of 6–10 mice were injected  
6 with 0.5 mg/kg bw methamphetamine and placed in either the dark or light area of the test apparatus for 3  
7 days. On the other 3 days, males were injected with saline and placed in the other compartment of the  
8 testing apparatus. On the 7<sup>th</sup> day, the divider in the apparatus was raised and the time spent in each  
9 compartment was measured. Activity was measured in groups of 9–10 mice for 3 hours following injection  
10 with saline or 2 mg/kg bw methamphetamine. Dopamine-induced binding of <sup>35</sup>S-guanosine-5' [ $\gamma$ -thio]-  
11 triphosphate in the limbic system was measured (n = 3 samples/group). Protein levels of dopamine and  
12 vesicle monoamine transporters in brain were determined by Western blot (n = 6 samples), and mRNA  
13 levels of dopamine receptor in brain were determined by RT-PCR. Data were analyzed by ANOVA with  
14 Bonferroni/Dunnett test. **[It was not clear if the litter or offspring was considered the statistical unit.]**  
15

16 In conditioned-preference testing, exposure to all 3 bisphenol A doses resulted in a significant and dose-  
17 related increase in preference for compartments associated with methamphetamine exposure. **[Control**  
18 **mice showed no compartment preferences while the times spent in the methamphetamine-associated**  
19 **compartment were ~150, 200, and 275 seconds by animals in each respective dose group.]** Preference  
20 for the methamphetamine compartment was eliminated by injecting the animals with SCH23390, A  
21 dopamine D<sub>1</sub> receptor antagonist. In mice exposed to the high dose of bisphenol A, activity was  
22 significantly increased **[by ~80% at peak]** compared to the control group following methamphetamine  
23 challenge, and sensitization to methamphetamine-induced activity was also enhanced. Dopamine-induced  
24 binding of <sup>35</sup>S-guanosine-5' [ $\gamma$ -thio]-triphosphate in the limbic system was potentiated **[increased by**  
25 **~15%; not clear if statistically significant]** and G-protein activation was increased **[by ~75%]** in mice  
26 exposed to the high bisphenol A dose. The effects on G-protein activation were eliminated following  
27 injection with SCH23390 or sulpiride, a dopamine D<sub>2</sub> receptor antagonist. No changes were observed for  
28 expression of dopamine and vesicle monoamine transporter proteins. Expression of dopamine D<sub>1</sub> receptor  
29 mRNA was significantly up-regulated to 130% of control levels in the high-dose bisphenol A group. **[For**  
30 **all endpoints except for conditioned preference, only the data from the high-dose bisphenol A group**  
31 **was shown. It was not clear if that was the only dose tested for those endpoints or if the high-dose**  
32 **data were shown because it was the only dose that resulted in a statistically significant effect.]** The  
33 study authors concluded that “prenatal and neonatal exposure to bisphenol A can potentiate central  
34 dopamine D<sub>1</sub> receptor-dependent neurotransmission, resulting in supersensitivity of methamphetamine-  
35 induced pharmacological actions related to psychological dependence on psychostimulants.”  
36

37 **Strengths/Weaknesses:** Strengths include a wide range of doses administered orally. Weaknesses include  
38 absence of adequate experimental details, inappropriate statistical procedures that did not account for litter  
39 or repeated measurement, inadequate presentation of body weight data, and use of high doses,  
40

41 **Utility (Adequacy) for CERHR Evaluation Process:** This report is inadequate for the evaluation process  
42 due to the reasons stated above.  
43

44 **Tando et al. (432)**, supported by the Japanese Ministries, investigated the effects of bisphenol A exposure  
45 in the maternal diet during the prenatal and lactational period on the long-term development of the cortex  
46 and substantia nigra. ddY mice were maintained under a 12 hour:12 hour light:dark cycle prior to mating.  
47 From GD 0 through weaning on PND 21, dams had free access to a diet containing bisphenol A (purity  
48 >99%) at 0, 3, or 8000 mg/kg feed. Pups were weaned on PND 21 to a diet without bisphenol A. **[The**  
49 **basal feed, cage, and bedding were not specified. Daily feed consumption was not reported. Assuming**  
50 **a pregnant mouse eats ~0.15 kg feed/kg bw/day and a lactating mouse eats ~0.45 kg feed/kg bw/day,**  
51 **bisphenol A intake would have been ~0, 4.5, or 1200 mg/kg bw/day during gestation and ~0, 1.35, or**

### 3.0 Developmental Toxicity Data

1 **3600 mg/kg bw/day during lactation.** ] At 8–11 weeks of age, male and female offspring (n= 4 and  
2 5/sex/treatment group) were killed and formalin-perfused. Brains were harvested and embedded in paraffin.  
3 Immunohistochemical detection for tyrosine hydroxylase, calbindin D-28 K, calretinin, and parvalbumin  
4 proteins were performed. In situ TUNEL was also performed. Statistical analyses use ANOVA and post-  
5 hoc test using the Bonferroni/ Dunn multiple comparison test. **[It was not clear if the litter or offspring**  
6 **was considered the statistical unit.]**  
7

8 No cytoarchitectural anomalies were seen in brain sections of either sex across treatment groups, based on  
9 hematoxylin-eosin and Kluver-Barrera stains. **[Data were not shown.]** The distribution and density of  
10 immunopositive staining for calbindin D-28K, calretinin, and parvalbumin showed no statistically  
11 significant differences in low or high-dose bisphenol A exposed groups. Female offspring exposed to the  
12 lower dose level of bisphenol A exhibited a significant decrease in the volume of the substantia nigra. The  
13 number of tyrosine hydroxylase-positive nuclei and fibers in this region was significantly reduced in low-  
14 bisphenol exposed female mice compared to control females and high dose bisphenol A-exposed females  
15 [**↓18%, and 16%, respectively, estimated from a graph**]. No significant differences in number of  
16 tyrosine hydroxylase positive cells were identified in bisphenol A-exposed males. Decreased values in  
17 immunopositive staining could not be attributed to apoptosis, based on TUNEL staining **[data not shown]**.  
18

19 The authors concluded that there were sex and dose-specific sensitivities of the developing substantia nigra,  
20 in the DDY mice with females exposed to a low but not a high dose of bisphenol A showing a significant  
21 reduction in the number of tyrosine hydroxylase-positive nuclei. They indicated that the functional  
22 significance of this reduction was unknown. The authors suggested a putative mechanism involving  
23 interaction of bisphenol A with ER $\beta$ , which is abundantly present in the developing substantia nigra.  
24

25 **Strengths/Weaknesses:** Strengths of this study are that BPA was delivered orally to the dams during the  
26 gestational and lactational period and the use of appropriate methods for assay of the anatomical and some  
27 molecular aspects of brain development. Weaknesses include the lack of specification of the feed, broad  
28 range of the two doses used, small sample size given high variability of endpoints (4 and 5/sex/treatment  
29 group), and absence of expected sexually dimorphisms in measures in the controls.  
30

31 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate and not useful for the  
32 evaluation process for the reasons stated above.  
33

34 **Mizuo et al. (433)**, supported by the Japanese Ministry of Health, Labor, and Welfare and the Ministry of  
35 Education, Culture, Sports, Science, and Technology, examined the effect of perinatal bisphenol A  
36 exposure on morphine-induced rewarding effects and hyperlocomotion in mice. Testing was conducted in  
37 offspring of ddY mice that received chow containing 0, 0.002, 0.5, or 2 mg bisphenol A/g feed **[0, 2, 500,**  
38 **or 2000 ppm]** during gestation and the neonatal period of pup development. **[No information was**  
39 **provided on the number of dams treated/group, purity of bisphenol A, or feed, caging, or bedding**  
40 **materials.]** In place-conditioning testing, 6–10 offspring/group were placed in one compartment of a  
41 testing apparatus following saline injection and in a second compartment of the apparatus following  
42 morphine injection; on the second day, mice were given free access to both compartments and the time  
43 spent in each compartment was measured. Locomotor activity was measured after injecting 9–10  
44 mice/bisphenol A group with saline or 10 mg/kg bw morphine. Guanosine-5'-diphosphate binding and  
45 expression of  $\mu$ -opioid receptor mRNA were measured in 3 independent samples/group. Statistical analyses  
46 included 2-way ANOVA with Bonferroni/Dunnett test. **[No information was given on the ages that**  
47 **testing was conducted and the sex of mice tested. It was not clear if the litter or offspring was**  
48 **considered the statistical unit.]**  
49

50 In place-preference conditioning testing, a dose-dependent increase was observed for the time spent in the  
51 compartment associated with morphine exposure and statistical significance was attained at the two highest

### 3.0 Developmental Toxicity Data

1 dose levels. [The time spent in the morphine-associated compartment was ~15 seconds for controls,  
2 **150 seconds for the mid-dose group, and 175 seconds for the high-dose group.**] Locomotion in the  
3 high-dose bisphenol A group was significantly increased following morphine injection [**~130 compared to**  
4 **10 activity counts in high-dose bisphenol A group compared to the control**]. Bisphenol A treatment had  
5 no effect on guanosine-5'-diphosphate binding (i.e.,  $\mu$ -opioid receptor mediated G-protein activation) or  
6 expression of  $\mu$ -opioid receptor mRNA. The study authors concluded that chronic exposure to bisphenol A  
7 induces morphine-induced rewarding effect and hyperlocomotion that does not occur through activation of  
8 the  $\mu$ -opioid receptor.

9  
10 **Strengths/Weaknesses:** Strengths of this study are that BPA was delivered orally to the dams during the  
11 gestational and lactational period. Weaknesses include the lack of specification of the feed, broad range of  
12 the two doses used, small sample size (n=6-10) and inappropriate statistics that do not account for litter or  
13 repeated measures.

14  
15 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate and not useful for the  
16 evaluation process.

17  
18 **Miyatake et al. (434)**, supported by the Japanese Ministry of Health, Labor, and Welfare and the Ministry  
19 of Education, examined the effects of developmental bisphenol A exposure on morphine-induced  
20 rewarding effects in male ddY mice. Maternal mice were orally exposed to olive oil vehicle, bisphenol A  
21 **[purity not indicated]** at 0.003 or 200 mg/kg bw/day, or 17 $\beta$ -estradiol at 3  $\mu$ g/kg bw/day by gavage. The  
22 compounds were administered 3 times a day from the mating period through weaning of offspring. Seven  
23 male offspring/group were examined in a place-conditioning test at 7 weeks of age. During the  
24 preconditioning period, mice were placed in one compartment of a cage following injection with saline and  
25 in another compartment of the cage following sc injection with morphine. During testing, the amount of  
26 time spent in each compartment of the cage was measured. Statistical analyses included ANOVA followed  
27 by Bonferroni/Dunnett test. **[It was not clear if the litter or offspring was considered the statistical**  
28 **unit.]**

29  
30 Developmental exposures to either bisphenol A dose resulted in a preference for the cage compartment  
31 associated with morphine exposure. Developmental exposure to 17 $\beta$ -estradiol at 3  $\mu$ g/kg did not affect  
32 place preference. Based on the findings of this study and in vitro studies described in Section 3.2.1.1, the  
33 study authors concluded that bisphenol A alters dopamine responsiveness in mouse neurons and astrocytes,  
34 which could potentially contribute to development of psychological dependence on drugs of abuse.

35  
36 **Strengths/Weaknesses:** Strengths include the use of a positive control and corresponding measurement of  
37 in vitro and behavioral endpoints. Weaknesses include the use of only 2 doses, 1 very low and 1 high (both  
38 had similar effects), inadequate experimental details regarding exposure and numbers of dams, small  
39 sample size for behavioral endpoints, inappropriate statistical procedures that did not account for litter of  
40 origin or repeated behavioral measurements.

41  
42 **Utility (Adequacy) for CERHR Evaluation Process:** This report is inadequate and not useful for the  
43 evaluation process.

44  
45 **Ryan and Vandenberg (435)**, supported by North Carolina State University and EPA, evaluated the  
46 effects in mice of prenatal and postnatal exposure to bisphenol A on sexually dimorphic behaviors.  
47 C57BL/6 mice were maintained in polycarbonate cages (checked frequently for condition) with chip  
48 bedding and were given Purina 5001 chow. Females were mated and the day a vaginal plug was identified  
49 was considered GD 1. Beginning on GD 3, dams were treated with bisphenol A **[purity not indicated]** 2 or  
50 200  $\mu$ g/kg bw/day, ethinyl estradiol 5  $\mu$ g/kg bw/day, or the tocopherol-stripped corn oil vehicle. The dose  
51 was placed in the back of the throat with a gavage needle. Daily dosing was continued to PND 21, when

### 3.0 Developmental Toxicity Data

pups were weaned. One female per litter was randomly selected for behavioral testing and was ovariectomized. Pup anogenital distance was measured at weaning. Non-ovariectomized mice were checked for vaginal opening and vaginal smears taken daily thereafter. Puberty was defined as the first day on which cornified cells were detected in 4–7 females/group. Fourteen mice/treatment group were tested in an elevated plus maze and a light-dark preference chamber. Sixteen mice/treatment group were tested in a radial arm maze and a modified Barnes maze. Testing occurred 2 weeks after ovariectomy. Statistical analysis used ANOVA with post-hoc Student *t*-test. The radial arm and Barnes mazes were run for 5 consecutive days and a repeated measures design was added to the ANOVA.

There was no effect of treatment on anogenital distance or anogenital distance divided by body weight. Other results are summarized in Table 80. Puberty was advanced by exposure to ethinyl estradiol or the high dose of bisphenol A. The results of the elevated plus and light-dark preference tests led the authors to conclude that bisphenol A and ethinyl estradiol increased anxiety. The improved performance in the radial arm and Barnes mazes led the authors to conclude that ethinyl estradiol masculinized spatial ability. [The results from the elevated plus maze also suggest masculinization of behavior, because males show more “anxiety” in this paradigm.] Bisphenol A 200 µg/kg bw/day resulted in a decrease in errors on earlier trials than the control in the radial arm maze, but this effect was not characterized by the authors as providing strong evidence of an alteration in spatial memory.

**Table 80. Behavior of Female Mice after Gestational and Lactational Exposures**

Endpoint <sup>a</sup>	Bisphenol A, µg/kg bw/day		Ethinyl estradiol
	2	200	
Puberty onset	↔	↓4.5 days	↓6.25 days
Time in open arms of plus maze	↔	↓41% (P = 0.06)	↓73%
Time in light part of light/dark preference box	↔	↓52%	↓69%
Errors in radial arm and Barnes mazes	↔	↔	↓

<sup>a</sup>The size of the difference from control was estimated from graphs.

↓ Statistically significant decrease from control value; ↔ no statistical difference from control value,

↓ Decrease identified by authors although statistical difference from control not shown.

From Ryan and Vandenberg (435)

**Strengths/Weaknesses:** Selection of established measurements of sexually dimorphic behaviors and replication of previous work by Howdeshell et al. (396), the use of positive controls, the appropriate evaluation of pubertal onset, adequate sample sizes for behavioral methods, weight, and AGD measures are all strengths of this work. A weakness is the small sample size for evaluating pubertal onset.

**Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the evaluation process with the exception of the pubertal data.

**Tyl et al. (436)**, sponsored by the American Plastics Council, conducted a 2-generation GLP study of bisphenol A in CD-1 mice. [This study is discussed in detail in Section 4.2.3.2. Results relevant to developmental toxicity are presented here.] Mice were fed Purina Certified Ground Rodent Diet No. 5002 containing 177–213 ppm genistein, 173–181 ppm daidzein, and 39–55 ppm glycitein. Mice were housed in polypropylene cages with Sani-Chip® bedding. F<sub>0</sub> and F<sub>1</sub> mice (28 sex/group/generation) were fed diets containing bisphenol A (99.70–99.76% purity) at 0.018, 0.18, 1.8, 30, 300, or 3500 ppm. Target intakes were 0.003, 0.03, 0.3, 5, 50, or 600 mg/kg bw/day. The study authors estimated bisphenol A intake in males at 0.0024–0.0038, 0.024–0.037, 0.24–0.37, 3.98–6.13, 39.1–60.8, or 529–782 mg/kg bw/day. Bisphenol A intakes (in mg/kg bw/day) by females were estimated at 0.0030–0.0041, 0.030–0.042, 0.32–0.43, 5.12–7.12, 54.2–67.8, 653–910 during the pre-mating period; 0.0027–0.0029, 0.027–0.028, 0.28–0.29, 4.65–4.80, 47.0–48.6, 552–598 during the gestation period; and 0.0087–0.0063, 0.062–0.091, 0.61–

### 3.0 Developmental Toxicity Data

1 0.89, 10.4–15.1, 103.2–146.4, 1264–1667 during the lactation period. In each generation, there were 2  
2 vehicle control groups with 28 mice/sex/group. A positive control group was given feed containing 17 $\beta$ -  
3 estradiol at 0.5 ppm (target intake of 0.08 mg/kg bw/day). **[The Expert Panel notes that a separate 2-  
4 generation study was used to characterize the dose-response relationship for 17 $\beta$ -estradiol.]**  
5 Homogeneity, stability, and concentration of bisphenol A in feed were verified. Exposure of F<sub>0</sub> mice began  
6 at ~6 weeks of age. Exposure of F<sub>1</sub> animals began at weaning, although it was noted that pups began eating  
7 the dosed feed in the late lactation period. F<sub>0</sub> and F<sub>1</sub> mice were fed the bisphenol A-containing diets for a  
8 minimum of 8 weeks prior to mating and during a 2-week mating period. Exposures of females continued  
9 through the gestation and lactation period.

10  
11 Live F<sub>1</sub> and F<sub>2</sub> pups and litters at birth, sex ratio, and survival during the lactation period were not affected  
12 and there were no clinical or gross signs of toxicity in F<sub>1</sub> or F<sub>2</sub> offspring. A non-dose-related decrease in  
13 PND 21 survival index and lactational index (pups surviving on PND 21/PND 4) was described in F<sub>2</sub> pups  
14 of the 300 ppm group. **[The biological significance of the effect was not discussed by the study authors,  
15 but because the effect was not dose-related it is unlikely to be of biological significance.]** In F<sub>1</sub> pups  
16 from the 3500 ppm group, body weights were reduced during PND 7, 14, and 21 in F<sub>1</sub> females and both  
17 sexes combined and on PND 7 and 21 in F<sub>1</sub> males. An increase in male pup body weight observed on PND  
18 7 in the 1.8 ppm group was not considered to be treatment related by the study authors because no dose-  
19 response relationship was observed. There was no effect on anogenital distance in F<sub>1</sub> or F<sub>2</sub> males or females  
20 on PND 0. Anogenital distance was also unaffected in F<sub>2</sub> males and F<sub>1</sub> and F<sub>2</sub> females on PND 21.  
21 Anogenital distance adjusted for body weight was reduced in F<sub>1</sub> males from the 300 and 3500 ppm groups  
22 on PND 21. Based on the lack of effect on anogenital distance at birth and inconsistencies between  
23 generations, the study authors did not consider the decreases in anogenital distance in F<sub>1</sub> males to be  
24 treatment-related. An increase in anogenital distance in F<sub>2</sub> females from the 0.018 ppm group on PND 0  
25 was not considered to be treatment related by the study authors. Preputial separation (absolute age and  
26 adjusted for body weight on day of acquisition) was delayed in parental and retained F<sub>1</sub> males of the 3500  
27 ppm group. When adjusted for PND 30 body weight, preputial separation was delayed in retained but not  
28 parental F<sub>1</sub> males from the 3500 ppm group. Body weights on day of vaginal opening were lower in F<sub>1</sub>  
29 females from the 3500 ppm group. Day of vaginal opening was accelerated in the 3500 ppm group if  
30 adjusted for PND 21 body weight, but not body weight on the day of acquisition. Due to the lack of effect  
31 when adjusted for body weight on day of acquisition, the study authors did not consider effects on vaginal  
32 opening to be treatment related.

33  
34 Dose-related organ weight changes in F<sub>1</sub> weanlings that were considered to be treatment-related by study  
35 authors included decreased absolute and relative (to body or brain weight) spleen and paired testes weights  
36 at 3500 ppm. Treatment-related absolute organ weight changes in F<sub>2</sub> weanlings included decreased weights  
37 of spleen, paired testes, and seminal vesicles with coagulating glands in the 3500 ppm group. Changes in  
38 organ weights relative to body weight in F<sub>2</sub> weanlings included decreased spleen weight in males and  
39 females and increased relative left kidney weight in 3500 ppm males. Treatment-related changes in organ  
40 weight relative to brain weight in F<sub>2</sub> weanlings were decreased spleen weight in both sexes and decreased  
41 paired testes weight at 3500 ppm and seminal vesicles with coagulating glands at 300 and 3500 ppm. Other  
42 organ weight effects (e.g., affecting epididymides, thymus, brain, ovaries, and/or uterus with cervix and  
43 vagina weights) were not considered to be dose-related due to lack of dose-response relationships or no  
44 consistent effects across generations. The study authors reported no gross findings in F<sub>1</sub> or F<sub>2</sub> weanlings.  
45 **[Although not clear because the number of animals examined for gross testicular effects was not  
46 reported in Tables 23 and 49 of the study, it appeared that the incidence of undescended bilateral  
47 testes may have been increased in F<sub>1</sub> and F<sub>2</sub> weanling males of the 3500 ppm group.]** The incidence of  
48 hepatic cytoplasm alteration (clear hepatocellular cytoplasm, slightly more basophilic cytoplasm, and/or  
49 minute vacuoles) was apparently increased in F<sub>1</sub> males from the 300 and 3500 ppm groups and F<sub>1</sub> females  
50 and F<sub>2</sub> males from the 3500 ppm group. The incidence of seminiferous tubule hypoplasia was increased in  
51 F<sub>1</sub> and F<sub>2</sub> weanlings from the 3500 ppm group. **[Another histopathological finding that appeared to be**

### 3.0 Developmental Toxicity Data

1 **possibly increased in weanlings from the 3500 ppm group was unilateral hydronephrosis in F<sub>1</sub> males.**  
2 **It did not appear that histopathological data were statistically analyzed.]**  
3

4 The study authors identified bisphenol A NOELs of 30 ppm (~5 mg/kg bw/day) for systemic effects and  
5 300 ppm (~50 mg/kg bw/day) for developmental toxicity. **[The lowest benchmark doses were obtained**  
6 **from F<sub>1</sub> body weight data on PND 21: BMD<sub>10</sub> 548 mg/kg bw/day, BMDL<sub>10</sub> 267 mg/kg bw/day,**  
7 **BMD<sub>1SD</sub> 580 mg/kg bw/day, BMDL<sub>1SD</sub> 370 mg/kg bw/day.]**  
8

9 **Strengths/Weaknesses:** Strengths include the large number and range of doses examined, the rigor with  
10 which the study was performed (including evaluation of phytoestrogen content of feed), the large sample  
11 size in each group, the number of additional animals per litter that were retained and examined, the use of a  
12 concurrent estrogenic positive control group, and the thoroughness of the histological evaluation.  
13

14 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
15 evaluation process. .  
16

#### 17 3.2.8 Mouse—parenteral exposure postnatally with or without prenatal exposure

18

##### 19 3.2.8.1 Female reproductive endpoints

20 **Suzuki et al. (437)**, supported by Japanese Ministry of Education, Culture, Sports, Sciences, and  
21 Technology, the Special Coordination Funds of Science and Technology Agency of the Japanese  
22 Government, and the Japanese Ministry of Health, Labor, and Welfare, conducted a study to examine the  
23 effects of bisphenol A exposure on the reproductive system of the female mouse. Two sets of studies were  
24 conducted, one with prenatal exposure, and one with postnatal exposure. In both studies, ICR/Jcl strain  
25 mice were fed a commercial diet (CE-2, CLEA, Tokyo, Japan). **[No information was provided about**  
26 **bedding or caging materials.]** Bisphenol A **[purity not reported]** was administered by sc injection in  
27 sesame oil vehicle. For histological examinations, organs were fixed in Bouin solution. Parametric data  
28 were analyzed by ANOVA, with post hoc Student *t*-test or Welch *t*-test. Data expressed as proportions  
29 were analyzed by Fisher exact probability test. For exposures occurring in the prenatal period, the litter was  
30 maintained as the statistical unit.  
31

32 In the prenatal exposure study, mice were administered bisphenol A by sc injection at 0 (vehicle), 10, or  
33 100 mg/kg bw/day on GD 10–18 (day of vaginal plug = GD 0). Other groups of mice were treated with  
34 diethylstilbestrol at 0.0067–67 µg/kg bw/day during the same period. **[Numbers of dams treated were not**  
35 **specified.]** On GD 19, fetuses were removed by cesarean section, weighed, adjusted to 7 pups/litter  
36 **[numbers for each sex not indicated]**, and fostered to untreated mothers. Pups were weaned at 22 days of  
37 age. Some pups were ovariectomized at 30 days of age, and some were killed at 30 or 40 days of age for  
38 histological examination of reproductive organs, polyovular follicle numbers, corpora lutea numbers, and  
39 mitotic index in uterine and vaginal cells. In the remaining pups, vaginal smears were examined from 41 to  
40 70 days of age. Fertility was then assessed by mating the mice with untreated males (2 or 3 females/male).  
41 Offspring were counted and sexed. The authors stated that 2 or 3 pups/litter were used in each analysis.  
42 Data tables list the sample size as 8–11/group/time period for the bisphenol A and control groups.  
43

44 Bisphenol A treatment did not affect the histology of the uterus or vagina in ovariectomized mice. The  
45 study authors stated there was no evidence of increased mitogenicity compared to controls in uterine cells  
46 of intact or ovariectomized mice exposed to bisphenol A. **[Figure 3 of the study indicated a higher**  
47 **mitotic index in epithelial cells of ovariectomized mice of the high-dose group.]** Mitotic indices were  
48 significantly lower in stromal cells of intact mice of both dose groups and in glandular cells of the low-dose  
49 group. There was no increase in mitogenicity of vaginal cells compared to the control group; in intact mice,  
50 the mitotic index was lower than control values in vaginal epithelial cells of the high-dose group and  
51 stromal cells of the low-dose group. Number of vaginal epithelial layers was increased in both bisphenol A

### 3.0 Developmental Toxicity Data

1 dose groups of intact mice compared to control mice. No effect was reported for uterine or vaginal  
2 epithelial stratification. There were no effects on numbers of polyovular follicles. **[Data were not shown  
3 by study authors.]** The number of mice with corpora lutea at 30 days of age was significantly reduced in  
4 the low-dose group (4 of 9 mice in low dose group compared to 7 of 9 mice in control group). Estrous  
5 cyclicity was not affected by bisphenol A treatment. In mating studies, bisphenol A exposure did not affect  
6 the number of mice giving birth, number of fetuses/litter, or sex ratio. Several effects were observed in  
7 mice prenatally exposed to diethylstilbestrol, and most of the effects occurred at the high dose of 67 µg/kg  
8 bw/day. In the high-dose diethylstilbestrol group, there were changes in vaginal and uterine histology,  
9 increases in mitotic indices in vaginal and uterine cells of ovariectomized animals, vaginal stratification and  
10 increased layers of epithelial cells in ovariectomized animals, disrupted estrous cycles, and complete  
11 infertility. The number of mice with corpora lutea at 30 days was decreased at the two highest  
12 diethylstilbestrol doses ( $\geq 6.7$  at µg/kg bw/day).

13  
14 In the postnatal exposure experiment, female mice (1.5 g bw) were sc injected with bisphenol A at 0.015 or  
15 0.150 mg/pup/day or diethylstilbestrol at 0.3 or 3 µg/pup/day for 5 days, beginning on the day of birth.  
16 **[The number of animals treated was not stated. Based on body weights provided by authors,  
17 bisphenol A doses were estimated at 10 and 100 mg/kg bw/day; diethylstilbestrol doses were  
18 estimated at 200 and 2000 µg/kg bw/day.]** Two-thirds of mice were ovariectomized at 30 days of age and  
19 then killed at 30, 40, or 90 days of age for histological examination of reproductive organs. Numbers of  
20 polyovular follicles were determined at 30 days of age, and number of corpora lutea were counted at 30 and  
21 90 days of age. Estrous cyclicity was monitored in the remaining mice at 61 to 90 days of age. The 90-day-  
22 old mice were sc injected with 5 mg/kg bw colchicine and killed 5 hours later. Mitotic rates of uterine and  
23 vaginal cells were determined, and histological examinations of reproductive organs were conducted.  
24 Sample sizes were 6–17/group/time period in analyses conducted in mice exposed postnatally.

25  
26 Vaginal stratification was observed at 40 days of age in 4 of 7 ovariectomized mice of the high-dose  
27 bisphenol A group, which was higher than in the control. The incidence of vaginal stratification in 90-day-  
28 old ovariectomized mice of the high-dose group (4 of 10) did not attain statistical significance compared to  
29 control. In ovariectomized mice, significant increases in the mitotic rate compared to controls were  
30 observed in uterine stromal cells and vaginal epithelial cells at the high dose. The number of vaginal  
31 epithelial layers was also increased in the high-dose bisphenol A group (~4 layers in treated group  
32 compared to 3.5 layers in control group). There were no significant changes in estrous cycles or number of  
33 mice with corpora lutea. In 30-day-old mice of the high-dose group, significant increases were observed in  
34 the number of mice with polyovular follicles (15 of 17 in exposed group compared to 6 of 15 in control  
35 group) and the numbers of polyovular follicles/mouse (mean  $\pm$  SE:  $0.8 \pm 0.2$  in the exposed group and  $0.2 \pm$   
36  $0.1$  in control group); polyovular follicles contained 2 oocytes in the control and bisphenol A groups.  
37 Effects observed in mice treated with both doses of diethylstilbestrol included increased stratification of  
38 vaginal cells in ovariectomized mice at 40 and 90 days of age, increased mitotic rates of vaginal and uterine  
39 cells in ovariectomized mice, disrupted estrous cycles, and increased polyovular follicles. The study  
40 authors concluded that high doses of bisphenol A induce ovary-independent vaginal stratification and  
41 polyovular follicles when administered during postnatal but not prenatal development.

42  
43 **Strengths/Weaknesses:** The use of diethylstilbestrol as a positive control is a strength as are an  
44 experimental design that appropriately examined litter effects. The use of relatively high doses by sc  
45 injection and small sample sizes for ovarian histopathology are weaknesses.

46  
47 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate but of limited utility due to  
48 the route and dose level.

49  
50 **Nikaido et al. (438),** supported by the Japanese Ministry of Health, Labor, and Welfare, examined the  
51 effects of bisphenol A exposure on the development of the reproductive system in female mice. Mice used

### 3.0 Developmental Toxicity Data

1 in this study were housed in polyisopentene cages with white pine chip bedding. The mice were fed a low-  
2 phytoestrogen diet (NIH-07 PLD; Oriental Yeast Co.) and provided water in polycarbonate bottles with  
3 rubber stoppers. At 15 days of age, 17–24 female CD-1 mice/group were sc injected with DMSO vehicle,  
4 10 mg/kg bw/day bisphenol A ( $\geq 99\%$  purity), or 10  $\mu\text{g}/\text{kg}$  bw/day diethylstilbestrol for 4 days. Additional  
5 groups were dosed with other compounds, but those results will not be discussed. **[No information was  
6 provided on the numbers of litters represented.]** Mice were weaned at 21 days of age. Body weights  
7 were measured weekly. Day of vaginal opening was determined and estrous cyclicity was assessed over 21-  
8 day periods beginning at 5, 9, and 21 weeks of age. Six mice/group/time period were killed and necropsied  
9 at 4, 8, 12, and 24 weeks of age. **[In contrast to the Materials and Methods section, there was no  
10 mention of animals killed at 12 weeks of age in the abstract or results section of the study.]** Ovaries,  
11 uteri, vaginas, and inguinal mammary glands were fixed in 10% neutral buffered formalin.  
12 Histopathological analyses were conducted of the ovary, uterus, and vagina. Mammary glands were  
13 examined as whole-mount preparations. It appears that all endpoints were assessed in every mouse.  
14 Statistical analyses included homogeneity of variance analysis and ANOVA or Kruskal-Wallis test. If  
15 statistical significance was obtained, data were further analyzed by Fisher protected least significant  
16 difference test.

17  
18 Exposure to bisphenol A resulted in no effects on body weight gain, age of vaginal opening, estrous  
19 cyclicity, histopathological changes in the uterus or vagina, or growth or development of the mammary  
20 gland. At 4 weeks of age, 33% of mice in the control group, 83% of mice in the bisphenol A group, and  
21 100% of mice in the diethylstilbestrol group lacked corpora lutea. **[It appears that the study authors  
22 considered the lack of corpora lutea to be normal based on the age of mice.]** No effects on corpora  
23 lutea numbers or numbers of polyovular follicles were observed at later ages. Mice treated with  
24 diethylstilbestrol experienced accelerated vaginal opening and increased time in estrus. In their conclusion,  
25 the study authors reiterated the lack of effects in the bisphenol A group.

26  
27 **Strengths/Weaknesses:** The use of diethylstilbestrol as a positive control is a strength, but the lack of  
28 information on sample size of dams, small sample size for postnatal endpoints, subcutaneous route, and  
29 high dose level are weaknesses.

30  
31 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate and not useful in the  
32 evaluation.

33  
34 **Markey et al. (439)**, supported by NIH, examined the effects of perinatal bisphenol A exposure on  
35 reproductive development in mice. CD-1 mice were fed Purina rodent chow that tested “negligible for  
36 estrogenicity in the E-SCREEN assay.” Cages and bedding tested negative for estrogenicity in the E-  
37 SCREEN assay. Tap water was supplied in glass bottles. From GD 9 (GD 1 = day of vaginal plug) through  
38 PND 4, 6–10 mice/group were exposed to bisphenol A **[purity not reported in the manuscript;  $97 \pm 2\%$   
39 per A. Soto, personal communication, March 2, 2007]** at 0 (DMSO vehicle), 0.000025, or 0.000250  
40 mg/kg bw/day through a sc pump. Offspring were culled to 10/litter on PND 7 and weaned on PND 20.  
41 One pup/litter from 6–10 litters/treatment group was killed on the day of proestrus at 3 months of age. The  
42 uterus and vagina were weighed and subjected to morphometric analysis. The uterus was also assessed for  
43 cell proliferation by bromodeoxyuridine (BrdU) incorporation, apoptosis by TUNEL method, and  
44 expression of ER $\alpha$  and progesterone receptor by an immunostaining procedure. Data that were normally  
45 distributed and showed homogeneity of variance were analyzed by ANOVA and least significant difference  
46 test. Other data were analyzed by Kruskal-Wallis and Mann-Whitney *U* test.

47  
48 Significant effects observed in 3-month-old offspring exposed to the high dose included decreased absolute  
49 and relative (to body weight) vaginal weight, decreased volume of uterine lamina propria, and increased  
50 percentage of proliferating uterine glandular epithelial cells. In mice of both dose groups, there were  
51 significant increases in expression of ER $\alpha$  and progesterone receptor in uterine luminal epithelial cells;

### 3.0 Developmental Toxicity Data

1 levels of both receptors were also increased in the subepithelial stroma. No treatment effects were observed  
2 for apoptosis in uterine luminal and glandular epithelial cells. No treatment effects were observed for  
3 vaginal morphometry or cell proliferation. The study authors concluded that environmentally relevant doses  
4 of bisphenol A affect the development of the genital tract at the gross and cellular level in the female  
5 offspring of mice exposed during pregnancy.

6  
7 **Strengths/Weaknesses:** The administration of very low doses is a strength. A critical weakness is the use  
8 of DMSO as a vehicle which is known to degrade the pump apparatus, and is inappropriate as a vehicle for  
9 in vivo studies. A critical weakness is the uncertainty of the DMSO concentration as a vehicle and therefore  
10 pump performance.

11  
12 **Utility (adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
13 given exposure uncertainties.

14  
15 **Muñoz-de-Toro et al. (418)**, supported by NIH and National University of Litoral (Argentina), examined  
16 the effect of perinatal bisphenol exposure on mammary gland development in mice. Food, caging, and  
17 bedding material were reported to test negligible for estrogenicity in the E-SCREEN. Water was provided  
18 in glass bottles. CD-1 mice (n = 6–10/group) were implanted with osmotic pumps designed to deliver  
19 bisphenol A [**purity not indicated**] at 0 (DMSO vehicle), 0.000025, or 0.000250 mg/kg bw/day from GD 9  
20 (GD 1 = day of vaginal plug) through PND 3 (not defined). Offspring were culled to 10 pups/litter on PND  
21 7. One female offspring/litter, from 6–10 litters/group, was killed on PND 20 and 30 and at 4 months of  
22 age. The 4-month-old mice were killed on proestrus. Another group of mice [**number not specified**] was  
23 killed on the first proestrus. Mammary glands were collected for evaluation of mammary structures at 20  
24 and 30 days and 4 months of age and day of first proestrus. Mammary glands were also collected from 30-  
25 day-old mice for analysis of DNA synthesis by BrdU incorporation, expression of ER $\alpha$  and progesterone  
26 receptor using immunohistochemistry techniques, apoptosis by TUNEL method, and *Wnt4* mRNA by RT-  
27 PCR. Plasma 17 $\beta$ -estradiol levels were measured in mice killed at first proestrus. In an experiment to  
28 monitor response to 17 $\beta$ -estradiol, one pup/litter (n = 10/group) was ovariectomized at 25 days of age and  
29 implanted with a sc pump supplying vehicle or 0.5  $\mu$ g 17 $\beta$ -estradiol/kg bw/day on PND 25–35. Mice were  
30 killed following 17 $\beta$ -estradiol treatment for examination of mammary structures. Statistical analyses  
31 included ANOVA and Dunn post hoc test. If the data were not normally distributed, statistical analyses  
32 were done by Kruskal-Wallis and Mann-Whitney test.

33  
34 In 30-day-old mice, bisphenol A exposure increased numbers of terminal end buds at both doses and area  
35 of terminal end buds at the high dose. Percentages of apoptotic cells were decreased on PND 30 in mice  
36 from both bisphenol A dose groups. The percentage of stromal cells undergoing proliferation on PND 30  
37 was reduced in the high-dose bisphenol A group. The number of epithelial cells expressing progesterone  
38 receptors was increased in both dose groups on PND 30, but there were no treatment-related changes in  
39 ER $\alpha$  receptor expression. Clusters of progesterone receptors were often observed in the ductal epithelium  
40 of bisphenol A-treated mice. Slopes of the correlation between age of first proestrus and mammary length  
41 were significantly reduced in the high-dose group, suggesting slower ductal invasion of stroma. There were  
42 no significant differences in plasma 17 $\beta$ -estradiol levels in mice killed at first proestrus. Trends for  
43 increasing expression of mRNA for *Wnt4*, a mediator of lateral branching downstream from progesterone  
44 receptors, did not attain statistical significance. The number of lateral branches in mammary gland at 4  
45 months of age was significantly increased at the low but not the high dose. In mice exposed to the high  
46 dose of bisphenol A during perinatal development and 17 $\beta$ -estradiol during postnatal development  
47 compared to mice who were exposed to 17 $\beta$ -estradiol but not bisphenol A, there were increases in  
48 numbers, area, and size of terminal end buds, terminal end bud numbers/ductal area, and terminal end bud  
49 area/ductal area. The study authors concluded that “. . . perinatal exposure to environmentally relevant  
50 [bisphenol A] doses results in persistent alterations in mammary gland morphogenesis.”

### 3.0 Developmental Toxicity Data

1 **Strengths/Weaknesses:** This study was a follow-up on the study of Markey et al. (439) and tested the  
2 same doses using a similar schedule for effects on mammary tissue. The administration of very low doses is  
3 a strength. The statistics appear to be inappropriate in not accounting for the significant number of  
4 comparisons made. A critical weakness is the uncertainty of the DMSO concentration as a vehicle and  
5 therefore pump performance.

6  
7 **Utility (adequacy) for CERHR Evaluation Process:** This paper is inadequate for the evaluation process  
8 given exposure uncertainties.

#### 9 3.2.8.2 Male reproductive endpoints

11 **Nakahashi et al.(440)**, supported by the Japanese Ministry of Education, Science, Sports, and Culture,  
12 examined the effect of neonatal bisphenol A exposure on adult sperm count in mice. On the first 5 days of  
13 life, 10–15 neonatal SHN mice/group were injected [**route not indicated**] with sesame oil/DMSO vehicle  
14 or with bisphenol A [**purity not reported**] in sesame oil at 0.0005 or 0.050 mg/day. [**Assuming a neonatal**  
15 **mouse weights 2 g, the mice received doses of 0.25 and 25 mg/kg bw/day**]. A group of 12 mice received  
16 0.050 mg/day bisphenol A in sesame oil in combination with 100 IU retinol acetate in DMSO vehicle. In a  
17 second exposure protocol, pregnant mice were fed a vitamin A-deficient diet (Low vitamin A diet; Clea  
18 Japan) from 3 days prior to gestation to PND 5. After PND 5, the dams were fed commercial diet (CE-7,  
19 Clea Japan). On the first 5 days of life, their pups (n = 7–9/group) were injected with bisphenol A at 0  
20 (sesame oil) or 0.0005 mg/day. Male offspring from both studies were weaned at 20 days of age and fed the  
21 CE-7 diet. Mice were killed at 14 weeks of age and epididymal sperm counts were obtained. [**No**  
22 **information was provided about caging and bedding materials. Numbers of litter represented were**  
23 **not indicated. Procedures for statistical analyses were not discussed.**]

24  
25 A 35% reduction in sperm counts was observed in mice from the 0.050 mg/day group compared to the  
26 control group. A significant reduction in sperm counts was not observed in the group co-treated with 0.050  
27 mg/day bisphenol A and retinol acetate. Administration of a vitamin A-deficient diet to dams had no effect  
28 on sperm counts in their offspring, but sperm counts were reduced in mice born to mothers fed a vitamin A-  
29 deficient diet and injected with 0.0005 mg/day bisphenol A in the neonatal period. The study authors  
30 concluded that vitamin protects infants from the effects of environmental xenoestrogens.

31  
32 **Strengths/Weaknesses:** The subcutaneous route of administration, lack of clarity on exposure issues, lack  
33 of husbandry and statistical information are weaknesses.

34  
35 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is inadequate for inclusion and not  
36 useful.

37  
38 **Aikawa et al. (441)**, supported by the Japanese Ministry of Education, Science, Sports, and Culture,  
39 examined the effects of neonatal bisphenol A exposure on sperm endpoints in adult mice. Unless otherwise  
40 specified, dams were fed CE-7 and CA-1 (Clea Japan Inc). [**No information was provided about caging**  
41 **or bedding materials.**] In the first experiment, SHN mice were sc injected with bisphenol A, bisphenol A  
42 plus retinol acetate, or vehicle for 5 days beginning on the day of birth. Doses of each compound were 0.5  
43 or 50 µg/day bisphenol A [**purity not reported**] (n = 10–14/group), 50 µg bisphenol A plus 100 IU retinol  
44 acetate/day (n = 5), and vehicle control (sesame oil for bisphenol A and or DMSO for retinol acetate; n =  
45 11). [**Assuming a neonatal mouse weights 2 g, these bisphenol A doses would be 0.25 and 25 mg/kg**  
46 **bw/day.**] In another group, pregnant mice were fed a low vitamin A diet from 3 days prior to gestation to  
47 PND 5 and were fed a normal vitamin A-containing diet (CE-7 and CA-1) beginning on the 6<sup>th</sup> day  
48 following parturition [**number/group not stated**]. Pups born to those dams (n = 7–8/group) were sc  
49 injected with 0.5 µg/day bisphenol A or vehicle for 5 days, beginning on the day of birth. In all groups,  
50 mice were weaned at 3 weeks of age, individually housed at 8 weeks of age, and killed at 10 weeks of age.  
51 Sperm were collected for analysis of motility and abnormalities. In pups not born to vitamin A-deprived

### 3.0 Developmental Toxicity Data

1 dams, testes were fixed in formalin for histopathological evaluation. Data were analyzed by ANOVA and  
2 Fisher least significant difference test.

3  
4 Sperm motility was significantly reduced in mice injected with 50 µg/day bisphenol A (~25 vs. 50% in  
5 controls) but was not affected in mice exposed to 50 µg/day bisphenol A plus retinol acetate. Sperm  
6 motility was not affected in mice born to mothers fed a normal diet and exposed to 0.5 µg/day bisphenol.  
7 Compared to the vehicle control group born to mothers fed a normal diet, the mice born to mothers fed a  
8 vitamin A-deficient diet and injected with 0.5 µg/day bisphenol A had significant reductions in sperm  
9 motility [**~19 compared to 50% in vehicle controls**]. Sperm motility was also reduced in the mice born to  
10 mothers fed a vitamin A-deficient diet but not exposed to bisphenol A. In groups born to mothers fed a  
11 vitamin A-deficient diet, there were no differences in sperm motility following exposure to vehicle or  
12 bisphenol A. Percentage abnormal sperm was 6.8% in the vehicle control group and was significantly  
13 increased in mice exposed to 0.5 µg/day bisphenol A [**~45%**], 50 µg/day bisphenol A (78.2%), 50 µg/day  
14 bisphenol A plus retinol acetate (27.8%), vehicle following birth to vitamin A-deficient mothers [**~45%**],  
15 or bisphenol A following birth to vitamin A-deficient mother [**~70%**]. No histopathological alterations  
16 were reported in testes of mice exposed to 0.5 or 50 µg/day bisphenol A or 50 µg/day bisphenol A plus  
17 retinol acetate. The study authors concluded that neonatal exposure to a relatively large dose of bisphenol A  
18 damages sperm motility and morphology, effects that are inhibited by vitamin A and enhanced by vitamin  
19 A-deficient diets.

20  
21 In a second experiment, 3 pups/group were sc injected with 20 µg 17β-estradiol/day, 20 µg 17β-estradiol  
22 plus 100 IU acetate retinol acetate/day, 50 µg bisphenol A/day, or vehicle (sesame oil for bisphenol A and  
23 17β-estradiol or DMSO for retinol acetate) for 5 days beginning on the day of birth. Mice were killed at 18  
24 days of age. Testis, efferent duct, epididymis, and vas deferens were fixed in formalin and analyzed for  
25 ERα using an immunohistochemical method. Data were analyzed by ANOVA and Fisher least significant  
26 difference test.

27  
28 In a second experiment, 3 pups/group were sc injected with 20 µg 17β-estradiol/day, 20 µg 17β-estradiol  
29 plus 100 IU acetate retinol acetate/day, 50 µg bisphenol A/day, or vehicle (sesame oil for bisphenol A and  
30 17β-estradiol or DMSO for retinol acetate) for 5 days beginning on the day of birth. Mice were killed at 18  
31 days of age. Testis, efferent duct, epididymis, and vas deferens were fixed in formalin and analyzed for  
32 ERα using an immunohistochemical method. Data were analyzed by ANOVA and Fisher least significant  
33 difference test. Bisphenol A exposure had no effect on ERα expression in male reproductive organs.  
34 Exposure to 17β-estradiol increased the numbers of ER-positive cells in vas deferens epithelium, but there  
35 was no increase when mice were treated with acetate retinol in addition to 17β-estradiol. The study authors  
36 concluded that the lack of effect of bisphenol A may be due to its weak estrogenic activity.

37  
38 **Strengths/Weaknesses:** This study provided follow-up information to that of Nakahashi et al.(440). The  
39 use of 17β-estradiol as a positive control in the testis histology study is a strength. Weaknesses include  
40 subcutaneous route of administration, lack of clarity on exposure issues, small sample sizes, lack of  
41 husbandry and statistical information.

42  
43 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate and not useful based on  
44 small sample sizes and inadequate presentation of statistical methods of analysis.

45  
46 **Toyama and Yuasa (381)**, supported in part by the Japanese Ministry of Environment and Ministry of  
47 Education, Science, Sports, and Culture, examined the effects of neonatal bisphenol A [**purity not**  
48 **reported**] exposure on spermatogenesis during puberty and adulthood in rats and mice. [**No information**  
49 **was provided about chow or bedding and caging materials. The rat data are reported in Section**  
50 **3.2.4.**] ICR mice were sc injected with bisphenol A in a DMSO and olive oil vehicle on PND 1, 3, 5, 7, 9,  
51 and 11 (PND 0 = day of birth). Bisphenol A doses were 0.0001, 0.001, 0.005, and 0.010 mg/kg bw in mice.

### 3.0 Developmental Toxicity Data

1 Additional animals were treated with 17 $\beta$ -estradiol and estradiol benzoate. Animals were killed weekly at  
2 2–10 weeks of age and some pups were also killed at 24 and 31 days of age. There were 5  
3 animals/dose/time point in bisphenol groups A groups and apparently 3–4 vehicle control mice. Testes  
4 were examined by light and electron microscopy. Males from each experimental group (a total of 12 mice)  
5 were mated with 2 females **[numbers tested in each dose group not reported]**. A total of 12 mouse dams  
6 were allowed to complete pregnancy. **[It does not appear that any statistical analyses were conducted.]**  
7

8 In mature spermatids of 7-week-old mice in the vehicle control group, incidences of deformed acrosome,  
9 deformed nucleus, and abnormal ectoplasmic specialization were below 0.3%. In 7-week-old mice treated  
10 with  $\geq 0.001$  mg/kg bw bisphenol A, the incidence of deformed acrosome was >50–60%, the incidence of  
11 deformed nucleus was >40%, and the incidence of abnormal ectoplasmic specialization was >60–70%.  
12 **[Data were not shown for individual dose levels.]** Similar effects were observed in the groups treated  
13 with 17 $\beta$ -estradiol and estradiol benzoate. No effects were reported at other ages. **[Data were not shown**  
14 **by study authors.]** The blood-testis barrier remained intact based on histologic observations. All tested  
15 males from the bisphenol A group were fertile, and sex ratio, litter sizes, and pup weights were reported to  
16 be normal. **[No results were shown for individual dose levels. Fertility data were presented in Table 4**  
17 **and 5 of the study, but it is not clear which dose level(s) were represented.]** The study authors  
18 concluded that bisphenol A acts as an estrogen and induces transient changes in the male reproductive  
19 system of rodents that resolve in adulthood.  
20

21 **Strengths/Weaknesses:** The strengths include the use of multiple doses of bisphenol A and the use of both  
22 rats and mice, allowing interspecies comparisons. Weaknesses include small sample size, unclear data  
23 analyses, and sc route of administration.  
24

25 **Utility (adequacy) for CERHR Evaluation Process:** This study is inadequate and not useful due to  
26 critically small sample size, route of administration, lack of clarity of design, and inappropriate statistical  
27 procedures.  
28

#### 3.2.9 Sheep

30 **Evans et al. (442)**, supported by the British Council, Irish Health Research Board, and the Royal Society,  
31 examined the effects of bisphenol A exposure on gonadotropin secretion on prepubertal female lambs. **[No**  
32 **information was provided about feed or composition of bedding or caging materials.]** Starting at 3  
33 weeks of age, female Poll Dorset lambs were weighed weekly, and blood samples were collected 2  
34 times/week for measurement of LH and FSH levels. At 4 weeks of age, lambs were randomly assigned to  
35 treatment groups according to body weight. From 4 to 11 weeks of age, 6 lambs/group received biweekly  
36 im injections with the 10:1 corn oil/alcohol vehicle, 3.5 mg/kg bw bisphenol A **[purity not reported]**,  
37 0.175 mg/kg bw diethylstilbestrol **[listed as 0.0175 in the legend for Figure 1 of the study]**, or 3.5 mg/kg  
38 bw octylphenol. Lambs were ovariectomized at nine weeks of age. **[The text of the methods sections**  
39 **reported ovariectomy at the beginning of treatment, but that statement appears to be an error since**  
40 **it is not indicated elsewhere in the paper.]** On the last day of treatment, blood was collected every 15  
41 minutes for 6 hours to assess pulsatile LH secretion. All lambs were then killed. Adrenal glands, kidneys,  
42 and ovaries were weighed. Uteri were examined as discussed in Morrison et al. (443). Data were analyzed  
43 by ANOVA, Dunnett multiple comparison post hoc test, regression analysis, Munro algorithm, and paired  
44 *t*-tests.  
45

46 Compared to the control group, the bisphenol A group did not experience significant changes in body,  
47 kidney, adrenal, or ovarian weights. **[No data were shown for body, kidney, and ovarian weights in the**  
48 **control versus bisphenol A group.]** Uteri from the bisphenol A group were reported to be visually larger,  
49 but no uterine weights were provided. Over the 7-week treatment period, bisphenol A did not significantly  
50 affect blood LH or FSH levels compared to controls. Compared to controls, the bisphenol A group  
51 experienced significant decreases **[% change compared to controls]** in concentration **[48%]**, amplitude

### 3.0 Developmental Toxicity Data

1 [77%], and frequency [66%] of pulsatile LH secretion. Octylphenol did not have any effect on the  
2 endpoints examined. Diethylstilbestrol treatment resulted in decreased blood levels of LH and FSH over the  
3 treatment period, including the period following ovariectomy. Concentration, amplitude, and frequency of  
4 pulsatile LH secretion were also lower in the diethylstilbestrol group, with a greater magnitude of effect  
5 compared to bisphenol A. The study authors concluded that the bisphenol A dose tested can inhibit LH  
6 secretion in lambs.

7  
8 **Strengths/Weaknesses:** The unique animal model and the use of LH pulsatile response are uncommon but  
9 interesting. The high dose level via im injection is a weakness as are small sample sizes (n = 6). The  
10 statistical tests for LH trends did not seem to take into account the repeated nature of the sampling leading  
11 to over stating the significance of trend effects.

12  
13 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate for inclusion but of limited  
14 utility for the evaluation process.

15  
16 **Morrison et al. (443)**, supported by the Wellcome Trust, Dr. Ferranti, and the Irish Health Research Board,  
17 examined the effects of bisphenol A exposure on the lamb uterus. [No information was provided on feed  
18 or composition of bedding or caging materials.] At 4 weeks of age, female Poll Dorsett lambs were  
19 randomly assigned to treatment groups according to body weight. Beginning at 4 weeks of age and  
20 continuing for 7 weeks, 6 lambs/group received biweekly im injections with the 10:1 corn oil:alcohol  
21 vehicle, 3.5 mg/kg bw bisphenol A [purity not reported], 0.175 mg/kg bw diethylstilbestrol, or 3.5 mg/kg  
22 bw octylphenol. Lambs were ovariectomized during the fifth week of exposure. Throughout the study,  
23 blood was collected for measurement of gonadotropin levels and the results of those analyses were reported  
24 in the study by Evans et al. (442). Lambs were killed following 7 weeks of exposure. Uteri and cervixes  
25 were fixed in Bouin solution for histopathological examination, morphometric measurement, and  
26 immunohistochemical detection of ER $\alpha$  and ER $\beta$ . Statistical analyses included ANOVA with Fisher  
27 protected least significant difference.

28  
29 Significant effects observed with bisphenol A treatment [% change compared to controls] were increased  
30 uterine/cervical tract weight [87%], endometrial area [154%], and endometrial/myometrial ratio [65%].  
31 Qualitative histopathological observations in uteri from bisphenol A-treated lambs included endometrial  
32 edema, decreased endometrial gland density compared to controls, and crowding of cells in the uterine  
33 epithelium, which contained substantial amounts of eosinophilic, non-vacuolated cytoplasm. In contrast to  
34 uteri from control lambs, mononuclear cell exocytosis was not a common observation in uteri from the  
35 bisphenol A group. The cervical epithelium was keratinized in the bisphenol A group. Qualitative analyses  
36 revealed that diffuse intracellular staining for ER $\alpha$  and ER $\beta$  in the uterine subepithelium was most  
37 pronounced in the bisphenol A and diethylstilbestrol groups. Similar to animals treated with bisphenol A,  
38 the diethylstilbestrol group had increased uterine weight, keratinized cervical epithelium, changes in uterine  
39 histology, and keratinized cervical epithelium, but there was no change in endometrial/myometrial ratios.  
40 No changes were observed following exposure to octylphenol. The study authors concluded that bisphenol  
41 A exposure altered the uterocervical environment of lambs.

42  
43 **Strengths/Weaknesses:** This is a companion to the study of Evans et al. (442) with similar strengths. The  
44 single high dose level via im injection is a weakness as is the exclusion of data from 2 lambs based on  
45 responses for E/M ratio endpoints, thus reducing the n to 5 and potentially biasing the data. The statistical  
46 analyses do not appropriately account for the number of multiple comparison made which can increase the  
47 probability of detecting an effect by chance.

48  
49 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate for inclusion but of limited  
50 utility in the evaluation process.

51

### 3.0 Developmental Toxicity Data

1 **Savabieasfahani et al. (444)**, supported by the U.S. Public Health Service, NIH, and the University of  
2 Michigan, used Suffolk ewes to investigate the effects of maternal exposure to bisphenol A or methoxychlor  
3 **[not discussed here]** during gestation. Pregnant Suffolk ewes used in this experiment were exposed to a  
4 natural photoperiod in the same pasture and fed a diet of 1.25 kg alfalfa/grass hay. Pregnant ewes (n = 10)  
5 of similar average weight were injected sc on GD 30–90 with 5 mg/kg bw day bisphenol A (99+% purity)  
6 dissolved in cottonseed oil. Control pregnant ewes (n = 16) were administered vehicle injections. Lambs  
7 were born over about a one month interval in early spring. Birth outcome measurements included number  
8 and gender of offspring, weight, height, chest circumference, genital development, and measurement of  
9 blood insulin and insulin-like growth factor-1. Lambs were cross-fostered and group housed on PND 3.  
10 Lactating ewes were fed a diet of corn and alfalfa hay. Lambs had free access to standardized Shur Gain  
11 feed pellets. **[The authors note the presence of phytoestrogens in the feed but did not provide**  
12 **quantification.]** At weaning, female were separated from male offspring, and the females were housed in  
13 open air pens under natural photoperiod with free access to feed pellets, as described above.

14  
15 Maternal blood samples were taken on GD 50, 70, and 90 for measurement of bisphenol A using HPLC.  
16 The number and sex of offspring in each treatment group, weight, height, chest circumference, and genital  
17 development were noted. Blood levels of insulin and insulin-like growth factor 1 were assayed by RIA on  
18 PND 1. In female offspring **[n not indicated]**, blood was drawn biweekly during the first 2 postnatal  
19 months for determination of LH by RIA. Timing of puberty onset was estimated through twice weekly  
20 blood draws for progesterone (n = 11/group). Estrus cycling patterns were determined by frequent  
21 measurement of FSH, LH, and progesterone by RIA in 3 female offspring/group after synchronization with  
22 prostaglandin F2 $\alpha$  at 40 weeks of age. Statistical analyses were performed using ANOVA, repeated  
23 measures ANOVA, or a linear mixed model. A cluster algorithm was used to identify LH pulses, with  
24 Student *t*-test to determine LH nadirs.

25  
26 Blood levels of bisphenol A were significantly higher in exposed pregnant ewes than controls at all  
27 sampling times. The levels reached ( $37.4 \pm 3.3 \mu\text{g/L}$ ) were compared to exposure levels reported in  
28 pregnant women [0.3–18.9  $\mu\text{g/L}$  (104)]. No statistical difference was reported in gestation length, number  
29 of offspring, or sex. There were no significant differences in female lambs in anogenital distance, insulin,  
30 or insulin-like growth factor levels on PND 1. In female offspring, prenatal bisphenol treatment  
31 significantly decreased birth weight **[by ~11%]**, height **[by ~5%]**, and chest circumferences **[by ~7%, all**  
32 **comparisons estimated from a graph]**. In male offspring exposed to bisphenol A, there were no  
33 significant differences from control in birth weight, height, chest circumference, or anogenital distance, but  
34 anoscrotal:anonaivel ratio was significantly increased **[by 21%]**. Bisphenol A treatment significantly  
35 increased levels of circulating LH **[by ~89%, estimated from a graph]** during the first 2 months of life in  
36 female offspring. Onset of puberty was not affected by treatment in bisphenol A-exposed female offspring,  
37 but these females had a significantly longer first breeding season **[by ~2 weeks]** and larger number of  
38 cycles during the first breeding season). Estrous cycle length and progesterone levels were not different  
39 from controls. The bisphenol A group had significantly lower peak and total LH, and the amplitude of LH  
40 pulses was significantly increased, while frequency showed no difference from control group. No  
41 differences in FSH were seen between groups. Progesterone secretion pattern showed no difference  
42 between groups, despite perturbations in LH patterns.

43  
44 The authors concluded that prenatal exposure to bisphenol A impairs growth in female fetuses and is  
45 associated with dampening of the LH surge. Although there was no apparent effect on progesterone  
46 production, the authors suggested that the changes induced by prenatal exposure of females could interfere  
47 with fertility.

48  
49 **Strengths/Weaknesses:** This study appears to have been well conducted with the utilization of multiple  
50 endpoints in sheep. Weaknesses are the use of a single dose level and the relatively small sample size. The

### 3.0 Developmental Toxicity Data

1 single time point for bisphenol A plasma determination at an unknown time relative to sc injection is a  
2 weakness.

3  
4 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate though of limited utility.

#### 5 6 3.2.10 Non-mammalian species

7 While these studies in non-mammalian species can be quite useful for understanding mechanisms and  
8 environmental impacts, the studies are not considered useful for the evaluation process, because of the  
9 uncertain relationship between human biology and that of the model species.

##### 10 11 3.2.10.1 Invertebrates

12 **Hill et al. (445)** supported by the Council on Undergraduate Research and the Association for Biological  
13 Laboratory Education, examined the effects of bisphenol A on the development of 2 freshwater sponge  
14 species. (*Heteromyenia* sp. and *Eunapius fragilis*). Sponge gemmules were incubated in tissue culture wells  
15 containing bisphenol A [**purity not indicated**] at 0, 0.16, 16, 80, or 160 ppm [**mg/L**]. The control group  
16 was incubated in the spring water vehicle. There were 5 replicates/treatment. Nonylphenol and  
17 ethylbenzene were also examined. Growth was measured on days 3, 6, and 9. Because growth patterns  
18 were similar at all 3 evaluation periods, statistical analyses were conducted only for day 6 data. Data were  
19 analyzed by ANOVA and Tukey multiple comparison test. In both species, abnormal development or  
20 malformation of the water vascular system was observed at a bisphenol A dose of 16 ppm and germination  
21 was completely inhibited at 80 and 160 ppm. Significantly reduced growth rates were observed in  
22 *Heteromyenia* sp. at 160 ppm. Similar effects were observed with nonylphenol and ethylbenzene. The study  
23 authors stated that sponges may prove useful for examining endocrine-disrupting compounds.

24  
25 **Strengths/Weaknesses:** This study used a unique model with a focus on the aquatic system.

26  
27 **Utility (Adequacy) for CERHR Evaluation Process:** This study may have utility for environmental  
28 assessment, but is not useful for human risk assessment.

29  
30 **Roepke et al. (446)**, supported by the National Oceanic and Atmospheric Administration, examined the  
31 effects of bisphenol A exposure on development of two species of sea urchin, *Strongylocentrotus*  
32 *purpuratus* and *Lytechinus anamesus*. In dose-response studies, sea urchin embryos were incubated from 1  
33 to 96 hours post-fertilization in media containing bisphenol A [**purity not indicated**] at 0, 250, 500, 750, or  
34 1000 µg/L [**culture ware not discussed**]. Development toxicity was assessed at 96 hours by examining  
35 larvae at the pluteus stage. The larvae were categorized as normal, delayed, abnormal, elongated, or  
36 hatched. Data were obtained in 3 replicates. Results were reported to be similar for the 2 species, and unless  
37 otherwise indicated, data were shown for *S. purpuratus*. In additional studies, sea urchin embryos were  
38 incubated in bisphenol A at 0–500 µg/L with and without addition of tamoxifen or bisphenol A at 0–750  
39 µg/L with and without the addition of ICI 182,780. Data were analyzed by ANOVA followed by Tukey-  
40 Kramer test or Tukey or Student-Newman-Keuls tests for pair-wise multiple comparison. An EC<sub>50</sub> of 226.6  
41 µg/L (lower limit: 121.6, upper limit: 323.5 µg/L) was estimated for developmental toxicity associated with  
42 bisphenol A exposure. Based on EC<sub>50</sub> values, 17β-estradiol was ~15 times more potent than bisphenol A.  
43 Tamoxifen inhibited developmental toxicity, and ICI 182,780 enhanced the developmental toxicity induced  
44 by bisphenol A; similar results were obtained for 17β-estradiol. The study authors concluded that bisphenol  
45 A induced developmental toxicity in sea urchins through a tamoxifen-sensitive mechanism at levels  
46 exceeding environmentally relevant concentrations.

47  
48 **Strengths/Weaknesses:** The use of 2 species and multiple concentrations are strengths.

49  
50 **Utility (Adequacy) for CERHR Evaluation Process:** This study may have utility for environmental  
51 assessment, but is not useful for human risk assessment.

### 3.0 Developmental Toxicity Data

1 **Andersen et al. (447)**, supported by the Danish Strategic Environmental Research Program, evaluated the  
2 effects of bisphenol A on female sexual maturation in the zooplanktonic crustacean *Acartia tonsa*. Eggs  
3 were grown in the presence of the algal food source for the organism after exposure of the algae to  
4 bisphenol A (>99% purity) for 3 hours to promote sorption by the algae of the test chemical [**culture ware  
5 not discussed**]. The treated algae were added to *Acartia tonsa* eggs to give nominal bisphenol A  
6 concentrations of 0.2, 2, and 20 µg/L. [**Actual concentrations were not reported. An untreated or  
7 vehicle-treated control appears to have been used.**] 17β-Estradiol 23 µg/L was used as a positive  
8 control, and 2,3-dichlorophenol 13.6 µg/L was used as a negative control. On the eighth day of incubation,  
9 10–25 juvenile *Acartia tonsa*/group were transformed to an egg-collection apparatus, in which exposure to  
10 treated algae continued. Eggs were collected daily and counted until day 12, at which time a stable adult  
11 level of egg production was established. Egg production by group was compared using Student *t*-test. [**A  
12 repeated-measures test appears not to have been used.**] A significant increase in egg production was  
13 shown on day 10 in animals treated with bisphenol A 20 µg/L and 17β-estradiol 23 µg/L compared to  
14 control. The authors concluded that bisphenol A accelerated female reproductive maturation in *Acartia  
15 tonsa* and that the effect appeared to be estrogenic.

16  
17 **Strengths/Weaknesses:** Strengths are the use of multiple exposure levels, the inventive method of feeding  
18 bisphenol A to the test organisms, and the use of 17β-estradiol as a positive control.

19  
20 **Utility (Adequacy) for CERHR Evaluation Process:** This study may have utility for environmental  
21 assessment, but is not useful for human risk assessment.

22  
23 **Watts et al. (448)**, supported by the European Union, examined development and reproduction in 2  
24 generations of non-biting midges (*Chironomus riparius*) exposed to bisphenol A. The study began with  
25 incubation of 4 egg ropes/group in media containing vehicle, bisphenol A, or ethinyl estradiol [**apparently  
26 at the same concentrations described below**]. Twenty 1<sup>st</sup>-instar larvae from the appropriate media were  
27 added to each exposure glass jar containing dechlorinated water and sediment spiked with bisphenol A  
28 [**purity not indicated**] at concentrations of 0 (ethanol vehicle control and dechlorinated tap water control),  
29 <0.010, 0.078, 0.55, 77, 750, or 10,400 µg/L. Four replicate jars were prepared for each dose level.  
30 Concentrations in sediment were verified. Numbers and sexes of adults emerging from each replicate jar  
31 were determined. Egg ropes produced by the first generation were counted and placed in media containing  
32 test solutions or vehicle controls. Four egg ropes/group were selected and used to reseed the sediments with  
33 the second generation of larvae. Adults emerging from the second generation were counted. Statistical  
34 significance was determined by ANOVA. In the first generation, adult emergence was delayed in females  
35 from the <0.010, 0.55, and 77 µg/L bisphenol A groups but was not affected in males. Males were reported  
36 to emerge significantly earlier than females. In the second generation, emergence of males and female  
37 adults was significantly delayed at ≥0.078 µg/L bisphenol A. At concentrations of 0.010–750 µg/L, there  
38 were no significant differences in the percentage of adults emerging in either generation. No second-  
39 generation adults emerged in the group exposed to 10,400 µg/L. There were no effects on sex ratio.  
40 Exposure to bisphenol A did not significantly affect the number of eggs produced by the first generation. In  
41 contrast to bisphenol A, exposure to ethinyl estradiol accelerated adult emergence. The study authors  
42 concluded that the endpoints evaluated indicated general sediment toxicity but were not useful for detecting  
43 estrogenic effects.

44  
45 **Strengths/Weaknesses:** The wide range of exposure levels and the use of ethinyl estradiol as a positive  
46 control are strengths.

47  
48 **Utility (Adequacy) for CERHR Evaluation Process:** This study may have utility for environmental  
49 assessment, but is not useful for human risk assessment.

50

### 3.0 Developmental Toxicity Data

1 **Watts et al. (449)**, supported by the European Union, examined the effects of bisphenol A exposure on  
2 moulting and mouthpart deformities in non-biting midge (*Chironomus riparius*) larvae. Four egg-  
3 ropes/group were incubated in glass jars in media containing bisphenol A [**purity not indicated**] at 0  
4 (ethanol vehicle or dechlorinated water group), 0.010, 0.1, 1, 10, 100, or 1000 µg/L. Concentrations of  
5 bisphenol A were verified in the 1000 µg/L group. Upon hatching, exposures were continued in 10  
6 larvae/group. Endpoints examined included survival, time of moulting to successive instars, wet weight 2  
7 days after moulting to fourth instar, and mouthpart morphology in fourth-instar head capsules. Statistical  
8 analyses included ANOVA, Tukey-Kramer multiple comparison test, and Kruskal-Wallis test. [**Effects**  
9 **were similar in ethanol and water controls.**]. Moulting was delayed and larval weights were significantly  
10 decreased in the 1000 µg/L bisphenol A group. Deformities of the mentum were significantly increased in  
11 the range of 0.010–1 µg/L bisphenol A. The effects of ethinyl estradiol were also examined, and the study  
12 authors noted similar patterns of malformations, with greater incidence following exposure to ethinyl  
13 estradiol than bisphenol A. The study authors concluded that exposure to bisphenol A delayed moulting  
14 and increased mouth part deformities at concentrations that were at opposite ends of the exposure range.

15  
16 **Strengths/Weaknesses:** This study is similar in its strengths to that of Watts et al. (448).

17  
18 **Utility (Adequacy) for CERHR Evaluation Process:** This study may have utility for environmental  
19 assessment, but is not useful for human risk assessment.

#### 20 21 3.2.10.2 Frog

22 **Iwamuro et al. (450)**, support not indicated, conducted a series of studies to examine the effects of  
23 bisphenol A exposure on development of the frog *Xenopus laevis*. In a study to assess survival and  
24 morphological abnormalities, 60–100 stage 7 embryos/group were exposed to bisphenol A [**purity not**  
25 **indicated**] at 0 (ethanol vehicle), 10, 20, 25, 30, 50, or 100 µM [**0, 2.3, 4.6, 5.7, 6.8, 11, or 23 mg/L;**  
26 **culture ware not discussed**]. Siblings were randomly distributed among different treatment groups.  
27 Survival was assessed at 48, 96, and 120 hours. At least 3 embryos/group were examined for malformations  
28 at 5–7 days following fertilization. Data were analyzed by chi-squared test. Survival of embryos was  
29 significantly reduced following exposure to  $\geq 25$  µM [**5.7 mg/L**] bisphenol A for 96 or 120 hours. Complete  
30 mortality was observed at concentrations  $\geq 50$  µM [**11 mg/L**]. The study authors calculated a median LD<sub>50</sub>  
31 for survival of 21 µM [**4.8 mg/L**]. The malformation rate was reported for the 10 and 25 µM [**2.3 and 4.6**  
32 **mg/L**] group, and significant increases in malformations occurred in the 25 µM [**4.6 mg/L**] group. The  
33 types of malformations were reported as scoliosis, swollen head, and shortened distance between eyes. The  
34 effects of 17β-estradiol were also examined. An increase in malformations was observed with exposure to  
35 10 µM 17β-estradiol, but there was no effect on survival.

36  
37 In a second study, metamorphosis was observed in 10–12 tadpoles (stage 52) placed in solutions containing  
38 10 or 25 µM [**2.3 or 5.7 mg/L**] bisphenol A [**purity not indicated**] with and without the addition of 0.1  
39 µM thyroxine for 21 days. Expression of thyroid hormone receptor-β gene was measured by RT-PCR in 3  
40 regions (head, trunk, and tail) of tadpoles that were exposed to 10 or 100 µM [**2.3 or 23 mg/L**] bisphenol A  
41 with and without the addition of 0.1 µM triiodothyronine or thyroxine. Negative controls were exposed to  
42 ethanol/DMSO vehicle. Metamorphosis data were analyzed by Duncan new multiple range test. Bisphenol  
43 A significantly inhibited both spontaneous and thyroxine-induced metamorphosis. All concentrations of  
44 bisphenol A reduced expression of thyroid hormone receptor-β hormone and inhibited increases in  
45 thyroxine- and triiodothyronine-induced expression.

46  
47 In a third study, tails were removed from 4 tadpoles/group and cultured for 4 days in media containing 10  
48 or 100 µM [**2.3 or 23 mg/L**] bisphenol A with and without the addition of 0.1 µM triiodothyronine.  
49 Negative controls were exposed to ethanol/DMSO vehicles. Data were analyzed by Duncan new multiple  
50 range test. Growth of the tails was measured over a 4-day period. Neither bisphenol A dose significantly  
51 affected tail growth. Both bisphenol A doses blocked tail shortening that was induced by triiodothyronine.

### 3.0 Developmental Toxicity Data

1 The study authors concluded that high doses of bisphenol A adversely affect development of *Xenopus*  
2 *laevis* embryos and larvae.

3  
4 **Strengths/Weakness:** The wide range of exposure levels is a strength.

5  
6 **Utility (Adequacy) for CERHR Evaluation Process:** This study may have utility for environmental  
7 assessment, but is not useful for human risk assessment.

8  
9 **Oka et al. (451)**, support not indicated, examined the effects of bisphenol A exposure on development of  
10 the frog *Xenopus laevis*. Embryos were exposed to the ethanol vehicle or 10–100  $\mu\text{M}$  [**2.3–23 mg/L**]  
11 bisphenol A from developmental stage 6 until the early tadpole stage (late stage 10) [**purity not indicated,**  
12 **and culture ware not discussed**]. Embryos were harvested at stages 19, 23, 33/34, and 40 and prepared for  
13 histological examination to determine the presence of apoptotic cells. Apoptosis was also assessed using a  
14 TUNEL staining method. Ten embryos were killed at the tail bud stage (stage 35/36, 37/38, and 40), and  
15 genomic DNA was isolated and examined by electrophoreses to determine if 180 base pair ladders  
16 indicative of apoptosis were present. [**No information was provided on the number of individual doses**  
17 **examined or the number of embryos exposed/dose. No quantitative data were presented by authors,**  
18 **and it does not appear that data were statistically analyzed.**] Embryos exposed to 40–100  $\mu\text{M}$  [**9.1–23**  
19 **mg/L**] bisphenol A died during the gastrula stage. Developmental abnormalities were observed in embryos  
20 exposed to 20  $\mu\text{M}$  [**4.6 mg/L**] bisphenol A. The abnormalities included open neural tubes at stage 19,  
21 morphological defects at stages 23 and 33/34, and crooked vertebrate, swollen abdomen, and malformed  
22 head at stage 40. Malformations persisted following stage 40, and death occurred during the tadpole stage.  
23 In stage 33/34 and 40 embryos of the 20  $\mu\text{M}$  [**4.6 mg/L**] group, apoptotic cells were observed in the  
24 prosencephalon, mesencephalon, rhombencephalon, and spinal cord. Apoptosis was confirmed using the  
25 TUNEL staining method. Using the DNA ladder method, it was found that apoptosis also occurred at  
26 stages 35/36, 37/38, and 40. The authors briefly stated that they tested stage 10, 19, or 23 embryos and  
27 found normal development following bisphenol A exposure. [**No additional details were provided.**] The  
28 effects of 17 $\beta$ -estradiol were also examined. Malformations were observed in embryos exposed to 10  $\mu\text{M}$   
29 17 $\beta$ -estradiol, but apoptotic cells were not observed in the nervous system. A very brief description was  
30 provided of a study in which embryos were simultaneously exposed to 20  $\mu\text{M}$  [**4.6 mg/L**] bisphenol A and  
31 1–10  $\mu\text{M}$  17 $\beta$ -estradiol. Co-exposure with 17 $\beta$ -estradiol did not inhibit bisphenol A-induced apoptosis. The  
32 study authors concluded that bisphenol A induced malformations and apoptosis in *Xenopus laevis* at  
33 concentrations exceeding environmental levels and that the effects did not appear to occur through an  
34 estrogenic mechanism.

35  
36 **Strengths/Weaknesses:** The use of 17 $\beta$ -estradiol exposure to suggest a non-estrogenic mechanism of  
37 bisphenol A toxicity is a strength. The omission of some important details and the high concentrations are  
38 weaknesses.

39  
40 **Utility (Adequacy) for CERHR Evaluation Method:** This study may have utility for environmental  
41 assessment, but is not useful for human risk assessment.

42  
43 **Sone et al. (452)**, supported by the Japanese Ministry of Environment and Ministry of Education, Culture,  
44 Sports, Science, and Technology, examined the effects of bisphenol A exposure on the development of  
45 *Xenopus laevis* embryos. Three different sets of experiments were conducted. Data were analyzed by  
46 ANOVA followed by Fisher protected least significant difference test. From 3 to 96 hours following  
47 fertilization, embryos were exposed to bisphenol A [**purity not indicated**] at 1, 2.5, 5, 10, 15, 20, 25, or 30  
48  $\mu\text{M}$  (0.3, 0.6, 1.1, 2.3, 3.4, 4.6, 5.7, or 6.8 mg/L). Each exposure was replicated 3 times. Negative control  
49 groups consisted of the ethanol vehicle, medium alone, or dilution medium. Rates of normal embryo  
50 development were equivalent in the 3 different negative control groups. In groups exposed to  $\geq 20 \mu\text{M}$

### 3.0 Developmental Toxicity Data

1 bisphenol A, there was a significant decrease in normal embryos and a non-significant increase in mortality  
2 rate. Teratogenicity was characterized by short body length, microcephaly, flexure, edema, and abnormal  
3 gut coiling. Increases in embryo abnormalities were also observed following exposure to  $\geq 10 \mu\text{M}$  17 $\beta$ -  
4 estradiol or nonylphenol.

5  
6 To determine sensitive stages, embryos were exposed to control media or 20  $\mu\text{M}$  [4.6 mg/L] bisphenol A  
7 for 45–48-hour periods ranging from 3 to 48 hours post fertilization, 12–60 hours post-fertilization, 24–72  
8 hours post-fertilization, 36–84 hours post-fertilization, or 48–96 hours post-fertilization. Body length, gross  
9 malformations, and distance between eyes were measured at 96 hours following exposure. [The methods  
10 section indicated that 59–71 embryos were examined in the bisphenol A group for each time period  
11 of exposure. However, a figure in the study reported the sample size as 3/time period.] During the  
12 period of 3–48 hours following fertilization, statistically significant effects in the bisphenol A group  
13 included decreased body length and increased incidences of microcephaly, flexure, edema, and abnormal  
14 gut coiling. No increases in abnormal effects were observed following exposure at later time periods.  
15 Abnormalities were observed following exposure to 17 $\beta$ -estradiol or nonylphenol at early or late stages.

16  
17 In the third part of the study, embryos were exposed to 20  $\mu\text{M}$  [4.6 mg/L] bisphenol A from 3 to 96 hours  
18 following fertilization. RNA was isolated from whole embryos and subjected to analysis by cDNA  
19 microarray. Results obtained in microarray analyses were confirmed by PCR analysis. The sample size was  
20 reported as 2. The microarray analysis revealed 179 up-regulated and 103 down-regulated genes following  
21 exposure of embryos to bisphenol A. The study authors identified 27 genes in which expression was  
22 changed following exposure to bisphenol A, nonylphenol, or 17 $\beta$ -estradiol. The identified genes included:  
23 *KNP-1a*, *CmaB*, *XIRG*,  $\alpha$ -skeletal tropomyosin, apelin, cyclin G1, *Ube213*, *HGF*, toponin C2, ribosomal  
24 protein L9, and *Rattus norvegicus* similar to *CG10042-PA*. The other genes were not identified. The study  
25 authors concluded that these findings might provide clues to deciphering mechanisms of teratogenic effects  
26 associated with bisphenol A and the other compounds examined in this study.

27  
28 **Strengths/Weaknesses:** The inclusion of 17 $\beta$ -estradiol as a comparator was a strength and the high  
29 bisphenol A concentration is a weakness.

30  
31 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is not useful for the evaluation process.

32  
33 **Pickford et al. (453)**, supported by the Bisphenol A Global Industry group, the Society of the Plastics  
34 Industry, the Bisphenol A Sector Group of the European Chemical Industry Council, and the Japan  
35 Chemical Industry Association, examined the effects of bisphenol A exposure on development of frog  
36 gonads. Beginning at stage 43/45 (~2 days post-hatching, 4 days post-fertilization, exposure day 0) and  
37 continuing through stage 66, *Xenopus laevis* larvae were exposed to bisphenol A [purity not indicated] at  
38 nominal concentrations of 0 (water control), 1.0, 2.3, 10, 23, 100, or 500  $\mu\text{g/L}$  in a flow-through test system  
39 [culture ware not discussed]. Actual concentrations were verified as 0.83, 2.1, 9.5, 23.8, 100, and 497  
40  $\mu\text{g/L}$ . A positive control group was exposed to 2.7  $\mu\text{g/L}$  17 $\beta$ -estradiol. There were 4 replicate test  
41 vessels/dose, with each containing 40 larvae (i.e., 160 larvae/test condition). Larvae were observed daily  
42 for mortality, behavior, and appearance. Growth and development were assessed on all larvae of a replicate  
43 tank on exposure days 32 and 62 (36 and 68 [66?] days post fertilization). Froglets were killed and  
44 observed at completion of metamorphosis (stage 66). Total length was measured, sex was determined, and  
45 testes and ovaries were assessed for abnormalities such as asymmetry, complete absence, presence of  
46 melanocytes, irregular shape, segmentation or fragmentation, vacuoles, and ambiguous sexual morphology.  
47 Data were analyzed by Fisher exact test, ANOVA, Wilcoxon rank sum test, *G* test, and chi-squared test.  
48 Following exposure to bisphenol A, there were no significant differences in survival, distribution of  
49 developmental stages on day 32 or 62, time to completion of metamorphosis (stage 66), or length of stage  
50 66 froglets. Bisphenol A exposure did not affect sex ratio or abnormalities in testis or ovary [data were not  
51 shown by authors for testis and ovary effects]. In contrast, exposure to 17 $\beta$ -estradiol resulted in an

### 3.0 Developmental Toxicity Data

1 increase in ratio of females to males and testicular and ovarian abnormalities. The study authors identified a  
2 no observed effect concentration of 500 µg/L for bisphenol A.

3  
4 **Strengths/Weaknesses:** The use of a wide range of exposure levels is a strength, but the incomplete data  
5 presentation with missing organ weight data and the lack of histological evaluations are weaknesses.

6  
7 **Utility (Adequacy) for CERHR Evaluation Process:** This study may have utility for environmental  
8 assessment, but is not useful for human risk assessment.

9  
10 **Levy et al. (454)**, supported by the Ministry of Environment and Traffic of Baden-Württemberg, evaluated  
11 the effect of bisphenol A on gonad development in *Xenopus laevis* tadpoles. Tadpoles (n = 40/group) were  
12 exposed beginning at stages 42/43 to ethanol vehicle or to bisphenol A (>99% purity) or 17β-estradiol, both  
13 at concentrations of 10<sup>-8</sup> or 10<sup>-7</sup> M [**bisphenol A concentrations 2.3 and 23 µg/L. Actual concentrations**  
14 **were 90–105% of target concentrations after addition of bisphenol A to the media but decreased to**  
15 **low levels by the end of the 48-hour period between media changes. Culture ware was not discussed.]**  
16 After completion of metamorphosis, froglets were killed for examination of gonads. Tadpoles not  
17 completing metamorphosis were killed after 120 days of chemical exposure for examination of gonads. In a  
18 second experiment, bisphenol A concentrations were 10<sup>-8</sup>, 10<sup>-7</sup>, or 10<sup>-6</sup> M [**2.3, 23, or 228 µg/L**] and the  
19 17β-estradiol positive control used a concentration of 10<sup>-7</sup> M. In a third experiment, 50 tadpoles/group were  
20 treated for 2 weeks with ethanol vehicle, bisphenol A 10<sup>-7</sup> M [**23 µg/L**], or 10<sup>-7</sup> M 17β-estradiol after  
21 which whole-body homogenates were used for extraction of RNA and determination of *ER* by RT-PCR.  
22 Statistical analyses were performed with Kruskal-Wallis *H* test followed by Mann-Whitney *U* test. The  
23 gonadal sex of control animals was 56% male and 44% female. 17β-Estradiol treatment increased the  
24 female ratio to 81% at 10<sup>-7</sup> M and 84% at 10<sup>-8</sup> M. Bisphenol A treatment resulted in a significant increase  
25 in females (69%) at 10<sup>-7</sup> M [**23 µg/L**]. At 10<sup>-8</sup> M bisphenol A, there were 65% females, which did not  
26 reach statistical significance. In the second experiment, a significant increase in females was seen after  
27 treatment with 10<sup>-7</sup> M [**23 µg/L**] (70%, compared to 48% in controls and 96% with 17β-estradiol  
28 treatment). There was no significant effect of bisphenol A at 10<sup>-8</sup> M [**2.3 µg/L**] (51% female) or 10<sup>-6</sup> M  
29 [**228 µg/L**] (53% female). Bisphenol A and 17β-estradiol both resulted in increased *ER* mRNA. The  
30 authors concluded that bisphenol A affects the sexual development of *Xenopus laevis*, probably through an  
31 estrogenic mechanism.

32  
33 **Strengths/Weaknesses:** The measurement of bisphenol A in the media is a strength, but its lack of stability  
34 is a weakness.

35  
36 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not useful for the evaluation process.

37  
38 **Yang et al. (455)**, supported by the Chinese Ministry of Science and Technology, examined the effects of  
39 bisphenol A exposure in black-spotted pond frog tadpoles. Thirty tadpoles/tank were exposed in duplicate  
40 to bisphenol A (≥95% purity) at concentrations of 0, 0 (+DMSO vehicle), 2, 20, or 200 µg/L [**ppb**] for up  
41 to 60 days [**culture ware not discussed**]. Tadpoles were also exposed to mixtures containing bisphenol A  
42 + nonylphenol at 2 + 2, 20 + 20, or 200 + 200 µg/L. Additional tadpoles were exposed to mixtures  
43 containing the same bisphenol A/nonylphenol mixtures in addition to *p,p'*-DDE 2 + 2 + 0.5, 20 + 20 + 5, or  
44 200 + 200 + 50 µg/L. Five tadpoles/tank were pooled at 15, 30, 45, and 60 days. The tadpoles were  
45 homogenized for measurement of testosterone and thyroxine levels by radioimmunoassay. Alkaline-labile  
46 phosphate was measured as a biomarker for vitellogenin. Data were analyzed by ANOVA.

47  
48 Malformations of tail flexure were observed in 10% of tadpoles exposed to 200 µg/L bisphenol for 45 days,  
49 and similar rates of malformation (13.3%) were observed in the mixtures containing 200 µg/L bisphenol A.  
50 A “decrease” (not statistically significant) in thyroxine levels was observed following 60 days of exposure

### 3.0 Developmental Toxicity Data

1 to all bisphenol A doses ( $\geq 2$   $\mu\text{g/L}$ ). “Increases” (not statistically significant) in testosterone levels were  
2 reported with all bisphenol A doses at 30 days of exposure. *p,p'*-DDE at  $\geq 5$   $\mu\text{g/L}$  inhibited increases in  
3 testosterone level observed with mixtures of bisphenol A and nonylphenol [**not statistically analyzed**].  
4 “Increases” (not statistically significant) in alkaline-labile phosphate levels were reported following 30 or  
5 more days of exposure to all bisphenol A doses. In animals exposed to bisphenol A and nonylphenol in  
6 combination compared to either compound alone, alkaline-labile phosphate levels were increased at 15  
7 days of exposure but decreased at 60 days of exposure [**not statistically analyzed**]. *p,p'*-DDE inhibited the  
8 increase in alkaline-labile phosphate levels induced by the bisphenol A + nonylphenol mixture on day 15 of  
9 exposure [**not statistically analyzed**].

10  
11 **Strengths/Weaknesses:** The lack of attention to statistical analysis is a weakness and makes the authors’  
12 conclusions unreliable.

13  
14 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not useful in the evaluation process.

15  
16 **Imaoka et al. (456)**, supported by the Japanese Ministry of Education, Science, Culture, Sports, and  
17 Technology, evaluated the effects of bisphenol A on development of the African clawed frog, *Xenopus*  
18 *laevis*. Embryos were cultured with bisphenol A from stage 10.5, formation of the neural plate, to stage 35  
19 at a bisphenol A (in DMSO) concentration of 25, 50, or 100  $\mu\text{M}$  [**5.8, 11, or 23 mg/L**]. Tadpoles were  
20 morphologically evaluated at stages 28–35. Total RNA was extracted and reversed transcribed and RT-  
21 PCR used to quantify the expression of specific genes. Expression levels relative to  $\beta$ -actin or histone H4  
22 were compared with Student *t*-test. Abnormalities in the head and eye region were described with a “minor  
23 effect” at 25  $\mu\text{M}$  and a “major effect” at 50  $\mu\text{M}$  bisphenol A. [**Data were not shown.**] There were no  
24 treatment-related effects on expression of *sox-2*, *nrp-1*, *myoD*, *sox17 $\alpha$* , or *notch*. Relative expression levels  
25 of *pax-6* declined in a concentration-related manner to about 56% of control at the high concentration  
26 [**estimated from a graph**]. Relative expression levels of *esr-1* decreased in a concentration-dependent  
27 manner to about 22% of control at the high concentration [**estimated from a graph**]. Microinjection into  
28 blastomeres of plasmids containing NICD (the intracellular domain of *notch*), but not of X-delta-1 (a *notch*  
29 ligand) corrected the decreased expression of *esr-1*. The authors concluded that bisphenol A decreased *esr-*  
30 *1* expression by disrupting *notch* signaling.

31  
32 **Strengths/Weaknesses:** This is an interesting study on the molecular alterations induced in frog embryos  
33 exposed to BPA. The study demonstrated alterations in several key developmental genes and malformed  
34 development at high concentrations. The high concentrations are, however, weaknesses and the effects of  
35 uncertain concern to human health because humans would not be exposed in this manner.

36  
37 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is not useful in the evaluation process.

#### 3.2.10.3 Fish

38  
39  
40 **Kishida et al. (457)**, supported by the National Science Foundation and USEPA, included bisphenol A in a  
41 study to test the utility of changes in CYP450 aromatase mRNA expression as a marker of xenoestrogen  
42 effects in the CNS of zebrafish (*Danio rerio*). Fish embryos were incubated in solutions containing  
43 bisphenol A [**purity not indicated**] at 0 (DMSO vehicle), 0.01, 0.1, or 10  $\mu\text{M}$  [**0, 2.3, 23, or 228  $\mu\text{g/L}$** ]  
44 from 2 to 48 hours post-fertilization [**culture ware not discussed**]. Expression of the CYP450 aromatase  
45 gene was determined in 50 embryos/treatment group using an RT-PCR/Southern blot technique. [**There**  
46 **was no mention of statistical analyses of data.**] The Southern blot analysis revealed a  $\sim 3$ -fold increase in  
47 the band intensity of CYP450 aromatase at the high concentration (10  $\mu\text{M}$ ) of bisphenol A. The potency of  
48 bisphenol A was determined to be lower than those of 17 $\beta$ -estradiol and diethylstilbestrol, which induced  
49  $\sim 3$ –4-fold increases in band intensity at concentrations up to 3 orders of magnitude lower than bisphenol A.  
50 In additional experiments with exposure to bisphenol at 2–48 hours post-fertilization, embryo mortality was  
51 increased by exposure to 10 and 20  $\mu\text{M}$  [**228 and 457  $\mu\text{g/L}$** ] bisphenol A and malformations (curved tails)

### 3.0 Developmental Toxicity Data

1 were increased by exposure to 20  $\mu$ M. The effects were similar to those observed with 17 $\beta$ -estradiol, but  
2 bisphenol A was less potent. **[Very few protocol details were provided, and no data were shown by**  
3 **study authors for mortality and malformation endpoints.]** The study authors concluded that bisphenol  
4 A could act as a developmental neurotoxicant by upregulating CYP450 aromatase expression but that  
5 further studies were needed to determine if there are changes in neural estrogen biosynthesis or CNS  
6 development.

7  
8 **Strengths/Weaknesses:** A weakness of this paper for the current evaluation is the lack of morphometric  
9 data. The significance of the observed change in aromatase is not clear.

10  
11 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not useful in the evaluation process.

12  
13 **Segner et al. (176)**, supported by the European Commission, examined estrogenicity responses and in vivo  
14 life cycle effects in zebrafish exposed to bisphenol A. Estrogenicity studies are discussed in Section 2. One  
15 hundred fertilized eggs/vessel were exposed to bisphenol A (98% purity) at 0, 94, 188, 375, 750, or 1500  
16  $\mu$ g/L under semistatic conditions **[culture ware not discussed]**. Exposures were continued until fish  
17 became sexually mature. The numbers of fish/vessel were adjusted to 50 following 42 days of exposure and  
18 30 following 75–78 days of exposure. Two replicates were examined. Bisphenol A concentrations were  
19 confirmed by GC/MS. Endpoints evaluated included survival, behavior, growth, time to first spawning, egg  
20 production, and fertilization success (percent fertilized eggs/vessel/day). Statistical analyses included  
21 ANOVA and William test. EC<sub>50</sub> values were calculated by probit analysis and analyzed by Kruskal-Wallis  
22 and Mann-Whitney *U* tests. 17 $\beta$ -Estradiol, ethinyl estradiol, and 4-tert-octylphenol were also examined  
23 using similar protocols. The authors only discussed results for reproductive success because they stated that  
24 it was the most consistent and reproducible effect following exposure of the fish to estrogenic substances.  
25 An EC<sub>50</sub> value of 6140 nM **[1.4 mg/L]** bisphenol A was obtained for fertilization success, and the study  
26 authors stated that the value exceeded concentrations typically found in the environment. Bisphenol A had  
27 a relative potency of 0.0000006 compared to 17 $\beta$ -estradiol and was 45 times less potent than 4-tert-octyl-  
28 phenol. The study authors concluded that the in vivo potency of the compounds was overestimated by in  
29 vitro estrogenicity assays (described in Section 2).

30  
31 **Strengths/Weaknesses:** This study was well-performed.

32  
33 **Utility (Adequacy) for CERHR Evaluation Process:** This study is useful in showing a lack of effect on  
34 fertilization at environmentally relevant concentrations of bisphenol A, but not useful to the evaluation  
35 process.

36  
37 **Metcalfe et al. (197)**, supported by the Environmental Science and Technology Alliance Canada, the  
38 Natural Sciences and Engineering Research Council of Canada, and Health Canada, in glass jars, exposed  
39 medaka (*Oryzias latipes*) from 1 day after hatching until 85–110 days after hatching to bisphenol A **[purity**  
40 **not indicated]** at 0, 10, 50, 100, or 200  $\mu$ g/L (n = 60 fish/treatment). Over the 48 hours between media  
41 change, actual concentrations were a mean 59.6% of nominal concentrations. Fish were killed and  
42 embedded in paraffin for section. Gonads were evaluated to determine the sex of the fish and whether testes  
43 contained ova, an intersex condition. Length and weight of the animals and sex ratio were not altered by  
44 treatment **[statistical methods not reported]**. There were 2 instances of intersex gonads in males exposed  
45 to bisphenol A 10  $\mu$ g/L and no instances at higher concentrations. Histologic changes in testes including a  
46 reduction in germ cells were noted at 50  $\mu$ g/L and higher. At 200  $\mu$ g/L, oogenesis in females was more  
47 advanced than in controls.

48  
49 **Strengths/Weaknesses:** Strengths of this study are the step-sectioning of gonads and the use of several  
50 positive control estrogens, which worked as expected.

51

### 3.0 Developmental Toxicity Data

1 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not useful to the evaluation process.

2  
3 **Yokota et al. (458)**, supported by the Japanese Environment Agency, exposed medaka (*Oryzias latipes*) to  
4 bisphenol A (>99% purity) at 0, 3.2, 16, 80, 400, or 2000 µg/L from fertilization until 60 days after  
5 hatching (n = 60/treatment) [**culture ware not discussed**]. Actual bisphenol A concentrations were  
6 generally within 3% of nominal concentrations prior to hatching. After hatching, the lower 2 concentrations  
7 were ~70–80% of nominal and the higher concentrations were ~90% of nominal. Fish were assessed for  
8 survival, time to hatching, and growth. Sixty days after hatching, 19 or 20 fish/treatment were killed and  
9 sectioned for examination of the gonads using hematoxylin and eosin staining of fixed specimens.  
10 Statistical analysis was performed using ANOVA and nonlinear regression. Hatchability was >90% in all  
11 treatment groups. Time to hatch and mortality were not affected by treatment, although there was a non-  
12 concentration dependent delay in hatching at 13 µg/L. Body length and weight 60 days after hatching were  
13 negatively correlated with bisphenol A concentration, and length and weight at 2000 µg/L were  
14 significantly lower than control values on pair-wise comparison. Based on external appearance and gonad  
15 examination, there were more females than males at 400 µg/L and there were no males at 2000 µg/L.  
16 Control sex ratio was 2:1 (male:female). There were 6 fish with intersex gonads among the 19 examined in  
17 the 2000 µg/L group. The authors concluded that bisphenol A adversely affects the early life stage of  
18 medaka with alteration of sexual differentiation.

19  
20 **Strengths/Weaknesses:** This study was well performed.

21  
22 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not useful to the evaluation process.

23  
24 **Pastva et al. (459)** support not indicated, examined the effects of bisphenol A exposure on development of  
25 medaka (*Oryzias latipes*). In a study examining abnormalities in embryos, 5 eggs were placed in individual  
26 glass vials containing bisphenol A [**purity not indicated**] at 0, 20, or 200 µg/L. There were 5 glass  
27 vials/exposure concentration, for a total of 25 embryos/group. The exposure period began 5 hours  
28 following fertilization and was continued for 9 days. Embryos were examined for malformations daily by  
29 observing them through the clear protective membrane of the egg. The severity of malformations was  
30 scored and severity indices were determined. In a second study examining mortality, newly hatched larvae  
31 were exposed for 96 hours to a method control solution, ethanol vehicle control solution, or 200 µg/L  
32 bisphenol A. Ten larvae were added to each jar, and there were 3 replicates/test solution (i.e., 30 larvae  
33 /concentration). Data were analyzed by *t*-test. The malformation severity index was significantly increased  
34 at 5–8 days following fertilization in embryos exposed to 200 µg/L bisphenol A, but the severity index did  
35 differ significantly from the control value on day 9. Abnormalities consisted of pericardial edema,  
36 hemorrhage, and hemostasis. Larval mortality was not affected by exposure to 200 µg/L bisphenol A. The  
37 study authors concluded that exposure to environmentally relevant concentrations of bisphenol A resulted  
38 in embryonic deformities in medaka, but that the embryos were able to repair the abnormalities prior to  
39 hatching.

40  
41 **Strengths/Weaknesses:** This study using medaka is similar in design to the FETAX assay, which uses  
42 *Xenopus*. These types of assays have not been demonstrated to have relevance for human risk assessment.

43  
44 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is not useful in the evaluation process.

45  
46 **Lee et al. (460)**, supported by Jeonnam Regional Environment Technology Development Center, exposed  
47 51-day-old Korean rockfish (*Sebastes schlegeli*) fry to bisphenol A in feed at 0, 0.05, 0.5, 5, 50, and 100  
48 mg/kg diet for 29 days [**purity of bisphenol A, stability in feed, and culture ware not indicated**]. At the  
49 end of the experiment, gonads were removed and sex determined by light microscopy of stained sections.  
50 There was no effect of bisphenol A on sex ratio compared to controls. [**The data presentation and**  
51 **statistical analysis are unclear: the number of female fish and number of male fish in each dose**

### 3.0 Developmental Toxicity Data

1 **group are presented as averages with an unspecified error and analyzed by Student *t*-test. Whole**  
2 **numbers would have been expected with chi-squared analysis.]** The authors concluded that there was no  
3 estrogenic effect of bisphenol A on sex differentiation in the Korean rockfish.

4  
5 **Strengths/Weaknesses:** The use of a positive control, which worked as expected, is a strength of this  
6 study. The inadequate presentation of data and statistical analysis is a weakness.

7  
8 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is not useful in the evaluation process.

9  
10 **Honkanen et al. (461)**, supported by the Finnish Graduate School of Environmental Science and  
11 Technology and the Academy of Finland, examined the effects of bisphenol A exposure on yolk-sac fry of  
12 landlocked salmon. Ten 8-day-old fry/beaker were exposed to bisphenol A [**99% pure**] at concentrations  
13 of 0, 10, 100, or 1000 µg/L for 42 days, in glass beakers. The ethanol vehicle and pure tap water were used  
14 as negative controls. There were 3–4 replicates/dose. One fry/beaker was photographed and killed  
15 following 6 days of exposure. After 6 weeks of exposure, all remaining fry were blotted and weighed.  
16 Three fry/beaker were photographed and 3 fry/beaker were examined histologically. Statistical analyses  
17 included ANOVA and Tukey test. Effects observed in fry exposed to the highest bisphenol A concentration  
18 included: yolk sac edema and hemorrhaging around gill arches and the front part of the yolk sac at 6 days  
19 of exposure; phlegmatic behavior (lack of activity during siphoning to renew solutions) on the 8<sup>th</sup> day of  
20 exposure; and darkening of color at 17 days of exposure. No increases in mortality were observed. At the  
21 end of the exposure period, wet weights were increased in fry exposed to the highest concentration, and the  
22 study authors stated that the effect was due to fluid accumulation. In fry exposed to the mid and high  
23 concentration of bisphenol A, strongly stained fragments were observed in nuclei and storage substances in  
24 liver were decreased. No abnormalities were observed in histological examinations of heart, kidney, and  
25 thyroid gland. The study authors concluded that bisphenol A induced toxicity in fry at concentrations rarely  
26 found in the environment.

27  
28 **Strengths/Weaknesses:** The range of concentrations used in this study is a strength.

29  
30 **Utility (Adequacy) for CERHR Evaluation:** The finding of an effect only at a high concentration of  
31 bisphenol A may have importance for environmental assessments but is not of utility in the current  
32 evaluation process.

#### 34 3.2.10.4 Reptile and bird

35 **Stoker et al. (462)**, supported by the Argentine National Agency for the Promotion of Science and  
36 Technology and Argentina Ministry of Health, examined the effects of in ovo bisphenol A exposure on  
37 sexual development of the crocodilian reptile *Caiman latirostris*. A preliminary experiment was conducted  
38 to determine the effects of temperature on sex determination, and it was established that incubation at 30°C  
39 resulted in production of females while incubation at 33°C resulted in the production of males. In the main  
40 experiment, eggs were collected from 5 nests in Argentina. Half the eggs were incubated at 30°C and the  
41 other half at 33°C. Care was taken to avoid exposing eggs to putative sources of estrogens such as spray  
42 paint, plastic, and nesting materials. At each incubation temperature, eggs from each nest were equally  
43 distributed among treatment groups. Twenty days following collection, 1 egg/nest/incubation temperature  
44 was opened for stage determination. At developmental stage 20, bisphenol A [**purity not indicated**] was  
45 applied topically to the eggshell at concentrations of 1.4 or 140 ppm (0.09 or 9 mg/egg). Other eggs were  
46 treated with 0.014 or 1.4 ppm 17β-estradiol. Control eggs were left untreated or exposed to the ethanol  
47 vehicle. Hatchlings were weighed and measured at birth. At 10 days of age, 4 animals/group/incubation  
48 temperature were killed for determination of sex by examination of internal genitalia. Sex determination  
49 was confirmed by histological evaluation of organs, which were fixed in 10% buffered formalin.  
50 Morphometric analysis of seminiferous tubules was also conducted in 10-day-old animals. The remaining  
51 animals (6–11/group/incubation temperature) were raised until 6 months of age, at which time they were

### 3.0 Developmental Toxicity Data

1 killed, measured, and sexed by examination of external genitalia. Evaluators were blinded to treatment  
2 conditions. Statistical analyses included Kruskal-Wallis ANOVA and Mann-Whitney *U* test.

3  
4 At 33°C, there was 100% sex reversal in the high-dose bisphenol A and high-dose 17β-estradiol groups at  
5 10 days and 6 months of age. Whereas 100% of control and low-dose animals in the 33°C group were male,  
6 100% of animals in the high-dose bisphenol A and 17β-estradiol group were female. Although there was no  
7 sex reversal in the low-dose bisphenol A or 17β-estradiol groups incubated at 33°C, morphometric  
8 evaluations at 10 days of age revealed significantly increased perimeter of seminiferous tubules, which had  
9 empty lumens. There were no significant effects reported for bisphenol A following incubation at 30°C.  
10 The study authors concluded that bisphenol A induced estrogenic effects in caiman as evidenced by  
11 reversed gonadal sex and disrupted gonadal histoarchitecture.

12  
13 **Strengths/Weaknesses:** This study appears to have been well performed and the use of a positive control  
14 is a strength. A weakness is the expression of exposure level in terms of total egg weight, which precludes  
15 easy comparison to human exposure levels.

16  
17 **Utility (Adequacy) for CERHR Evaluation Process:** This study has no utility in the evaluation process.

18  
19 **Berg et al. (463)**, supported by the Foundation for Strategic Environmental Research and the Swedish  
20 Council for Forestry and Agricultural Research, examined the effects of bisphenol A exposure on  
21 development of sex organs in quail and chicken embryos. The effects of tetrabromobisphenol A were also  
22 examined but will not be discussed. Bisphenol A (99.4% purity) was injected into yolk of Japanese quail  
23 eggs on the third day of incubation and into chicken (domestic fowl) eggs on the fourth day of incubation at  
24 doses of 0 (propylene glycol vehicle), 67, and 200 μg/g egg. Eggs were also injected with diethylstilbestrol  
25 at doses of 2, 20, and 200 ng/g egg [**culture ware not discussed**]. Two days before the anticipated hatching  
26 date, embryos were examined for mortality (32–43 quail embryos and 34–91 chicken embryos/group  
27 examined) and müllerian duct abnormality or testicular histopathology (8–15 quail embryos/group and 7–  
28 30 chicken embryos/group examined). Testes were fixed in 4% formalin. Data were analyzed by Fisher  
29 exact probability test.

30 Exposure to bisphenol A did not increase mortality in quail embryos. Incidence of females with abnormal  
31 müllerian ducts was increased in quail embryos exposed to the high bisphenol A dose but the incidence of  
32 ovotestis in males was not increased by bisphenol A exposure. Mortality of chicken embryos was increased  
33 following exposure to both bisphenol A dose levels. The incidence of male chicken embryos with ovotestis  
34 was increased at the high dose of bisphenol A but there was no effect on females with abnormal müllerian  
35 ducts. Effects observed in one or more diethylstilbestrol groups included increased incidence of females  
36 with abnormal müllerian ducts in quail embryos and males with ovotestis in quail and chicken embryos.  
37 Based on study findings, the study authors concluded that bisphenol A can cause estrogen-like  
38 malformations in reproductive organs of birds.

39  
40 **Strengths/Weaknesses:** The detailed evaluation of genital tract morphology is a strength, but the  
41 expression of exposure level in μg per g egg makes it difficult to compare to human exposure levels.

42  
43 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not useful to the evaluation process.

44  
45 **Halldin et al. (137, 464)**, supported by the European Union and numerous Swedish agencies, examined the  
46 effect of in ovo exposure to bisphenol A on sexual behavior of male Japanese quail. On day 3 of  
47 incubation, the yolks of an unspecified number of quail eggs were injected with vehicle (emulsion of  
48 peanut oil, lecithin, and propylene glycol) or Bisphenol A (> **99% purity**) at 67 or 200 μg/g egg, and eggs  
49 were incubated at 37.5°C at 60% relative humidity. After hatching, male and female chicks were housed  
50 together. Males were individually housed at 7 weeks of age. At 9 weeks of age, 17 control and 4–7 treated  
51 males/group were examined for sexual behavior. Behavior with a sexually receptive female was evaluated

### 3.0 Developmental Toxicity Data

1 by observing actions such as neck grab, mount attempt, mounts, and cloacal contact movement. Testing  
2 was conducted for 2 minutes/day over 5 consecutive days. At the completion of testing, testis weight was  
3 measured, gonado-somatic index was determined, and plasma testosterone levels were measured by RIA.  
4 Females exposed in ovo (n = 5–8/group) were evaluated for numbers of eggs laid over 5 days and oviduct  
5 morphology. Statistical analyses included Kruskal-Wallis test, or chi-squared test for trend. No effects of  
6 bisphenol A exposure were reported for any of the effects examined including sexual behavior of males,  
7 testicular weight, gonado-somatic index in males, plasma testosterone levels, or numbers of eggs produced.  
8 Numbers of females with retained right oviduct were increased in the bisphenol A groups (2 of 5 and 4 of 7  
9 in each respective bisphenol A group versus 1 of 8 in controls) but the effect did not achieve statistical  
10 significance. Sexual behavior was reportedly affected at an ethinyl estradiol dose of 0.006 µg/g egg and  
11 diethylstilbestrol doses of 0.019 and 0.057 µg/g egg. The study authors concluded that, with the possible  
12 exception of a trend for retained right oviduct in females exposed to 200 µg/g egg, bisphenol A was not  
13 shown to affect any of the endpoints examined in Japanese quail, which were demonstrated to be a well  
14 suited model for studying effects of estrogenic compounds.

15  
16 **Strengths/Weaknesses:** The use of 2 positive controls and the attention to sexual behavior are strengths.  
17 Weaknesses are the expression of exposure level in µg per g egg, making it difficult to compare to human  
18 exposure levels, the lack of detail in the reporting of methods and results, and the lack of apparent  
19 statistical analysis.

20  
21 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not useful to the evaluation process.

22  
23 **Panzica et al. (465)**, supported by the University of Torino and Region Piemonte, conducted a study that  
24 intended to examine the effects of in ovo bisphenol A exposure on the vasotocin system and sexual  
25 behavior of Japanese quail. In 2 sets of experiments, quail eggs were injected with bisphenol A [**purity not**  
26 **indicated**] at 50, 100, or 200 µg/egg following 3 days of incubation [**culture ware not discussed**].  
27 Exposure to bisphenol A resulted in a dramatic decrease in the number of live chicks hatching (8–11%  
28 versus 55–60% in controls). Chicks that hatched survived less than a week. Dissection of non-hatched  
29 embryos indicated that development was blocked immediately following injection in most embryos. A high  
30 rate of malformations was observed in chicks that died following hatching. [**No further information was**  
31 **presented for methods, and no data were presented for individual doses.**]

32  
33 **Strengths/Weaknesses:** Weaknesses are the expression of exposure level in µg per g egg, making it  
34 difficult to compare to human exposure levels, and the lack of data presentation.

35  
36 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is not useful in the evaluation process.

37  
38 **Furuya et al. (466)**, supported by the Japanese Ministry of Education, Science, Sports, and Culture,  
39 examined the effects of bisphenol A exposure on growth of testes and combs of male chickens. Beginning  
40 at 2 weeks of age, male white Leghorn chicks were orally dosed weekly with corn oil vehicle (n = 5) or 200  
41 mg bisphenol A [**purity not indicated**] (n = 12). [**The specific method of oral dosing was not reported.**  
42 **It is assumed that birds were dosed until they were killed.**] Chickens were killed at 16 weeks of age.  
43 Combs and testes were weighed. Testes were fixed in 4% paraformaldehyde and examined histologically.  
44 [**Statistical methods were not discussed, and the levels of statistical significance were not reported.**]  
45 Bisphenol treatment did not affect body weight, but comb and testis weight were significantly lower in the  
46 chickens exposed to bisphenol A. Spermatogenesis was disturbed in the chickens of the bisphenol A group,  
47 as observed by small seminiferous lumen and scarcity of spermatids and mature sperm. Diameter of  
48 seminiferous tubules and incidence of seminiferous tubules with mature sperm were significantly lower in  
49 the bisphenol A group. The study authors concluded that bisphenol A might disturb the growth of comb  
50 and testes in male chickens, possibly through an endocrine mechanism.

51

### 3.0 Developmental Toxicity Data

1 **Strengths/Weaknesses:** The study of male puberty in chickens is a strength. Weaknesses are the use of a  
2 single dose level and the lack of information on dosing and statistical analysis. The paper would have been  
3 strengthened by measurement of hormone levels.  
4

5 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not useful to the evaluation process  
6

7 **Sashihara et al (467)**, supported by the Japan Ministry of Education, Science, and Culture and the Uehara  
8 Memorial Foundation, examined the effects of early life exposure to bisphenol A on growth and behavior  
9 in male chicks. Layer type (Julia) chicks were obtained from a local hatchery, housed in windowless rooms  
10 **[no further housing details provided]**, given ad libitum access to water and feed (Toyohashi Feed and  
11 Mills Co.), and provided continuous lighting. Birds were group housed based on weight. At 4 days of age,  
12 0, 100 or 200 µg of bisphenol A **[purity not given]** dissolved in 10% ethanol and sesame oil, was injected  
13 into the brain (n = 12 or 13 per group). Chicks were followed for growth up to 20 days after treatment. A  
14 subset of 7 chicks/group was used for behavioral testing 8 days after treatment. Birds were placed under  
15 isolation distress condition and for a 5-minute period were observed in a cage for motor activity and  
16 vocalization. At 20 days of age, birds were killed and liver, kidney, testis, and brain were weighed.  
17 Statistical analyses were performed using ANOVA and Duncan multiple range tests.  
18

19 There were no treatment effects on food intake 6 hours after injection or on body weight gain measured 3  
20 days after exposure. In the behavioral test, there were no treatment effects on jumping, locomotor activity,  
21 and duration of crouching. There was a statistically significant dose-dependent increase in the frequency of  
22 distress vocalizations. There were no treatment effects at 20 days on body or organ weights. The authors  
23 concluded that an acute early life exposure of the chick brain to 100 or 200 µg bisphenol A may affect  
24 stress-induced behavior, which may involve an estrogen-mediated pathway.  
25

26 **Strengths/Weaknesses:**

27 The rationale for the selection of the test animal and dosing procedures are not provided. Given that acute  
28 doses were injected directly into the brain, specific rationale for the method and selection of dose are  
29 critical to understanding the relevance of the study to human health or to wildlife or livestock concerns.  
30 This provides a vacuum for the interpretation of the dose-related increase in vocalizations that were  
31 reported.  
32

33 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not useful to the evaluation process  
34

35 **Furuya et al. (468)**, supported by the Japanese Ministry of Education, Science, Sports, and Culture,  
36 examined the effects of bisphenol A exposure on development of male chicks. Beginning at 2 weeks of age,  
37 male white Leghorn chicks were orally dosed every 2 days with bisphenol A at 0 (alcohol/corn oil vehicle)  
38 0.002, 0.020, 0.200, 2, or 200 mg/kg bw. The high-dose level was considered to be a positive control based  
39 on previous observations in the laboratory. **[No information was provided about the specific method of  
40 oral dosing, number of birds treated, purity of bisphenol A, or the type of feed or caging and bedding  
41 materials used. It was implied but not clearly stated that exposures were continued until the birds  
42 were killed.]** The birds were killed at 5, 10, 15, 20, and 25 weeks of age. The comb, wattle, and testes were  
43 weighed. Part of the testicular tissue was used to isolate mRNA for evaluation of *ERα* and aromatase  
44 expression by RT-PCR. Additional testicular tissue was fixed in 10% buffered formalin for histopathology  
45 analysis and assessment of spermatogenesis by using immunohistochemistry techniques to measure  
46 proliferating cell nuclear antigen levels. **[Methods for statistical analyses were not reported.]**  
47

48 Although responses were not dose-related, significant decreases in weight (doses at which effects were  
49 observed) were reported for comb and wattle at 10 weeks of age ( $\geq 0.002$  mg/kg bw), testis at 10 weeks of  
50 age (200 mg/kg bw), comb and testis at 15 weeks of age ( $\geq 0.020$  mg/kg bw), wattle at 15 weeks of age ( $\geq$   
51 0.2 mg/kg bw), comb at 20 weeks of age ( $\geq 0.200$  mg/kg bw), testis at 20 weeks of age (200 mg/kg bw), and

### 3.0 Developmental Toxicity Data

1 comb and testis at 25 weeks of age (200 mg/kg bw). There were no effects on body weight.  
2 Histopathological observations in testis (doses at which effects were observed) included significant and  
3 dose-related reductions in the number of spermatogonia at 5 weeks of age ( $\geq 2$  mg/kg bw) and number of  
4 spermatogonia, spermatocytes, and spermatids at 10–25 weeks of age ( $\geq 0.02$  mg/kg bw, except for  
5 decreases in spermatocytes at 10 weeks of age, which occurred at  $\geq 0.200$  mg/kg bw). Seminiferous tubule  
6 diameter was significantly reduced at all ages in groups exposed to  $\geq 0.020$  mg/kg bw. Significant and dose-  
7 related reductions in testicular proliferating cell nuclear antigen levels were observed at  $\geq 0.200$  mg/kg bw  
8 at 10 weeks of age and  $\geq 0.020$  mg/kg bw at 15–25 weeks of age. *ER $\alpha$*  mRNA was significantly increased  
9 according to dose (doses at which effects were observed) at 10 weeks of age ( $\geq 0.020$  mg/kg bw), 15 and  
10 20 weeks of age ( $\geq 0.200$  mg/kg bw/day), and 25 weeks of age (200 mg/kg bw). Significant and dose-  
11 related increases were also observed for aromatase mRNA expression (doses at which effects were  
12 observed) at 5 weeks of age ( $\geq 0.002$  mg/kg bw), 10 weeks of age (0.200 mg/kg bw), and 15 weeks of age  
13 (200 mg/kg bw). The study authors concluded that exposure to bisphenol A at environmentally relevant  
14 levels may affect male chicken phenotypes and result in unbalanced gene expression in the testis.  
15

16 **Strengths/Weaknesses:** This paper is a more detailed follow-up of the previous paper by these authors  
17 (466), and replication of these results is a strength. Additional strengths are the use of multiple exposure  
18 levels and the oral route of administration. The lack of information on statistical methods is a weakness.  
19

20 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not useful to the evaluation process  
21

22 While the in vitro studies are useful for mechanistic insights, cellular evaluation, and endpoint  
23 identification, *inter alia*, the studies as a group were considered not useful for the evaluation process.  
24

#### 25 3.2.11 In vitro

26 **Takai et al. (469)**, supported by the Japanese Ministry of Education, Science, and Culture, the Ministry of  
27 Health and Welfare, and the Science and Technology Agency, examined the effects of in vitro bisphenol A  
28 exposure on preimplantation mouse embryos. Two-cell embryos were obtained from B6C3F<sub>1</sub> mice and  
29 incubated for 48 hours in media containing bisphenol A [**purity not indicated**] at concentrations ranging  
30 from 100 pM to 100  $\mu$ M [**23 ng/L to 23 mg/L**] [**culture ware not discussed**]. A negative control group  
31 was exposed to the ethanol vehicle and the effects of tamoxifen were also tested. Cell numbers were  
32 counted, and trophoblast spreading was evaluated in blastocysts. Statistical analyses included chi-squared,  
33 Fisher post hoc, and Student *t*-tests. The number of embryos or samples/group ranged from 14 to 400 for  
34 each endpoint evaluated. Significant effects observed with bisphenol A exposure (percent change vs.  
35 control) included increased rate of development from 2- to 8-cell embryos following 24 hours exposure to 3  
36 nM [**0.68  $\mu$ g/L**] (94% vs. 88%), increased development to the blastocyst stage following 48 hours exposure  
37 to 1 and 3 nM [**0.23 and 0.68  $\mu$ g/L**] (69% in both dose groups vs. 58.7%), and decreased development to  
38 the blastocyst stage following 48 hours exposure to 100  $\mu$ M [**23 mg/L**] bisphenol A (31.2 vs. 58.7%). No  
39 effects were observed at concentrations between 10 nM and 10  $\mu$ M [**23  $\mu$ g/L and 2.3 mg/L**] bisphenol A.  
40 [**Data were not shown by study authors.**] Addition of 100 nM tamoxifen to cultures decreased  
41 development to the blastocyst stage at 1 and 3 nM [**0.23 and 0.68  $\mu$ g/L**] bisphenol A and increased  
42 development to blastocyst stage at 100  $\mu$ M [**23 mg/L**] bisphenol A. Trophoblast spreading was increased in  
43 blastocysts exposed to 100  $\mu$ M [**23 mg/L**] bisphenol A. Bisphenol A exposure did not affect morphology of  
44 or cell numbers in blastocysts. The study authors concluded that environmentally relevant concentrations of  
45 bisphenol A may affect early embryonic development through the ER and may also affect subsequent  
46 development.  
47

48 **Strengths/Weaknesses:** The wide range of bisphenol A concentrations is a strength. The postulated  
49 involvement of the ER in bisphenol A activity could have been more convincingly demonstrated with a  
50 positive control such as 17 $\beta$ -estradiol and with a more specific estrogen antagonist than tamoxifen. The use  
51 of serum-free and phenol red-free media is an appropriate way to avoid estrogenic contamination but is an

### 3.0 Developmental Toxicity Data

1 artificial environment compared to the estrogen-rich milieu in which preimplantation embryos normally  
2 develop.

3  
4 **Utility (Adequacy) for CERHR Evaluation Process:** This study provides some mechanistic information  
5 but is not useful in the evaluation process.

6  
7 **Takai et al. (470)**, supported by the Japanese Ministry of Education, Science, Sports, and Culture, the  
8 Ministry of Health and Welfare, and the National Institute for Environmental Studies, examined the effects  
9 of in vitro preimplantation exposure of mice to bisphenol A. Two-cell embryos were obtained from B6C3F<sub>1</sub>  
10 mice and incubated for 48 hours in media containing bisphenol A [**purity not indicated**] at 0 (ethanol  
11 vehicle), 1 nM [**0.23 µg/L**] or 100 µM [**23 mg/L**] [**culture ware not discussed**]. Embryos were assessed  
12 for number developing to the blastocyst stage, and then blastocysts were transferred to uterine horns of  
13 pseudopregnant mice (7/mouse). The dams were allowed to deliver and nurse the litters until weaning on  
14 PND 21 (day of birth not defined). Pups were randomly culled to maintain litter sizes at no more than 6.  
15 Body weight of pups was measured at birth and at weaning. Litters and pups were considered the  
16 experimental unit for statistical analyses. Statistical analyses included chi-squared and Fischer protected  
17 least significant difference tests. The number of embryos developing to the blastocyst stage was  
18 significantly increased by exposure to bisphenol A at 1 nM [**0.23 µg/L**] but decreased by exposure to 100  
19 µM [**23 mg/L**] (72.2 and 33.3% at each respective concentration versus 62.1% in controls). Developing  
20 embryos appeared morphologically normal and there were no significant differences in the numbers of  
21 cells. Birth weight, number of pups/litter, and sex ratio were not affected by treatment. At weaning, pups in  
22 both dose groups weighed more than controls (34–39% greater) and the effect was significant on a litter  
23 and pup basis. The study authors concluded that bisphenol A may affect early embryonic and postnatal  
24 development at low, environmentally relevant concentrations.

25  
26 **Strengths/Weaknesses:** This study was cleverly designed as a follow-up to the previous study and appears  
27 to show that a low concentration of bisphenol A stimulates early embryo development while a high  
28 concentration inhibits early embryo development. This study did not evaluate the effect of exogenous  
29 bisphenol A under physiologic conditions. The use of serum-free and phenol red-free media is an  
30 appropriate way to avoid estrogenic contamination but is an artificial environment compared to the  
31 estrogen-rich milieu in which preimplantation embryos normally develop. The trophic effects of bisphenol  
32 A at low concentration may have been compensating for the estrogen deprivation of the control culture. It  
33 would have been interesting to compare physiologic concentrations of 17β-estradiol to the control culture  
34 conditions.

35  
36 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not useful in the evaluation process.

37  
38 **Li et al. (471)**, support not indicated, examined the effect of in vitro bisphenol A exposure on  
39 postimplantation mouse and rat embryos. A limited amount of information was available for the study,  
40 which was published in Chinese, but included an abstract and data tables presented in English. GD 8.5  
41 mouse embryos and GD 9.5 rat embryos were cultured for 48 hours in media containing bisphenol A  
42 [**purity not indicated**] at 0, 40, 60, 80, or 100 mg/L [**culture ware not discussed**]. Exposure of rat  
43 embryos to bisphenol A concentrations ≥60 mg/L resulted in reduced crown-rump length and yolk sac  
44 diameter and affected yolk sac circulation and morphologic differentiation of the nervous system, heart,  
45 and forelimbs. Additional effects observed in rats at ≥80 mg/L included reductions in head length, number  
46 of somites, and flexion and changes in morphologic differentiation of the otic and optic system and tail.  
47 Exposure of mouse embryos to ≥60 mg/L bisphenol A resulted in reductions in flexion, yolk sac diameter,  
48 and yolk sac circulation and changes in morphologic differentiation of the olfactory system and branchial  
49 arches. In mouse embryos exposed to ≥80 mg/L bisphenol A, there were reductions in head and crown-  
50 rump length and number of somites and changes in morphologic differentiation of the visual system, heart,

### 3.0 Developmental Toxicity Data

1 brain, auditory system, and fore- and hindlimb buds. The study authors concluded that high concentrations  
2 of bisphenol A are toxic to rat and mouse embryos in vitro.

3  
4 **Strengths/Weaknesses:** The use of excessively high concentrations of bisphenol A is a weakness.

5  
6 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is not useful in the evaluation process.

7  
8 **Monsees et al. (472)**, supported by the Federal Environmental Agency of Germany, examined the effects  
9 of bisphenol A exposure on rat Sertoli cell cultures. Sertoli cell cultures were prepared using testes from  
10 18–21-day-old Sprague Dawley rats. The cultures were exposed for 24 hours to bisphenol A or ethinyl  
11 estradiol at 0 or 10–50  $\mu\text{M}$  [**2.3–11 mg/L**] [**culture ware not discussed**]. The effects of pesticides and  
12 heavy metals were also examined but will not be discussed. Endpoints assessed following the incubation  
13 period included viability by measurement of mitochondrial enzyme activity and lactate and inhibin B  
14 production. There were 8 replicates/experiment, and the experiment was repeated 3 times. Data were  
15 analyzed by Student *t*-test or unpaired Mann-Whitney test. Exposure of cells to bisphenol A resulted in  
16 increased lactate production (up to 30%) at  $\sim 25$   $\mu\text{M}$  [**5.7 mg/L**] bisphenol A and increased inhibin B  
17 production at  $\sim 10$   $\mu\text{M}$  [**2.3 mg/L**] and greater. There was no effect on cell viability following exposure to  
18 bisphenol A. Effects of ethinyl estradiol included increased mitochondrial dehydrogenase activity and a  
19 biphasic effect on inhibin B production, with an increase at  $\sim 10$   $\mu\text{M}$  and decreases at higher doses. The  
20 study authors concluded that secretion of lactate and inhibin B by Sertoli cells appeared to be sensitive  
21 markers for exploring possible Sertoli cell toxicants.

22  
23 **Strengths/Weaknesses:** The use of high concentrations of bisphenol A is a weakness. It is not clear how  
24 the increased lactate and inhibin B production would correlate with reproductive capacity.

25  
26 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not useful in the evaluation process.

27  
28 **Iida et al. (473)**, supported by an unnamed grantor and by Takeda Science Foundation, examined the  
29 effects of in vitro bisphenol A exposure on cultured rat Sertoli cells. The cell cultures were prepared using  
30 testes of 18-day-old rats and were exposed for up to 48 hours to bisphenol A [**purity not indicated**] at  
31 concentrations ranging from 50 to 100  $\mu\text{M}$  [**11–23 mg/L**] [**culture ware not discussed**]. Control cells were  
32 incubated in the DMSO-containing media. Morphology was examined by phase-contrast microscopy, and  
33 viability was assessed using the CellTiter 96 system in cells exposed to 0, 50, 100, 150, 200, and 300  $\mu\text{M}$   
34 [**0, 11, 23, 34, 46, and 68 mg/L**]. Immunochemistry analyses were conducted to detect transferrin and  
35 caspase-3 and apoptosis was assessed using a TUNEL method in cells exposed to 0, 100, and 200  $\mu\text{M}$  [**0,**  
36 **23, and 46 mg/L**] bisphenol A for 48 hours. A fluorescence staining technique was used to examine actin  
37 structure in cells incubated with 200  $\mu\text{M}$  [**46 mg/L**] bisphenol A. Experiments were performed in triplicate  
38 and repeated at least 3 times. Data were analyzed by ANOVA.

39  
40 Bisphenol A concentrations of  $\geq 150$   $\mu\text{M}$  [**34 mg/L**] increased detachment of Sertoli cells from substrate  
41 and reduced viability. In a time-response study, cell viability was reduced following exposure to 200  $\mu\text{M}$   
42 [**46 mg/L**] bisphenol A for  $\geq 12$  hours. Transferrin secretion by Sertoli cells was decreased following  
43 incubation with bisphenol A [**apparently at  $\geq 100$   $\mu\text{M}$  (23 mg/L); statistical significance not indicated**].  
44 Following incubation with 200  $\mu\text{M}$  [**46 mg/L**] bisphenol A, observations included solitary cells with a  
45 cortical ring of actin filaments and underdeveloped stress fibers, cells with membrane blebs consisting of  
46 protruding actin filaments, and round cells with a disorganized actin cytoskeleton and chromatin  
47 condensation. The study authors indicated that the observations were consistent with apoptosis. Expression  
48 of capsase-3 was observed in the round Sertoli cells. Capsase-3-positive cells were rarely observed in  
49 control cells, but were observed at incidences of  $<1\%$  in the 100  $\mu\text{M}$  [**23 mg/L**] group and  $\sim 9\%$  in the 200  
50  $\mu\text{M}$  group. Further examinations revealed that most and possibly all of TUNEL-positive cells were stained

### 3.0 Developmental Toxicity Data

1 with the caspase-3 antibody. The study authors concluded that decreased viability of Sertoli cells was most  
2 likely due to apoptosis and not necrosis.

3  
4 **Strengths/Weaknesses:** The evaluation of multiple endpoints is a strength; however, the concentrations of  
5 bisphenol A were much higher than are likely to be achieved with human exposures.

6  
7 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is not useful for the evaluation process.

8  
9 **Miyatake et al. (434)**, supported by the Japanese Ministry of Health, Labor, and Welfare, and the Ministry  
10 of Education, Culture, Sports, Science, and Technology, conducted a series of studies to examine the effect  
11 of bisphenol A exposure on cultures of mouse neuron/glia cells and astrocytes. Cell cultures were obtained  
12 from midbrains of ICR mice on PND 1. Statistical analyses included ANOVA followed by Student *t*-test.

13  
14 In the first 2 studies, astrocyte and neuron/glia cultures were incubated for 24 hours in media containing  
15 bisphenol A [**purity not indicated**] or 17 $\beta$ -estradiol at 0 or 10 fM to 1  $\mu$ M [**bisphenol A concentrations of**  
16 **2.3 pg/L–0.23 mg/L**] for 24 hours, and intensity of glial fibrillary acidic protein immunoreactivity was  
17 measured [**culture ware not discussed**]. In astrocyte cultures activation of cells, as determined by stellate  
18 morphology and significantly increased glial fibrillary acidic protein, occurred with exposure to bisphenol  
19 A at 100 fM [**23 pg/L**], 1 pM [**0.23 ng/L**], 10 pM [**2.3 ng/L**], 10 nM [**2.3  $\mu$ g/L**], 100 nM [**23  $\mu$ g/L**], and 1  
20  $\mu$ M [**0.23 mg/L**], but the effect was not observed in cells exposed to bisphenol A at 10 fM [**2.3 pg/L**], 100  
21 pM [**23 ng/L**], or 1 nM [**0.23  $\mu$ g/L**]. In neuron/glia cultures, a significant increase in glial fibrillary acidic  
22 protein was observed at bisphenol A concentrations of 100 fM [**23 pg/L**], 1 pM [**0.23 ng/L**], 10 pM [**2.3**  
23 **ng/L**], 100 nM [**23 ng/L**], and 1  $\mu$ M [**0.23 mg/L**], but not at bisphenol A concentrations of 10 fM [**2.3**  
24 **pg/L**], 100 pM [**23 ng/L**], 1 nM [**0.23  $\mu$ g/L**] or 10 nM [**2.3  $\mu$ g/L**]. Increases in glial fibrillary acidic protein  
25 immunoreactivity were not observed in astrocyte or neuron/glia cultures following treatment with 17 $\beta$ -  
26 estradiol. The study authors concluded that exposure of cell cultures to bisphenol A results in biphasic  
27 activation of astrocytes.

28  
29 In a third study, the role of steroid hormone receptors in bisphenol A-induced astrocyte activation was  
30 examined. Astrocyte and neuron/glia cell cultures were pretreated with an ER antagonist (ICI 182,780), an  
31 ER agonist/antagonist (tamoxifen), a progesterone receptor antagonist (mifepristone), or an androgen  
32 receptor antagonist (flutamide) for 24 hours. The cultures were then incubated with bisphenol A at 0, 1 pM  
33 [**0.23 ng/L**], or 1  $\mu$ M [**0.23 mg/L**], with and without the receptor ligands, for another 24 hours. None of the  
34 ligands attenuated astrocyte activation, and the study authors concluded that bisphenol A-induced  
35 activation of astrocytes was not mediated by estrogen, progesterone, or androgen receptors.

36  
37 In a fourth study, mouse midbrain astrocyte or neuron cultures were incubated for 24 hours in media  
38 containing bisphenol A at 0, 1 pM [**0.23 ng/L**], 1 nM [**0.23  $\mu$ g/L**], or 1  $\mu$ M [**0.23 mg/L**]. A fluorescent  
39 technique was used to measure calcium levels following treatment of cells with 1–100  $\mu$ M dopamine. In  
40 astrocyte and neuron cultures, dopamine-induced increases in intracellular calcium were enhanced  
41 following pretreatment with bisphenol A at 1 pM [**0.23 ng/L**], but not at 1 nM [**0.23  $\mu$ g/L**] or 1  $\mu$ M [**0.23**  
42 **mg/L**]. In neuron cells, pretreatment with 1  $\mu$ M [**0.23  $\mu$ g/L**] bisphenol A suppressed dopamine-induced  
43 increases in intracellular calcium. The study authors concluded that in vitro bisphenol A exposure results in  
44 altered dopamine responsiveness in astrocytes and neurons.

45  
46 In a fifth study, neuron/glia cultures were incubated in media containing bisphenol A or 17 $\beta$ -estradiol at 1  
47 pM, 1 nM, or 1  $\mu$ M for 24 hours [**bisphenol A concentrations of 0.23 ng/L, 0.23  $\mu$ g/L, and 0.23 mg/L**].  
48 An immunohistochemistry technique was used to identify apoptotic cells by the presence of caspase-3.  
49 Treatment with 1  $\mu$ M [**0.23  $\mu$ g/L**] bisphenol A activated caspase-3 in neurons. No increase in caspase 3  
50 was observed following exposure to cells to 17 $\beta$ -estradiol. The study authors concluded that high in vitro  
51 exposures to bisphenol A may result in toxicity to neurons.

### 3.0 Developmental Toxicity Data

1 **Strengths/Weaknesses:** The use of multiple concentrations of bisphenol A over a wide range, the  
2 evaluation of multiple endpoints, and the comparison to known receptor ligands are strengths.

3  
4 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is interesting in suggesting a non-  
5 hormonal mechanism of bisphenol A activity. Although the paper contains suggestive mechanistic  
6 information, it is not useful for the evaluation process.

7  
8 **Yamaguchi et al. (474)**, supported by the Promotion and Mutual Aid Corporation for Private Schools of  
9 Japan, examined the effects of low-level bisphenol A exposure on the differentiation of serum-free mouse  
10 embryo astrocyte progenitor cells into astrocytes. Astrocyte progenitor cells were grown on fibronectin-  
11 coated petri dishes under standard incubator conditions. Differentiation of astrocyte progenitor cells was  
12 induced with leukemia inhibitory factor (LIF) and bone morphogenetic protein-2 (BMP2) [**culture ware**  
13 **not discussed**]. Cells were additionally exposed to bisphenol A [**purity not provided**] at concentrations of  
14 0.1 ng/L to 100 mg/L with or without tamoxifen for 24, 48, 72, or 120 hours, to establish optimal  
15 experimental parameters. A tetrazolium salt based colorimetric assay was used to assess cell viability and  
16 dot-blot or Western blot detection of glial fibrillary acidic protein production was used as a marker of  
17 differentiated astrocytes. Subsequent assays were performed using bisphenol A treatments of 0.1 ng/L [**4**  
18 **pM**] or 1 mg/L [**40 mM**]. Controls were treated with LIF and BMP-2 for 48 hours. ANOVA and Tukey test  
19 were used for statistical analyses.

20  
21 Bisphenol A 0.11 ng/L had no effect on astrocyte progenitor differentiation; However, bisphenol A at 1, 10,  
22 and 100 ng/L induced significant differentiation compared to controls based on dot-blot assays of glial  
23 fibrillary acidic protein production. The highest glial fibrillary acidic protein levels were induced with 10  
24 ng/L bisphenol A exposure. At bisphenol A concentrations  $\geq 1 \mu\text{g/L}$ , there were no differences in astrocyte  
25 progenitor differentiation compared to control. Bisphenol A 10 ng/L induced significantly higher levels of  
26 phosphorylated signaling transducer and activator protein 3 (pSTAT3) and phosphorylated mothers against  
27 *decapentaplegic* homolog 1 (pSmad1), the activated forms of both proteins, which are induced to form a  
28 protein complex by BMP-2 and LIF, and in turn, promote glial fibrillary acidic protein expression.  
29 Addition of  $10^{-6}$  M tamoxifen resulted om glial fibrillary acidic protein, pSTAT3, and pSmad1 comparable  
30 to control levels. Bisphenol A at 10 ng/L and 1  $\mu\text{g/L}$  only marginally increased levels of Smad6 and  
31 oligodendrocyte lineage transcription factor 2, inhibitors of pSTAT3-p300 and pSmad1-Smad4 protein  
32 complex formation, which induce glial fibrillary acidic protein expression.

33  
34 The authors suggested that low levels of bisphenol A may alter brain development through a mode of  
35 action involving elevated levels of glial fibrillary acidic protein production through estrogen receptor  
36 regulation of glial fibrillary acidic protein expression and through a stimulatory BMP-2/LIF signaling  
37 pathway that induces the formation of pSmad and pSTAT3 coactivator complexes of glial fibrillary acidic  
38 protein expression.

39  
40 **Strengths/Weaknesses:** This study is interesting, but the in vitro system is not useful for predicting in vivo  
41 effects in humans.

42  
43 **Utility (Adequacy) for CERHR Evaluation Process:** This paper is not useful in the evaluation process.

### 44 **3.3 Utility of Developmental Toxicity Data**

#### 45 **3.3.1 Human**

46  
47 There are no human data on developmental effects of bisphenol A.  
48  
49

## 3.0 Developmental Toxicity Data

### 3.3.2 *Experimental animals*

There are 21 studies in which bisphenol A was given at a single dose level to rats and 6 studies in which bisphenol A was given at a single dose level to mice. These studies explored various aspects of bisphenol A developmental effects but are not useful in establishing dose-response relationships. The lowest dose level evaluated in these studies was 0.0024 mg/kg bw/day in rats (350) and 0.002 mg/kg bw/day in mice (404). There are 25 rat and 30 mouse studies in which bisphenol A was given at multiple dose levels. These studies included oral and subcutaneous administration routes; due to pharmacokinetic considerations, studies using the oral route are of greater utility in estimating human risk.

### 3.4 Summary of Developmental Toxicity Data

The studies summarized here are those considered by the panel to be the most important and relevant for the assessment of the effects of Bisphenol A on the human population. Evaluation of the scientific literature was made on the scientific quality of the study and also on its relevance to the assessment of the level the concern about potential effects of BPA on human health. The judgment was based on the criteria the Panel adopted which focused on the potential for providing information for the evaluation process. Several excellent studies have been placed in the “adequate-but-limited-utility” category with regard to the evaluation process. The panel did not consider the source of funding of any of the studies in any of their deliberations.

It is highly unlikely that humans would ever experience the very high internal levels of bisphenol A that are produced after an injection of bisphenol A. While it would be possible to measure levels of parent compound and metabolite after injections, no parenteral exposure studies in this data set have done so. Section 1 and (32) indicate that ca. 99% of human exposure comes from dietary sources, and bisphenol A is subject to efficient first-pass metabolic conversion in the gut and liver to the inactive glucuronide conjugate in humans and rats (70, 109, 119). In contrast, bisphenol A injected subcutaneously or intraperitoneally circulates as much higher proportion of the unconjugated parent compound (119). Because oral exposure is so relevant to the human situation, and the uncertainties associated with the altered internal metabolite profile and the abundant data from oral studies, the Panel puts greater weight on studies using the oral route of exposure for formulating levels of concern about human exposures.

The hypothesis has been advanced that the Charles River SD rat is insensitive to estrogens and other EDCs and therefore it should not be used for developmental studies of potential endocrine disruptors, and the studies of the effects of BPA which used this strain should be discounted. In order to address this important issue the Panel members reviewed the literature on estrogen-sensitivity across rat strains and suppliers, the following is a summary of our findings:

Different strains of rats show clear, robust reproducible differences in response to potent estrogens and antiandrogens. Several traits have been shown to be estrogen sensitive in rats including prolactin regulation in the pituitary, thymic involution, uterine pyometra, and liver carcinogenesis to name a few. It is evident that there are strain differences in respect to specific estrogen induced endpoints. However there is no clear pattern in which one strain can be considered to be more or less sensitive than another. The results of BPA studies with the SD rat cannot therefore be ignored.

#### 3.4.1 *Human*

There are no human data on developmental effects of bisphenol A. A study of the association between miscarriage and mean serum bisphenol A levels is discussed in section 4.4.1.

### 3.0 Developmental Toxicity Data

#### 3.4.2 Experimental animal

Studies considered by Expert Panel members to be of utility in evaluating developmental toxicity in mice are summarized in [Table 82 -Table 85](#). Rat and mouse studies with behavioral endpoints are summarized in [Table 86](#). The discussion of developmental toxicity is arranged according to general endpoints evaluated.

#### *General developmental toxicity (growth, survival, malformations)*

##### *Rat Studies*

Prenatal studies with oral dosing of rats consistently demonstrated an absence of malformations at doses up to 1000 mg/kg bw/day (316, 319). Reduced fetal survival and body weights at birth or during the postnatal period were reported in studies with oral exposures occurring throughout the entire gestation and/or lactation periods (319, 338, 475). LOAELs for decreased numbers of live fetuses or pups ranged from 475 to 1000 mg/kg bw/day (319, 338, 475). LOAELs for decreased pup body weight at birth were estimated at 300–1000 mg/kg bw/day (319, 338, 475). The LOAEL for reduced body weight during the postnatal period was 475 mg/kg bw/day (338, 475).

##### *Mouse Studies*

No increase in malformations was observed in mice with oral gavage of bisphenol A at doses of  $\leq 1250$  mg/kg bw/day (316). Prenatal developmental toxicity reported for mice included increased resorptions (LOAEL 1250 mg/kg bw/day) and decreased fetal body weight (LOAEL 1250 mg/kg bw/day) (316). Decreased body weight during the postnatal period was also reported in offspring of mouse dams exposed to bisphenol A during the entire gestation and lactation period (LOAEL 600 mg/kg bw/day), but the effect was not observed in a second generation exposed according to the same protocol (436). An increase in hepatic histopathologic findings (cytoplasmic variation) at weaning was also observed in offspring of mouse dams exposed during gestation and lactation (LOAEL 50–600 mg/kg bw/day) (436). A single dose level study with gestational exposure in mice reported increased lactational body weight gain and decreased postnatal pup survival at 0.0024 mg/kg bw/day (396).

#### *Reproductive system development*

##### *Rat studies*

Delays in vaginal opening were observed in offspring of rat dams receiving high oral doses of bisphenol A on GD 6–15 or during the entire gestational and lactational period (321, 338, 475). No delays in vaginal opening were observed with doses of bisphenol A  $\leq 1.2$  mg/kg bw/day administered to dams during gestation or lactation (337, 338, 475).

Estrous cycle alterations were not reported in rat oral exposure studies covering a wide range of doses ( $<1$ –475 mg/kg bw/day) administered during all or part of the gestational or lactational periods (337, 338, 342, 475).

Studies suggest that preputial separation is delayed following oral administration of high bisphenol A doses (LOAELs 47.5–475) to male rat offspring in the post weaning period (338, 352, 475). No effects on preputial separation were observed when treatment of rat dams with high doses (50–384 mg/kg bw/day) ended during the gestation or lactation period (321). Oral doses of bisphenol A  $\leq 1$  mg/kg bw/day also had no effect on preputial separation (337, 338, 475).

Effects on rat sperm parameters were inconsistent. Decreased sperm count and daily sperm production were reported in offspring of dams exposed during gestation (LOAEL 50 mg/kg bw/day for sperm count/g testis, LOAEL 50 mg/kg bw/day for daily sperm count/g testis) (321). A single dose level study reported decreased numbers of rats undergoing spermatogenesis following postweaning exposure of males to 100 mg/kg bw/day (352). In contrast, no consistent effects on sperm parameters were observed in rats following

### 3.0 Developmental Toxicity Data

1 exposures with up to 475 mg/kg bw/day during the prenatal, lactational, and post-weaning periods (338,  
2 475). Other rat studies with gestational and lactational doses ranging from <1 to 4 mg/kg bw/day also  
3 reported no effects on sperm parameters (337, 339). Testicular histopathology (multinucleated giant cells in  
4 seminiferous tubules and absent spermatogenesis) was only reported in a single dose level study at a  
5 bisphenol A dose of 100 mg/kg bw/day administered in the post-weaning period (352).

6  
7 Although some sporadic effects were reported for anogenital distance in male and female rats, study  
8 authors concluded that the endpoint was not affected by prenatal, lactational, and/or post-weaning exposure  
9 to bisphenol A (321, 337, 338, 476).

10  
11 No effects on rat prostate weight were observed with bisphenol A doses of <1–475 mg/kg bw/day  
12 administered during the gestational, lactational, and/or post-weaning periods (321, 338, 339, 342, 476). The  
13 study of Timms et al (402) in mice raise a level of concern.

#### 14 *Mouse studies*

15  
16 Exposure of mice to bisphenol A during pre- and postnatal development delayed preputial separation  
17 (LOAEL 600 mg/kg bw/day)(436). Effects reported for anogenital distance were inconsistent. A single  
18 dose study reported an increase in anogenital distance in male mice at 0.050 mg/kg bw/day (398). A second  
19 study with a wide dose range (0.003–600 mg/kg bw/day) reported no consistent or dose-related effects on  
20 anogenital distance (436).

21  
22 One group of investigators reported increased prostate weight at 0.002 and 0.020 mg/kg bw/day in  
23 offspring of mouse dams exposed during pregnancy (275). These prostate effects were consistent with  
24 findings in single dose level studies with gestational exposure of mice, however, it is noted that the studies  
25 had differing periods of exposure and ages of evaluation. One of these studies demonstrated increased  
26 prostate weight at 0.050 mg/kg bw/day (398). Another study demonstrated increased numbers of prostate  
27 ducts and proliferating cell nuclear antigen staining in dorsolateral prostate and increased prostate duct  
28 volume in dorsolateral and ventral prostate at 0.010 mg/kg bw/day (402). However, no effects on prostate  
29 or sperm production were observed in more robust studies with multiple dose levels and larger group sizes.  
30 A third mouse study with exposures occurring during gestation, lactation, and post-lactational periods also  
31 reported no effects on prostate weight, daily sperm production, or efficiency of daily sperm production at  
32 doses of 0.003–600 mg/kg bw/day (436). A fourth mouse study demonstrated no effect on sperm density  
33 following low-dose exposure ( $\leq 0.200$  mg/kg bw/day) during gestation or the post weaning period (428).

34  
35 Seminiferous tubule hypoplasia in association with undescended testes in mouse weanlings was reported  
36 following exposure during pre- and postnatal development (LOAEL 50–600 mg/kg bw/day; BMD<sub>10</sub> 283–  
37 591 mg/kg bw/day) but the effect was not observed in mice examined in adulthood (436). The findings  
38 were similar to those in studies reporting no testicular histopathology or lesions in reproductive organs  
39 following pre- and postnatal exposure to bisphenol A at  $\leq 0.2$  mg/kg bw/day (428).

40  
41 Following exposure of mice during pre- and postnatal development; no effect on age of vaginal opening,  
42 estrous cyclicity, or numbers of ovarian primordial follicles were observed at doses ranging from 0.003–  
43 600 mg/kg bw/day (436). No effect on age of vaginal opening was reported but there was a shortened  
44 period between vaginal opening and first estrus following gestational exposure to 0.0024 mg/kg bw/day in  
45 a single dose level study (396).

#### 46 *Body Weight*

47  
48 All rat and mouse multigenerational studies have measured body weight as an endpoint. No consistent  
49 differences have been detected in the weights of offspring of animals exposed to low to moderate doses of  
50 BPA (Table 81).

51

### 3.0 Developmental Toxicity Data

#### 1 *Hormone Levels*

2 Several studies have measured testosterone and LH levels in rats, there have also been investigations of  
3 thyroid hormone (T4) levels. TABLE. No consistent effects on the levels of these hormones have been seen  
4 (337).

#### 6 *Fertility and ability to raise pups to weaning following developmental exposure*

7 Multigenerational studies in both rats and mice have shown that BPA over a wide dose range does not  
8 compromise the ability of animals exposed during development to successfully produce offspring, raise  
9 them to weaning and for those offspring to successfully give rise to a subsequent generation of animals  
10 (337, 338, 436).

#### 12 *Neural and Behavioral Endpoints Following Oral Administration*

13 Several studies addressing effects on neural and behavioral endpoints have been conducted following  
14 gestational and lactational exposure [rats: (326, 361, 477)]; mice: [(403-405, 413, 435)], pubertal exposure  
15 [rat: (350, 369, 370)], and exposure during adulthood [gerbils: (478)].

16  
17 Gestational and lactational exposures in rats have reported subtle effects upon sexually-dimorphic brain  
18 nuclei (326), hormonal receptors in brain (404, 405), and certain sexually-dimorphic or reproductively  
19 relevant behaviors (361, 413, 435). Most of this work has utilized single doses across the range of 2 to 40  
20 micrograms/kg, and none has been confirmed or linked to other functional or clearly adverse effects. No  
21 effects on the volume of the SDN-POA of the hypothalamus were observed in offspring of rats orally  
22 exposed to bisphenol A doses ranging from 3.2 to 320 mg/kg bw/day during the gestation and lactation  
23 period (342). Single dose level rat studies demonstrated reduced sexually dimorphic difference in  
24 corticotropin-releasing hormone neurons in anterior stria terminalis at 2.5 mg/kg bw/day (326). No changes  
25 in sexual behavior were reported for female rats exposed to 0.3–320 mg/kg bw/day or males exposed to  
26 ≤0.3 mg/kg bw/day during the gestation and/or lactation period (342).

27  
28 Maternal behavior of dams has also been suggested to be altered in two studies of dams exposed during  
29 gestation and lactation (403, 477).

30  
31 One study involving exposures during puberty (477) suggested alterations in exploratory and sexual  
32 behavior of males following 40 microgram/kg on PND 23-30. Certain changes in hypothalamic estrogen  
33 receptors (370) following 40 microgram/kg exposures on PND 23-30 have been reported. Akingbemi (350)  
34 reported effects on gonadal hormonal and receptor endpoints in the pituitary following 2.4  
35 micrograms/kg/day on PND 21-35.

#### 37 *Other endpoints*

38 Following oral exposure of mice to bisphenol A during gestation, changes were observed for mRNA  
39 expression of arylhydrocarbon receptors, receptor repressor, or nuclear translocator and retinoic acid and  
40 retinoid X receptors in brain, testes, and/or ovary at 0.00002–20 mg/kg bw/day (404-406). The strongest  
41 effects were found at the lowest doses following exposures during organogenesis (GD 6.5-13.5 or 6.5-17.5)  
42 (405, 406). The study authors suggested those changes as possible mechanisms for bisphenol A-induced  
43 toxicity.

44  
45 A summary of LH and testosterone effects observed in humans and in bisphenol A-exposed experimental  
46 animals is included in Section 4.4.

#### 48 **Summary and Conclusion of Developmental Hazards**

### 3.0 Developmental Toxicity Data

1 There are sufficient data to conclude that bisphenol A does not cause malformations or birth defects in  
2 fetuses exposed during gestation at levels up to 640 mg/kg/d (rats) and 1000 mg/kg/d (mice) (316). This is  
3 consistent with the lack of malformations seen in offspring in multigenerational studies (338, 436).

4  
5 There are sufficient data to conclude that bisphenol A does not alter male or female fertility in rats or mice  
6 after gestational exposure up to doses of 450 mg/kg/d (337-339, 475).

7  
8 There are sufficient data to conclude that bisphenol A does not change the age of puberty in male or female  
9 rats [NOAELs of 0.2 mg/kg/d (337) and 1823 mg/kg/d (338)]. While limited data available suggest an  
10 effect on the onset of female puberty in mice [LOAEL 0.2 mg/kg/d (435), 0.002 mg/kg/d, (396)], the data  
11 are insufficient to conclude that Bisphenol A accelerates puberty in female mice. The limited data available  
12 suggest, but are insufficient to conclude, that Bisphenol A slightly delays the age of puberty in male mice at  
13 a LOAEL of ca. 550-800 mg/kg/d (436).

14  
15 There are sufficient data to conclude that bisphenol A exposure during development does not permanently  
16 affect prostate weight in adult rats or mice [NOAELs of: 1823 mg/kg/d (338), 600 mg/kg/d (436), 4  
17 mg/kg/d (339), 0.2 mg/kg/d (337), 50 mg/kg/d (321), and 320 mg/kg/d (342)]. There are sufficient data to  
18 conclude that Bisphenol A does not cause prostate cancer in rats or mice after adult exposure [calculated  
19 dose ranges of 25 – 400 mg/kg/d for rats, 600 – 3000 mg/kg/d, mice (157)]. There are slight suggestions,  
20 but insufficient data to conclude, that Bisphenol A might predispose towards prostate cancer in rats in later  
21 life following developmental exposure [at 10 µg/kg (479)]. There are slight suggestions, but insufficient  
22 evidence to conclude, that fetal exposure to Bisphenol A can contribute to urinary tract deformations in  
23 mice (10 µg/kg (402)).

24  
25 There are sufficient data to suggest that developmental exposure to Bisphenol A causes neural and  
26 behavioral alterations related to sexual dimorphism in rats and mice ( ca. 2.5 mg/kg/d, gestation and  
27 lactation in rats, (326); LOEL 0.00002 mg/kg/d, fetal mice, (406); 0.0002 mg/kg/d, fetal mice, (404), 0.04  
28 mg/kg/d, weaning to puberty, rats, (370); 0.1 mg/kg/d, GD3 – PND 20, rats, (361); 0.2 mg/kg/d, GD3 –  
29 PND20, mice, (435); 0.01 mg/kg/d, GD11-18, mice, (413), although other studies report no change in a  
30 related measure, the size of the sexually dimorphic nucleus of the pre-optic area (SDN-POA) [300 µg/kg/d,  
31 rats (372); NOEL of 320 mg/kg/d, rats, (342)].  
32

3.0 Developmental Toxicity Data

**Table 81. Adult Body Weights of Offspring Exposed during Gestation and/or Lactation**

Strain	Period of Dosing	Route	Dose (mg/kg/d)	Measured on PND	Finding (NE=No Effect)	Weights	SE	Sample Size	Reference
CR:Long Evans	GD12- PND21	Gavage	0.0, 2.4	90	M: ↑ 90d @2.4	450, 494	~14	12-14/group	Akingbemi et al. (350)
CR:Long Evans	GD21- PND90	Gavage	0.0, 2.4	90	NE	407, 412	~11	12-14/group	Akingbemi et al. (350) Negishi et al. (360)
F344/N	GD10- PND20	Gavage	0.0, 4.0, 40.0, 400.0	(7, 21, 28, 56), 84	M: ↓ 7d, 28d @40, ↓ 7d, 21d, 28d, 56d@400 F: ↓ 7d, 28d @4 and 40, ↓ 7d, 21d, 28d @400	M 303, 303, 303, 297; F: 186, 187, 185, 184	~3	27+, 27+, 27+, 15+, 9+	
Fisher	GD1- PND21	Gavage	0.0, 7.5, 120	(23, 28) 91	NE	259, 267, 259	~9	5, 5, 5	Yoshino et al. (347) Ichihara et al. (348)
Fisher	GD1- PND21	Gavage	0.05, 7.5, 30, 120	455	NE	427, 427, 420, 428	~21	12,12, 12, 12	
SD	GD6- GD21	Gavage	0.0, 0.1, 50	44, 50, 44	F: ↓ ~47@0.1	F: 246, 227, 242	~15	20, 20, 20	Talsness et al. (320)
SD	GD6- PND21	Water	0.0, 0.1, 1.2	(22, 28, 37, 56, 87) 110	M: ↑ 28d, 37d, 56d@0.1 and 28d, 37d, 56d@1.2 F: ↑22d, 28d, 37d, 56d, 87d, 110d @0.1	M: ~510, ~540, ~540; F: ~310, ~325, ~310 (from graph)	M: ~12 F: ~12	F 23, 18, 19; M 27, 19, 19	Rubin et al. (241)
SD	GD6- PND20	Gavage	0.0, 4.0, 40.0	63	NE	M: ~380, ~385, ~381; F: ~250, ~245, ~237	M: ~4; F: ~3	M: 5, 4, 4, 0; F: 5, 5, 5, 0	Kobayashi et al. (344)
SD	GD6- GD21	Gavage	0.0, 0.023, 0.049, 0.108	98	NE	F: 260, 261, 258, 250	~17.5	31, 21, 25, 25	Tinwell et al. (321)
SD CD All F1 gens-	Mating1- PND21	Diet	0.0, 0.015, 0.3, 4.5, 75, 750, 7500	>168	M: ↓ 84d@7500; F: ↓84d@7500;	M: 501, 505, 493, 506, 518, 476, 369; F: 290, 383, 287, 294, 295, 283, 234	M: ~8; F: ~5	30,10,10,10,10,10,10	Tyl et al. (338)
SD Crj IGS- F1 generation	GD11- GD17	Gavage	0.0, 0.0002, 0.002,	M 40; F 30	NE	M 241,237, 245, 228, 236; F 114, 120, 113,	~18 M, ~12 F	25, 25, 25, 25, 25	Ema et al. (337)

### 3.0 Developmental Toxicity Data

Strain	Period of Dosing	Route	Dose (mg/kg/d)	Measured on PND	Finding (NE=No Effect)	Weights	SE	Sample Size	Reference
SD Crj IGS- F2 generation	GD11- GD17	Gavage	0.02, 0.2 0.0, 0.0002, 0.002, 0.02, 0.2	M 41; F 31	NE	114, 113 M 240, 241, 237, 236, 237; F 116, 115, 113, 113, 117	~18 M ~13 F	25, 25, 25, 25, 25	Ema et al. (337)
SD Crj:CD IGS	GD15- PND10	Diet	0.0, 60, 3000	77	M: ↓ 77d@3000	M: 465, 452, 468, 421; F: 279, 272, 299, 254	M: ~33; F: ~25	8 litters	Takagi et al.(349)
SD CrI:CD BR	GD11- PND20	Gavage	0.0, 3.2, 320	~47	NE	F: 691, 736, 683, 668, 697	~19	44, 51, 47, 28, 38	Kwon et al. (342)
Wistar-Hans	14d before Mating- PND21	Water	0.0, 0.01, 0.1, 1.0, 10.0	(22, 29, 36, 43, 50, 57, 64, 71, 78, 85) 90	NE	331, 321, 328, 328, 328, 332	~20	51, 26, 26, 28, 27, 25 (litters)	Cagen et al. (339)
Wistar	GD1- PND21	Gavage	0.0, 0.03, 0.3	~87	NE	455.2, 460.8, 454.1	~7	13, 15, 13	Kubo et al. (356)
Wistar-AP	GD6- GD21	Gavage	0.0, 0.024, 0.051, 0.109	98	NE	F: 228, 241, 237, 237	~15	26, 26, 27, 26	Tinwell et al. (321)

### 3.0 Developmental Toxicity Data

**Table 82. Summary of High Utility Developmental Toxicity Studies (Single Dose Level)**

Model (Route)	Dose (mg/kg bw/day) & Dosing Period	Significant Developmental Findings	Reference
<b>High Utility Developmental Toxicity Studies (Single Dose Level)</b>			
<b>Rat</b>			
Sprague Dawley (oral by pipette)	0.04, PND 23–30 and animals evaluated at PND 37 or 90	↑ ER $\alpha$ expression in females vs. males in medial pre-optic area (also seen with positive control).	Ceccarelli et al, 2007 (370)
Sprague Dawley males (oral by pipette)	0.040, PND 23–30	↓ testosterone in males at PND 37 but not PND 90 ↓ Investigation of new object, ↓ intromission latency, ↓ serum testosterone.	Della Seta et al. (369)
F344/N dams (gavage)	0.1, GD 3 – PND 20	↓ Correct avoidance responses and ↑ failure of avoidance in active avoidance testing; no ↑ in locomotion following trans-2-phenylcyclopropylamine hydrochloride challenge in males	Negishi et al. (361)
Sprague Dawley males (gavage)	100, PND 23–53	↑ Age of preputial separation; ↑ kidney and thyroid weights; ↓ liver weight; ↓ cortical thickness of the kidney; ↑ hydronephrosis; ↑ multinucleated giant cells in seminiferous tubules; ↓ no. undergoing spermatogenesis	Tan et al. (352)
<b>Mouse</b>			
CD-1 dam (oral)	0.050, GD 16–18	↑ Anogenital distance adjusted for body weight on PND60; ↑ prostate weights on PND 3, 21, and 60; ↓ relative (to body weight) epididymis weight in the bisphenol A group on PND 60; ↑ androgen receptor binding on PND 21 and 60	Gupta (398)
CD-1 dam (oral from syringe)	0.010, GD 11–18	↓ Place preference associated with d-amphetamine in females	Laviola et al. (413)
CD-1 dam (oral by pipette)	0.010, GD 14–18; offspring mated and dosed with 0 or 0.010 on GD 14–18.	In mice exposed only during gestational development or in adulthood during pregnancy: ↓ time nursing and in nest and ↑ time nest building, resting alone, grooming, and out of nest In mice exposed during both gestational development and in adulthood during pregnancy: ↑ time resting alone	Palanza et al. (403)
CD-1 dam (oral by pipette)	0.010, GD 14–18	↑ No. of prostate ducts and proliferating cell nuclear antigen staining in dorsolateral prostate; ↑ prostate duct volume in dorsolateral and ventral prostate	Timms et al. (402)

↑, ↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

### 3.0 Developmental Toxicity Data

**Table 83. Summary of High Utility Developmental Toxicity Studies (Multiple Dose Levels)**

Model (Treatment)	Endpoint	Bisphenol A Dose Level (mg/kg bw/day)						Reference
		NOAEL	LOAEL	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>	
<b>High Utility Developmental Toxicity Studies (Multiple Dose Levels)</b>								
<b>Rat</b>								
Han-Wistar (drinking water from prior to mating through gestation and lactation)	Male reproductive organ weights, sperm production, testicular histopathology.	≥0.775–4.022 (high dose)						Cagen et al. (339)
CD (gavage, 2-generations exposure including pre-and postnatal development periods)	Prenatal or postnatal growth or survival, developmental landmarks, anogenital distance, age of puberty, fertility, estrous cyclicity, or sperm counts.	≥0.2 (high dose)						Ema et al. (337)
Sprague Dawley dam (gavage GD 1–20)	↓ Live fetuses/litter ↓ Male body weight ↓ Female body weight ↓ Ossification	300 100 300 300	1000 300 1000 1000	929 456 439	348 339 328	982 694 682	713 497 490	Kim et al. (319)
Sprague Dawley dam (gavage GD 11– PND 20)	Volume of SDN-POA, age or weight at vaginal opening or first estrous, estrous cyclicity, mean lordosis intensity, prostate weight, or histopathology in ventral prostate, ovary, or uterus.	≥320 (high dose)						Kwon et al. (342)
CD dams (gavage GD 6–15)	Implantation sites, resorptions, body weight, viability, sex ratio, and malformations.	≥640 (high dose)						Morrissey et al. (316)
Sprague Dawley dam (gavage GD6 – PND 21)	↑ Uterine epithelial cell nuclei ↑ Uterine epithelial nuclei with condensed chromatin ↑ Uterine epithelial cells with cavities ↓ ERβ-positive cells in uterine tissue	0.1 (low dose) 0.1 (low dose) 0.1 (low dose) 0.1 (low dose)						Schönfelder, et al(322)

### 3.0 Developmental Toxicity Data

Model (Treatment)	Endpoint	Bisphenol A Dose Level (mg/kg bw/day)						Reference
		NOAEL	LOAEL	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>	
<b>High Utility Developmental Toxicity Studies (Multiple Dose Levels)</b>								
	↓ Thickness of uterine luminal epithelium	0.1	50					
	↑ ERα-positive cells in uterine epithelium	0.1	50					
Wistar-derived Alderley Park dams (gavage GD 6–21)	Delayed vaginal opening	0.1	50	68	51	35	16	Tinwell et al. (321)
	↓ Sperm count/testis	0.1	50	55	30	57	31	
	↓ Sperm count/g testis	0.1	50	81	41	68	34	
	↓ Daily sperm count/testis	0.1	50	56	31	59	31	
	↓ Daily sperm count/g testis	0.1	50	83	42	70	34	
Sprague Dawley (dietary, multiple generations with exposure during pre-and post natal development)	Live F1 pups/litter	47.5	475	268	192	559	394	Tyl et al. (338, 476)
	Live F2 pups/litter	47.5	475	422	152	459	294	
	Live F3 pups/litter	47.5	475	236	174	376	286	
	F1 body weight, PND 4	47.5	475	406	283	561	400	
	F1, F2, or F2 body weight, PND 7	47.5	475	217–328	183–257	265–410	218–313	
	F1, F2, or F2 body weight, PND 14	47.5	475	183–243	163–209	177–227	153–191	
	F1, F2, or F2 body weight, PND 21	47.5	475	208–252	166–226	223–267	175–220	
	↑ Age at F1 vaginal opening	47.5	475	394	343	206	176	
	↑ Age at F2 vaginal opening	47.5	475	404	336	277	228	
	↑ Age at F3 vaginal opening	47.5	475	471	401	396	203	
	↑ Age at F1 preputial separation	4.75	47.5	466	411	188	163	
↑ Age at F2 preputial separation	47.5	475	300	255	241	203		
↑ Age at F3 preputial separation	47.5	475	547	473	222	189		
Mating, fertility, pregnancy, or gestational indices; precoital interval, postimplantation loss, estrous cyclicity, and reproductive organ histopathology; sperm count, morphology or motility; anogenital distance in males or females; areolas/nipples in males.	≥475 (high dose)							

### 3.0 Developmental Toxicity Data

Model (Treatment)	Endpoint	Bisphenol A Dose Level (mg/kg bw/day)						Reference
		NOAEL	LOAEL	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>	
<b>High Utility Developmental Toxicity Studies (Multiple Dose Levels)</b>								
<b>Mouse</b>								
CD-1 dam (gavage GD 6–15)	↑ Resorptions/litter	1000	1250	817	377	1245	1162	Morrissey et al. (316)
	↓ Fetal body weight/litter	1000	1250	1079	785	1249	1024	
C57BL/6N males (gavage GD 11–17 or PND 21–43)	Sperm density or lesions in reproductive organs	≥0.200 (high dose)						Nagao et al. (428)
	↓ Absolute seminal vesicle weight in group exposed during gestation		≤0.002 (low dose) <sup>a,b</sup>					
CF-1 (oral by pipette, GD 11–17)	↑ Prostate weight		≤0.002 (low dose)					Nagel et al. (275);
C57BL/6 dam (gavage GD 3 – PND 21)	no effect AGD or AGD corrected for body weight	≥0.2 (high dose)						Ryan and Vandenberg (435)
	No effect on errors in radial arm and Barnes mazes	≥0.2 (high dose)						
	↓ Time in open arms of plus maze	0.002	0.2					
	↓ Time in light part of light/dark preference box	0.002	0.2					
CD-1 (dietary, multiple generations with exposure during pre- and postnatal development)	↓ F1 body weight on PND 7, 14, and 21	50	600	548–560	267–313	580–617	370–506	Tyl et al. (436)
	↓ F1 male body weight at PND 21 necropsy	50	600	564	313	640	599	
	↓ F1 female body weight at PND 21 necropsy	50	600	387	254	776	598	
	Hepatic cytoplasmic variation, F1 male	5	50	124	92.5			
	Hepatic cytoplasmic variation, F2 male	50	600	224	178			
	Hepatic cytoplasmic variation, F1 female	5	50	333	200			
	Seminiferous tubule hypoplasia, F1 male	50	600	591	406			

### 3.0 Developmental Toxicity Data

Model (Treatment)	Endpoint	Bisphenol A Dose Level (mg/kg bw/day)						Reference
		NOAEL	LOAEL	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>	
<b>High Utility Developmental Toxicity Studies (Multiple Dose Levels)</b>								
	Seminiferous tubule hypoplasia, F2 male	5	50	283	233			
	Age of preputial separation, F1 parental or non-mated males	50	600	727–754	572–576	491–551	364–414	
	Anogenital distance per body weight, F1 male on PND 21	5	50	1373	607	1769	616	
	Postnatal survival; daily sperm production; efficiency of daily sperm production; sperm motility or morphology; estrous cyclicity; numbers of ovarian primordial follicles; mating or fertility indices; or adult prostate weight	≥600 (high dose)						

<sup>a</sup>There was little-to-no evidence of a dose-response relationship.

<sup>b</sup>No effects were observed at one or more higher dose levels.

### 3.0 Developmental Toxicity Data

**Table 84. Summary of Limited Utility Developmental Toxicity Studies (Single Dose Level)**

Model (Route)	Dose (mg/kg bw/day) & Dosing Period	Significant Developmental Findings	Reference
<b>Limited Utility Developmental Toxicity Studies (Single Dose Level)</b>			
<b>Rat</b>			
Long Evans male offspring (gavage) Experiment 3	0.0024, PND 21–90	↑ Serum LH level; ↓ weight of seminal vesicles; ↓ testicular testosterone level; and ↓ basal and LH-induced ex vivo testosterone production.	Akingbemi et al. (350)
Wistar male pup (sc injection)	100, PND 2–12.	Advanced testicular lumen formation, ↑testis weight, ↑Sertoli cell volume/testis, ↑ spermatocyte nuclear volume/unit Sertoli cell, and ↑ plasma FSH on PND 18; ↑ plasma FSH on PND 25; ↑ testicular weight in adulthood	Atanassova et al. (374)
Wistar dam (drinking water)	~2.5, gestation <sup>a</sup> – PND21	In rats 4-7 months of age: no effect on the number of corticotropin-releasing hormone neurons in the preoptic areas of males, a loss in sex difference in the anterior and posterior bed nuclei of the stria terminalis.	Funabashi et al. (326)
Sprague Dawley male pup (sc injection)	0.010, PND 1, 3, and 5; half the rats exposed to 17β-estradiol and testosterone in adulthood	In rats with no 17β-estradiol and testosterone exposure in adulthood: no effects on dorsal prostate weight, histopathology alterations, proliferation index, or apoptotic index.  In rats with 17β-estradiol and testosterone exposure in adulthood: ↑ incidence and severity of prostatic intraepithelial neoplasia; ↑ proliferation and apoptosis in regions of prostatic intraepithelial neoplasia	Ho et al. (384)
Sprague Dawley pup (sc injection)	300, PND 1–5	No effects on age of vaginal opening or preputial separation, copulation or fertility indices, sexual behavior of males, histopathologic alterations in males, or female reproductive organs, or effects on SDN-POA. <b>[Panel noted possible ↑ number of apically located nuclei in prostate, but a definitive conclusion could not be made based on 1 photograph]</b>	Nagao et al. (372)

### 3.0 Developmental Toxicity Data

Model (Route)	Dose (mg/kg bw/day) & Dosing Period	Significant Developmental Findings	Reference
<b>Limited Utility Developmental Toxicity Studies (Single Dose Level)</b>			
Wistar male pup (sc injection)	50, PND 22–32	<p>↑ Serum prolactin levels on PND 29 but not PND 120; ↑ lateral but not ventral prostate weight; ↑ focal luminal polymorphonuclear cellular infiltrate in prostate</p> <p>No histological evidence of prostate inflammation</p>	Stoker et al. (373)
<b>Mouse</b>			
CF1 (oral)	0.0024, GD 11–17	<p>↑ Body weight at weaning; ↓ postnatal pup survival; ↓ period between vaginal opening and first estrus</p> <p>No effect on age of vaginal opening</p>	Howdeshell et al. (396)
ICR/Jc1 mouse dams (sc injection)	0.02, GD 0 to GD10.5, GD12.5, GD14.5 or GD16.5	<p>↑ Tuj1 in the intermediate zone at GD14.5 and GD16.5; ↑ PDI immunoreactivity in the neocortex from GD12.5 until GD16.5 and in subplate cells at GD14.5; variable changes in BrdU labeling depending on when labeled and location; ↑ gene expression of <i>Math3</i>, <i>Ngn2</i>, <i>Hes1</i>, <i>LICAM</i>, and <i>THR-alpha</i> at GD14.5; ↓ gene expression of <i>Hes1</i> and <i>Hes5</i> at GD12.5</p> <p>No effect on immunoreactivity pattern for KI-67, nestin, Musashi and histone H<sub>3</sub></p>	Nakamura et al. (421)
ICR (oral)	0.002 mg/kg bw/day from 6.5–11.5, 6.5–13.5, 6.5–15.5, and 6.5–17.5 days post coitum	Variable changes in retinoic acid retinoid X receptors $\alpha$ mRNA expression in brain, ovary, and testis, depending on brain region and day of exposure	Nishizawa et al. (404)
<b>Other</b>			
Prepubertal Poll Dorset female lambs (im injection)	3.5 biweekly, at 4–11 weeks of age (ovariectomy at 9 weeks of age)	↔ on blood levels during treatment; ↔ on body, kidney, adrenal, or ovarian weights; ↓ pulsatile LH secretion	Evans et al. (442)

### 3.0 Developmental Toxicity Data

Model (Route)	Dose (mg/kg bw/day) & Dosing Period	Significant Developmental Findings	Reference
<b>Limited Utility Developmental Toxicity Studies (Single Dose Level)</b>			
Prepubertal Poll Dorset female lambs (im injection)	3.5 biweekly, at 4–11 weeks of age (ovariectomy at 9 weeks of age)	<p>↑ uterine/cervical tract weight, endometrial area, and endometrial/myometrial ratio</p> <p>Qualitative observations included endometrial edema, decreased endometrial gland density, crowding of cells in the uterine epithelium, keratinized cervical epithelium, ↑ intracellular staining for ER<math>\alpha</math> and ER<math>\beta</math> in the uterine subepithelium</p>	Morrison et al. (443)
Suffolk ewes (sc injection)	5 GD 30-GD90	<p>↓ birth weight, height and chest circumference in female offspring at birth</p> <p>↑ anoscrotal:anona vel ratio in male offspring at birth</p> <p>↑ LH and first breeding season in female offspring at PND 60</p>	Savabieasfahani et al. (444)

↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

<sup>a</sup> Implied but not stated that exposure occurred during the entire gestation period.

3.0 Developmental Toxicity Data

**Table 85. Summary of Limited Utility Developmental Toxicity Studies (Multiple Dose Levels)**

Model (Treatment)	Endpoint	Bisphenol A Dose Level (mg/kg bw/day)						Reference	
		NOAEL	LOAEL	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>		
<b>Limited Utility Developmental Toxicity Studies (Multiple Dose Levels)</b>									
<b>Rat</b>									
Long Evans males (gavage PND 21 to 35) Experiment 1	↓ Serum 17β-estradiol ↓ Serum LH and testosterone		0.0024 (low dose) <sup>a,b</sup> 0.0024 (low dose) <sup>a,b</sup>					Akingbemi et al. (350)	
Sprague Dawley (dietary for 17 weeks)	↓ pup weight at weaning (PND 21)	70	200					General Electric, 1976 (335)	
Sprague Dawley (dietary for 18 weeks)	No adverse effects reported	60 (high dose)						General Electric, 1978 (336)	
Sprague Dawley female pups (sc injection PND 0–9)	↓ Body weight in lactation period	105	427	286	200	233	156	Kato et al. (380)	
	↑ Age of vaginal opening	26	105	345	267	159	116		
	↓ No. with normal estrous cycles	105	427	81	28				
	↑ No. with cleft clitoris	26	105	299	failed				
	↓ Ovary weight	105	427	85	59	140	93		
	↓ Uterus, wet weight	105	427	66	55	128	96		
	↓ Uterus, blotted weight	105	427	273	128	318	168		
	↓ Uterine fluid weight	26	105	42	34	139	104		
	↑ No. with polycystic ovaries		≤105	81	24				
				(lowest dose examined)					
		↓ No. corpora lutea	105	427	238	90			
	↓ No. with corpora lutea	105	427	65	38	137	83		
	↓ Corpora lutea area		≤105	42	37	84	66		
			(lowest dose examined)						
Sprague Dawley female pups (sc injection PND 0–9)	No adverse effects reported	≥97 (high dose)						Kato et al. (382)	
Sprague Dawley (feed GD15 – PND 10)	No adverse effects reported	3000 ppm <sup>c</sup>						Masutomi et al. (351)	
<b>Mouse</b>									

### 3.0 Developmental Toxicity Data

Model (Treatment)	Endpoint	Bisphenol A Dose Level (mg/kg bw/day)						Reference
		NOAEL	LOAEL	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>	
<b>Limited Utility Developmental Toxicity Studies (Multiple Dose Levels)</b>								
CF-1 (oral by pipette, GD 11 to 17)	Prostate weight and sperm production.	≥0.020 (high dose)						Ashby et al. (394)
ICR/Jcl (sc GD 11–17)	↓ Female body weight at weaning		≤0.002 (low dose) <sup>a</sup>	0.065	0.017	0.088	0.021	Honma et al. (419)
	↓ Male body weight at birth	0.002	0.020	0.054	0.020	0.031	0.015	
	↑ Anogenital distance of females at weaning		≤0.002 (low dose) <sup>a, b</sup>					
	↑ Anogenital distance of males on PND 60		≤0.002 (low dose)	0.035	0.020	0.035	0.020	
	↓ Age at vaginal opening	0.002	0.020					
	↓ Body weight at vaginal opening		≤0.002 (low dose)					
	↓ Age at 1st estrus	0.002	0.020					
	↑ Estrous cycle length		≤0.002 (low dose) <sup>a</sup>	0.021	0.007	0.12	0.021	
	↑ Cornified cells		≤0.002 (low dose) <sup>b</sup>	0.17	0.020	0.44	0.021	
	↓ Lymphocytes in vaginal smear		≤0.002 (low dose) <sup>b</sup>	0.26	0.020	0.26	0.020	
ICR (oral GD 6.5–13.5 or 6.5–17.5)	↑ mRNA expression for arylhydrocarbon receptor in brain, testis, and ovary.		≤0.00002 (low dose) <sup>b</sup>					Nishizawa et al. (405)
	↑ mRNA expression for retinoic acid $\alpha$ receptor in brain and ovary.		≤0.00002 (low dose) <sup>b</sup>					
	↑ mRNA expression for retinoic acid $\alpha$ receptor in testis.	0.20	20					
	↑ mRNA expression for retinoid X $\alpha$ receptors in brain.		≤0.00002 (low dose) <sup>b</sup>					
	↑ mRNA expression for retinoid X $\alpha$ receptor in testis and ovary.	0.002	0.020 <sup>b</sup>					

### 3.0 Developmental Toxicity Data

Model (Treatment)	Endpoint	Bisphenol A Dose Level (mg/kg bw/day)						Reference
		NOAEL	LOAEL	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>	
<b>Limited Utility Developmental Toxicity Studies (Multiple Dose Levels)</b>								
ICR (oral GD 6.5–13.5 or 6.5–17.5)	↑ mRNA expression for arylhydrocarbon receptor, arylhydrocarbon receptor repressor, and arylhydrocarbon receptor nuclear translocator in brain, testis, and ovary.			≤0.00002 (low dose) <sup>b</sup>				Nishizawa et al. (406)
ICR/Jcl (sc GD 10–18; female offspring ovariectomized)	↑ No. of vaginal epithelial layers			≤10 (low dose)				Suzuki et al. (437)
	↓ No. with corpora lutea			≤10 (low dose) <sup>b</sup>				
ICR/Jcl (sc for 5 days beginning at birth; mice later ovariectomized except those used to monitor estrous cycles)	↑ Mitotic rate in uterine stromal cells and vaginal epithelial cells	10		100				Suzuki et al. (437)
	↑ Vaginal epithelial layers	10		100				
	↑ No. with polyovular follicles and no. polyovular follicles/mouse	10		100				
	Estrous cyclicity	100 (high dose)						
CF-1 (oral by pipette, GD 11–17)	↓ Body weight			≤0.002 (low dose) <sup>b</sup>				vom Saal et al. (392)

<sup>a</sup>There was little-to-no evidence of a dose-response relationship.

<sup>b</sup>No effects were observed at one or more higher dose levels.

<sup>c</sup>Feed consumption and dam weight not reported-dose not calculable.

### 3.0 Developmental Toxicity Data

**Table 86. Summary of Behavioral Studies in Rats and Mice Treated with Bisphenol A**

Treatment, mg/kg bw/day	Treatment age	Age at assessment	Results	Reference
<b>High Utility</b>				
<b>Rat</b>				
<i>Treatment of dam</i>				
3.2, 32, or 320, gavage	GD 11–PND 20	6 months	Lordosis behavior not affected by treatment	Kwon et al. (342)
0.1, gavage	GD 3–PND 20	Open field: 8 weeks Spontaneous motor activity: 12 weeks Passive avoidance: 13 weeks Elevated plus maze: 14 weeks Active avoidance: 15 weeks	Open field: No treatment effect Spontaneous motor activity: No treatment effect Passive avoidance: No treatment effect Elevated plus maze: No treatment effect Active avoidance: Fewer correct avoidance responses	Negishi et al. (361)
<i>Treatment of offspring</i>				
0.04, micropipette	PND 23–30	45 days	No treatment effect on environmental exploration, social investigation, play, or social interaction ↓Response to novel object ↓Intromission latency	Della Seta et al. (369)
<b>Mouse</b>				
0.010, syringe feeding	GD 11–18	60 days	↓Conditioned place preference (reinforced with amphetamine) in females	Laviola et al. (413)
0.010, micropipette (treatment of F <sub>0</sub> and F <sub>1</sub> females)	GD 14–18	Maternal behavior of F <sub>1</sub> assessed	Altered maternal behaviors when exposure was either prenatal or as an adult; however, exposure prenatally plus as an adult was not effective	Palanza et al. (403)
2 or 200, placed in back of dam's throat	GD 3–PND 21	5 weeks, ovariectomized female offspring	No effect on errors in radial arm and Barnes mazes Effects in high dose group: Puberty advanced ↓Time in open arms of plus maze ↓Time in light part of light/dark preference box	Ryan and Vandenberg (435)

↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

## 4.0 REPRODUCTIVE TOXICITY DATA

### 4.1 Human

#### 4.1.1 Female

**Takeuchi and Tsutsumi (90)**, supported by the Japanese Ministry of Education, Science, Sports, and Culture, the Ministry of Health and Welfare, and the Science and Technology Agency, measured bisphenol A in the blood serum of 14 healthy women, 11 healthy men, and 16 women with polycystic ovary syndrome [**diagnostic criteria not discussed**]. The healthy women were evaluated in the mid-follicular phase of the menstrual cycle. Bisphenol A was measured using a competitive ELISA. Serum was also evaluated for total and free testosterone,  $17\beta$ -estradiol, androstenedione, dehydroepiandrosterone sulfate, LH, FSH, and prolactin. Statistical analysis was by ANOVA. Correlation coefficients were obtained from a linear regression analysis. Mean  $\pm$  SEM bisphenol A serum concentrations (ng/mL) were  $0.64 \pm 0.10$  in normal women,  $1.49 \pm 0.11$  in normal men, and  $1.04 \pm 0.10$  in women with polycystic ovary syndrome. Bisphenol A serum concentrations were significantly correlated with total testosterone ( $r = 0.595$ ) and free testosterone ( $r = 0.609$ ) in all subjects and in all female subjects ( $r = 0.559$  for total testosterone and  $0.598$  for free testosterone). Bisphenol A serum concentrations were not significantly correlated with any other hormone measures. The authors concluded that either bisphenol A stimulates testosterone production or metabolism of bisphenol A is inhibited by testosterone. They further suggested that displacement of sex steroids from sex-hormone binding globulin by bisphenol A might disrupt the estrogen-androgen balance.

**Strengths/Weaknesses:** Quality assurance for the hormone radioimmunoassays appeared adequate; however, there was no standardization for time of day for the serum samples, which may result in variable testosterone levels. ELISA has not been standardized for human sera, and may over-estimate bisphenol A due to nonspecific binding (see Section 1.1.5). Very little descriptive information was given on any of the groups beyond mean age and body-mass index. No information was given on recruitment methods and participation rates/exclusions. The lack of diagnostic criteria for polycystic ovary syndrome is a weakness. No potential confounders or effect modifiers were identified except mean age and body-mass index. Mean values appear to have been similar between groups. The positive correlations between bisphenol A level and total/free testosterone levels in all women and in entire study group were noted, but these analyses were not adjusted for potential confounders or effect modifiers. No information was given on whether the data were normally or lognormally distributed. The study was limited by small numbers in each group and the results should be regarded as descriptive epidemiology.

**Utility (Adequacy) for CERHR Evaluation Process:** This paper is adequate but has limited utility given its small size, and limited design. The study provides some insight for potential mechanisms affecting the levels of bisphenol A in the body.

**Takeuchi et al. (94)**, supported by the Japanese Ministry of Education, Science, Sports, and Culture, the Ministry of Health, Labor, and Welfare, the National Institute for Environmental Studies, and the Science and Technology Agency, examined relationships between serum sex hormone and bisphenol A concentrations in women with ovarian dysfunction and obesity. Fasting blood samples were collected during the midfollicular phase from 19 non-obese and 7 obese healthy women with normal menstrual cycles. Blood samples were also obtained from 7 women with hyperprolactinemia, 21 patients with hypothalamic amenorrhea, and 13 non-obese and 6 obese patients with polycystic ovary syndrome. [**It not known whether any of these subjects were the same as those reported earlier by this group (90).**] Mean ages for the subgroups ranged from 25 to 29 years old. Blood serum was analyzed for bisphenol A levels using an ELISA technique, and total and free testosterone,  $17\beta$ -estradiol,

#### 4.0 Reproductive Toxicity Data

1 androstenedione, dehydroepiandrosterone sulfate, LH, FSH, prolactin, and insulin levels were measured  
2 using by RIA. Statistical analyses included ANOVA and linear regression analysis.  
3

4 Compared to non-obese healthy women, concentrations of bisphenol A in serum were significantly higher  
5 in non-obese women with polycystic ovary syndrome [**48% higher**], obese women with polycystic ovary  
6 syndrome [**65% higher**], and obese healthy women [**46% higher**]. Statistically significant positive  
7 correlations were found between bisphenol A level in serum and body mass index ( $r = 0.500$ ) and serum  
8 levels of total testosterone ( $r = 0.391$ ), free testosterone ( $r = 0.504$ ), androstenedione ( $r = 0.684$ ), and  
9 dehydroepiandrosterone sulfate ( $r = 0.514$ ). The study authors concluded that there is a strong  
10 relationship between serum levels of bisphenol A and androgens, possibly due to androgen effects on  
11 metabolism of bisphenol A.  
12

13 **Strengths/Weaknesses:** Quality assurance for the hormone radioimmunoassays appears adequate. In  
14 contrast to the 2002 article by these authors (90), blood draws were time-standardized to 9:00–10:00 AM  
15 after overnight fasting. As noted in Section 1.1.5, ELISA may over-estimate bisphenol A. It was not clear  
16 whether any of the women in this study were also included in their 2002 publication. No potential  
17 confounders or effect-modifiers were identified except mean age and body-mass index, and neither of  
18 these was controlled in the analyses. Positive correlations were observed for bisphenol A level with body-  
19 mass index, total testosterone, free testosterone, androstendione, and dehydroepiandrosterone sulfate for  
20 all study groups. These correlations are also found (with the exception of total testosterone) in the control  
21 (“normal women”) group as well. Normality of the distributions of the hormones were not reported, and  
22 not transformed prior to analysis. The study was limited by small numbers and results should be regarded  
23 as descriptive epidemiology.  
24

25 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate but has limited utility in  
26 assessing possible relationships of bisphenol A with androgens (testosterone, free testosterone,  
27 androstenedione, dehydroepiandrosterone sulfate) and conditions that may promote hyperandrogenism  
28 (obesity, polycystic ovarian syndrome).  
29

30 **Hiroi et al. (95)**, supported by the Japanese Ministry of Health, Labor, and Welfare, the National Institute  
31 for Environmental studies, and the Japan Science and Technology Agency, compared blood bisphenol A  
32 levels in women with and without endometrial hyperplasia. Volunteers were recruited from an outpatient  
33 clinic in Japan. Women included in the study consisted of 11 controls with normal endometrium, 19 with  
34 endometrial hyperplasia, and 7 with endometrial carcinoma. The hyperplasia group was further divided  
35 according to severity: 10 with simple hyperplasia and 9 with complex hyperplasia. Mean ages were 48.4–  
36 48.9 years in groups without cancer, and the mean age was 63.1 years in the group with endometrial  
37 cancer. Blood samples were collected at the time of endometrial examination. Serum bisphenol A levels  
38 were measured by ELISA. Data were analyzed by Student *t*-test, with the exception of gravidity and  
39 parity, which were analyzed by chi-squared test. There were no significant differences in age, gravidity,  
40 parity, or body height, weight, or mass index between the groups without endometrial cancer. Women  
41 with endometrial cancer were significantly older and had significantly lower values for gravidity, parity,  
42 height, and weight. Mean  $\pm$  SD serum bisphenol A levels were reported at  $2.5 \pm 1.5$  ng/mL in controls,  
43  $2.2 \pm 1.6$  ng/mL in women with hyperplasia, and  $1.4 \pm 0.5$  ng/mL in women with endometrial cancer.  
44 When the group with hyperplasia was divided according to severity, serum bisphenol A blood levels were  
45 reported at  $2.9 \pm 2.0$  ng/mL in the group with simple hyperplasia and  $1.4 \pm 0.4$  ng/mL in the group with  
46 complex hyperplasia. Serum bisphenol A levels were significantly lower in women with complex  
47 endometrial hyperplasia or endometrial cancer than in controls. The study authors concluded that their  
48 preliminary findings demonstrated a possible link between bisphenol A exposure and endometrial  
49 hyperplasia or cancer. It was noted that modes of action for bisphenol A may be more complex than  
50 expected and that these contradictory results might provide a clue about mechanisms of production of  
51 estrogen-dependent diseases.

#### 4.0 Reproductive Toxicity Data

1 **Strengths/Weaknesses:** Because this was a small, cross-sectional study, it is not possible to determine  
2 whether this association preceded disease, or could have been associated with the disease process. As  
3 noted in Section 1.1.5, ELISA may over estimate bisphenol A.  
4

5 **Utility (Adequacy) for CERHR Evaluation Process:** The cross sectional study design is adequate but  
6 of limited utility for this evaluation, but raises research questions regarding mechanisms of production of  
7 estrogen-dependent diseases.  
8

9 **Sugiura-Ogasawara et al. (93)**, supported by the Japanese Ministry of Health, Labor, and Welfare,  
10 conducted a study to determine if there is an association between recurrent miscarriage and bisphenol A  
11 levels in blood. The cases in this study were 45 patients with a history of 3 or more (3–11) consecutive  
12 first trimester miscarriages. Mean  $\pm$  SD age of the cases was  $31.6 \pm 4.4$ . None of the cases had a history  
13 of live birth. All were seen at a Japanese hospital between August, 2001 and December, 2002. Half of the  
14 cases were housewives and half were employed in various occupations. A hysterosalpingography  
15 analyses was conducted in cases, and chromosome analyses were conducted for both cases and their  
16 partners. Women were excluded from the study if uterine anomalies were observed or chromosomal  
17 abnormalities were detected in either partner. Serum bisphenol A levels were determined by ELISA.  
18 Immunological endpoints examined included antinuclear antibodies, antiphospholipid antibodies, and  
19 natural killer cell activity. Blood testing for hypothyroidism, diabetes mellitus, and hyperprolactinemia  
20 was conducted. Blood samples were obtained 5–9 days following ovulation in at least 2 cycles. Blood  
21 samples to determine progesterone and prolactin levels were taken at 3 months following the last  
22 miscarriage and prior to the next conception. For subsequent pregnancies, ultrasounds were conducted,  
23 and spontaneously aborted embryos/fetuses were karyotyped. Serum levels of bisphenol A in cases were  
24 compared to those of 32 healthy non-pregnant hospital employees with no history of live birth, infertility,  
25 or miscarriage. Mean  $\pm$  SD age of controls was  $32.0 \pm 4.8$ . None were taking oral contraceptives. Like the  
26 cases, the controls lived near Nagoya City. Statistical analyses included Welch test, Mann-Whitney test,  
27 and Pearson correlation coefficient.  
28

29 Bisphenol A levels (mean  $\pm$  SD) were reported to be significantly higher in women with recurrent  
30 miscarriages ( $2.59 \pm 5.23$  ng/mL) compared to healthy controls ( $0.77 \pm 0.38$  ng/mL). In the 45 cases,  
31 incidences of abnormal conditions were 15.6% for hypothyroidism, 13.3% for antiphospholipid  
32 antibodies, 22.2% for antinuclear antibodies, 11.1% for hyperprolactinemia, and 20.5% for luteal phase  
33 defect. Serum levels of bisphenol A were significantly higher in patients who tested positive versus  
34 negative for antinuclear antibodies (Mean  $\pm$  SD  $7.382 \pm 9.761$  vs.  $1.222 \pm 1.54$  ng/mL). Thirty-five of the  
35 patients became pregnant and 48.6% had another miscarriage. Serum bisphenol A levels in patients who  
36 miscarried were  $4.39 \pm 8.08$  ng/mL, and serum bisphenol A in patients with successful pregnancies were  
37  $1.22 \pm 1.07$  ng/mL (not statistically significant). The study authors concluded that exposure to bisphenol  
38 A is associated with recurrent miscarriage.  
39

40 In a letter to the editor, Berkowitz (480) stated that this study did not support an association between  
41 bisphenol A blood levels and recurrent miscarriage. Several limitations were noted for the study. Timing  
42 and numbers of blood samples collected were not clearly defined. It was noted that because bisphenol A  
43 has a short half life, it would be critical to know if blood samples were obtained in a timeframe relevant to  
44 the occurrence of miscarriage. Although differences in serum bisphenol A levels in cases compared to  
45 controls achieved statistical significance, it was noted that median levels of bisphenol A in serum were  
46 nearly identical in patients with recurring miscarriages ( $0.71$  ng/mL) and controls ( $0.705$ ). The similarities  
47 in median values suggested there were no differences between the two groups, and it was suggested that  
48 apparent differences in mean serum levels of bisphenol A were due to a few individuals, as was  
49 demonstrated in Figure 1 of the Sugiura-Ogasawara et al. (93) report. Berkowitz stated that the Welch test  
50 was inappropriate for statistical analyses and noted that the 2 evaluation groups could not be considered  
51 comparable because of differences in occupation (housewives compared to medical workers) and

#### 4.0 Reproductive Toxicity Data

1 unknown fertility of controls. Because the controls were not evaluated for factors such as hypothyroidism  
2 and systemic lupus erythematosus (associated with antinuclear antibodies), the conditions may have been  
3 overrepresented in cases and may have been the cause of the reported differences between the 2 groups.  
4 Although mean bisphenol A levels were (non-significantly) lower in women who subsequently became  
5 pregnant and had a successful pregnancy compared to those who miscarried, Berkowitz noted that the  
6 median level of bisphenol A was actually higher in women with the successful pregnancies. Lastly, the  
7 ELISA method for measuring bisphenol A levels has not been validated and is subject to inaccuracy due  
8 to extensive cross-reactivity.

9  
10 In a response to the comments by Berkowitz (480), Sugiura-Ogasawara (481) stated that although  
11 measurement of bisphenol A levels at various time points would have been ideal, obtaining samples every  
12 day during pregnancy would have been difficult. Sugiura-Ogasawara clarified that bisphenol A values  
13 were based on a single sample in each individual, but that similar tendencies were observed for a second  
14 blood sample. With respect to the use of women with live births as controls, Sugiura-Ogasawara  
15 explained that the same blood samples were used for measurements of other environmental compounds,  
16 some of which are known to decrease after delivery. It was noted that none of the cases had systemic  
17 lupus erythematosus, and that use of controls with hypothyroidism or antinuclear antibodies was not  
18 considered important for the study. Superiority of the HPLC method compared to the ELISA method for  
19 measuring serum bisphenol A levels was acknowledged, but the authors stated that the ELISA method  
20 was used because of limited funding, reiterated that the study was preliminary and used a small number of  
21 volunteers, and that additional studies using a larger sample and more appropriate analytical methods  
22 were needed.

23  
24 **Strengths/Weaknesses:** The letter from Berkowitz (480) summarizes many of the weaknesses of this  
25 study. No quality assurance information was given for the biomarker/hormone measurements. As the  
26 Berkowitz letter points out, the ELISA method is not standardized for human sera (and may over-estimate  
27 bisphenol A due to nonspecific binding), the distribution of exposure was not normal, and median values  
28 of the two groups were similar, with two women skewing the mean. Little information was provided on  
29 the characteristics of the two study groups or response rates. Age and body-mass index were controlled in  
30 the analyses, but other potential confounders and effect modifiers were not. The time between exposure  
31 and observation was not appropriate. Spontaneous abortions have been associated with many factors  
32 which have not been addressed here. The authors' conclusions require the assumption that bisphenol A  
33 measurement levels represent those present during the important time frame for the spontaneous abortion.  
34 The authors do not report the time frame for collection of the blood samples. Non-normal data were not  
35 appropriately transformed for analysis. Welch's test was used "...to compare bisphenol A  
36 levels...because the distribution of the two groups might have differed." Welch's test is a *t*-test for groups  
37 with unequal variance, not different distributions (both should be normal, which was probably not the  
38 case).

39  
40 **Utility (Adequacy) for CERHR Evaluation Process:** Because of limitations in the design and analysis  
41 of this work, this study is inadequate has no utility in this evaluation.

42  
43 **Yang et al. (99)**, supported by the Korean FDA, measured urine bisphenol A in 172 Korean men and  
44 women and evaluated the relationship of these values with UDP-glucuronosyl- and sulfotransferase  
45 polymorphisms, with sister-chromatid exchange testing, and with self-reported symptoms of possible  
46 endocrine origin. First-morning urine samples were collected at the time of a routine physical  
47 examination, as was a blood sample, and a questionnaire was completed. Urine bisphenol A was  
48 measured using reverse phase HPLC. DNA was isolated from blood samples and polymorphisms were  
49 determined at *SULT1A1* and *UGT1A6*. Sister chromatid exchange in response to N-methyl-N'-nitro-N-  
50 nitrosoguanidine (MNNG) was evaluated in blood cells [not otherwise specified]. The relationship

## 4.0 Reproductive Toxicity Data

1 between urine bisphenol A and continuous variables was assessed with simple or multiple regression  
2 analysis and the relationship with categorical variables assessed with the Wilcoxon test.

3  
4 None of the subjects reported occupational exposure to bisphenol A. The median urine bisphenol A  
5 concentration was 7.86 µg/L. Urine bisphenol A was not different in men and women. Urine bisphenol A  
6 was associated with body-mass index ( $P = 0.06$ ) and self-reported frequency of alcohol consumption ( $P =$   
7  $0.08$ ). *SULT1A1* and *UGT1A6* polymorphisms were not significantly associated with urine bisphenol A  
8 concentrations. No significant associations were observed between urine bisphenol A and MNNG-  
9 induced sister-chromatid exchange, although they were associated when lower levels of MNNG were  
10 used. There were no significant associations between urine bisphenol A and self-reported symptoms of  
11 possible endocrine origin, including thirst/frequent urination, dizziness, neck mass, heat intolerance,  
12 sweating, hot flashes, swelling of lymph nodes, dysmenorrhea, menstrual irregularity, or menorrhagia.  
13 The authors concluded that even though they had been unable to associate an endocrine disorder with  
14 urine bisphenol A, continuous biologic monitoring of bisphenol A would be prudent.

15  
16 **Strengths/Weaknesses:** Bisphenol A was measured in urine using HPLC. No information was given  
17 regarding any selection criteria or response rates and some outcome measures were self-reported.

18  
19 **Utility (Adequacy) for CERHR Evaluation Process:** While small, this paper is useful for providing  
20 descriptive exposure information on BPA urinary levels (see section I). This paper does not have utility  
21 for evaluation of reproductive endpoints.

### 22 23 4.1.2 Male

24 **Luconi et al. (482)**, supported by the Italian Public Health Project, examined the effects of in vitro  
25 exposure of human spermatozoa to bisphenol A. Semen was collected from normozoospermic men, and  
26 spermatozoa were separated. Intracellular calcium was measured using a spectrofluorimetric method in  
27 cells treated with 1 µM bisphenol A, 1 µM 17β-estradiol, 10 µM progesterone, [17 β-estradiol is noted  
28 as 10µM in Figure 6, text states 1µM] or the same concentrations of bisphenol A in combination with  
29 17β-estradiol or progesterone. Effects on acrosome reaction were examined using a fluorescent staining  
30 method in cells exposed to 1 µM [0.23 mg/mL] bisphenol A for 2 hours, with and without exposure to 10  
31 µM progesterone. [In the study figures summarizing results, sample numbers in studies involving  
32 bisphenol A were listed at 5–11. It is not known if the sample numbers represented total numbers of  
33 sperm donors. Very few protocol details were provided in the methods section and many of the  
34 limited details presented above were obtained from the results section.] Data were analyzed by  
35 Student *t*-test and 1-way ANOVA. Treatment of spermatozoa with bisphenol A resulted in a modest  
36 influx of calcium, but bisphenol A had no effect on calcium responses induced by 17β-estradiol or  
37 progesterone. Bisphenol A exposure did not affect basal acrosome reaction or acrosome reaction induced  
38 by progesterone. Results were in contrast to those observed with 17β-estradiol, which inhibited the  
39 acrosome reaction induced by progesterone. The study authors concluded, BPA did not exert any direct  
40 effect on calcium fluxes and acrosomal reaction in human spermatozoa either in basal conditions or in  
41 response to P challenge.

42  
43 **Strengths/Weaknesses:** Strengths of this paper include examining human spermatozoa and use of a  
44 concurrent control (E2) to demonstrate the responsiveness of the system. Weaknesses include limited  
45 information on the spermatozoa samples, the single concentration of BPA used, and lack of clarity of  
46 concentrations of E2 versus bisphenol A administered.

47  
48 **Utility (Adequacy) for CERHR Evaluation Process:** This paper did not demonstrate that BPA-altered  
49 P-mediated acrosomal reaction and is not useful in the evaluation process.

#### 4.0 Reproductive Toxicity Data

1 **Hanaoka et al. (116)**, supported by the Japanese Ministry of Health and Welfare and Ministry of  
2 Education, Science, Sports, and Culture, examined possible relationships between bisphenol A exposure  
3 and hormone levels in male workers. Exposed workers included 42 men in 3 Japanese plants who sprayed  
4 an epoxy hardening agent consisting of a mixture of bisphenol A diglycidyl ether (10–30%), toluene (0–  
5 30%), xylene (0–20%), 2-ethoxyethanol (0–20%), 2-butoxyethanol (0–20%), and methyl isobutyl ketone  
6 (0–30%). The workers were said to wear “protection devices” during spraying. Controls consisted of 42  
7 male assembly workers from the same plants who did not use bisphenol A diglycidyl ether, were within 3  
8 years of age to exposed workers (37 years vs. 38 years), and smoked the same number of cigarettes/day as  
9 exposed workers (21/day). Percentages of smokers were 86% in both groups, but percentages of alcohol  
10 drinkers were significantly lower in the exposed workers (43%) than in controls (57%) ( $p=0.03$ ). Urine  
11 and blood samples were obtained during periodic health examinations performed in June and July, 1999.  
12 Urinary bisphenol A was measured by HPLC, and urinary organic solvent metabolites were measured by  
13 GC or HPLC. Plasma LH, FSH, and free testosterone levels were measured by immunosolvent assay in a  
14 commercial laboratory. Data were log transformed and compared by paired *t*-test, Pearson correlation  
15 coefficient, and chi-squared test. Adjustments were made by linear regression for age and drinking habits,  
16 which were considered possible confounders.

17  
18 Urinary bisphenol A concentrations were significantly higher in exposed workers (median: 1.06  
19  $\mu\text{mol/mol}$  creatinine [**0.043  $\mu\text{g/kg bw}$** ]; range: <0.05 pmol to 11.2  $\mu\text{mol/mol}$  creatinine) than in controls  
20 (median: 0.52  $\mu\text{mol/mol}$  creatinine [**0.021  $\mu\text{g/kg bw}$** ]; range: <0.05 pmol to 11.0  $\mu\text{mol/mol}$  creatinine).  
21 Average difference was reported as 2.5 (95% CI 1.4–4.7;  $P = 0.002$ ). Bisphenol A was not detected in 3  
22 exposed workers and 1 control. Urinary solvent metabolites were detected more frequently in exposed  
23 workers than controls. No differences in plasma testosterone or LH concentrations were observed  
24 between exposed workers and controls. Plasma FSH concentrations were significantly lower in exposed  
25 workers (median: 5.3 mIU/mL; range: 4.0–8.3 mIU/mL) than in controls (median 7.6 mIU/mL; range  
26 5.4–11.0 mIU/mL; average difference = 1.3; 95% CI –1.5 to –1.0). A “mild correlation” was reported  
27 between urinary bisphenol A and FSH ( $r = -0.20$ ,  $P = 0.071$ ) but was not observed for urinary solvent  
28 levels. A statistically significant relationship was observed between FSH and bisphenol A following  
29 adjustment for alcohol intake ( $r = -0.23$ ;  $P = 0.045$ ). The study authors concluded that bisphenol A may  
30 be generated endogenously following exposure to bisphenol A diglycidyl ether, and bisphenol A may  
31 disrupt gonadotropic hormone secretion in men.

32  
33 **Strengths/Weaknesses:** Quality assurance for the hormone radioimmunoassays appeared adequate.  
34 Blood draws and urine samples were time standardized between 10 AM and 12 noon. Reference values  
35 were given and population values were considered in the discussion. Use of HPLC for bisphenol A and  
36 standard methods for the other urinary metabolites with creatinine-adjustment are strengths. The epoxy  
37 sprayer workers were matched to coworkers from other parts of the process. All selected workers  
38 participated in the study. Analyses were adjusted for age and alcohol use, and workers were matched on  
39 age ( $\pm 3$  years) and cigarette use. A plausible ( $P = 0.07$ ) correlation between bisphenol A and decreasing  
40 FSH was reported. The authors took care to note that all FSH levels were within the clinical normal range.  
41 Correlations between other workplace exposures and hormones were not observed. Blood and urine  
42 samples were collected concurrently, but not on the first day of the week. Statistical methods were  
43 appropriate to the study size and distribution of the data. Non-normal distributions were transformed or  
44 treated as non-normal. Biomarker data were handled appropriately in analysis.

45  
46 **Utility (Adequacy) for CERHR Evaluation Process:** This survey was methodologically sound and  
47 mechanistically thoughtful. This study is adequate and of high utility for the evaluation.  
48

## 4.0 Reproductive Toxicity Data

### 4.2 Experimental animal

Studies in this section examine reproductive endpoints after administration of bisphenol A to sexually mature animals. Reproductive endpoints after administration of bisphenol A during pregnancy, the neonatal period, or puberty are discussed in Section 3.2.

#### 4.2.1 Female

##### 4.2.1.1 Rat

**Goloubkova et al. (240)**, supported by the Brazilian National Council of Scientific and Technological Development and the National University of Rio Grande Do Sul, examined the effects of bisphenol A exposure on the uterus and pituitary of ovariectomized rats. Wistar rats (60–67 days old) were fed a standard certified rodent diet. **[No information was provided on housing or bedding materials.]** Rats were subjected to bilateral ovariectomy or sham surgery. At 14 days post-surgery, rats were randomly assigned to groups of at least 6 animals. Rats were sc injected with bisphenol A in DMSO vehicle (>99% purity) at doses of 11, 78, 128, or 250 mg/kg bw/day for 7 days. An ovariectomized vehicle control group was exposed to the 50% DMSO vehicle. A sham-operated control group was not exposed to the vehicle. Rats were killed following the dosing period, and body and uterine weight were measured. Trunk blood was collected for measurement of serum prolactin level by RIA. The anterior pituitary was weighed and preserved in 10% formalin. An immunohistochemical technique was used to identify pituitary cells expressing prolactin. A total of 3 or 4 rats/group were evaluated for prolactin-positive cells in the pituitary and 6–8 rats were evaluated for the other endpoints. Data were analyzed by ANOVA followed by post hoc Student-Neuman-Keuls test or Kruskal-Wallis ANOVA followed by post hoc Dunn test.

In the 250 mg/kg bw/day group, final body weight was 7% lower than in the ovariectomized vehicle control group, and body weight gain was lower compared to the ovariectomized vehicle and sham controls. There was no effect of treatment on food intake. A dose-related increase in uterine weight occurred in all groups of rats exposed to bisphenol A compared to the ovariectomized vehicle controls, but uterine weight in the bisphenol A groups was lower than in the sham controls. Ovariectomy resulted in decreased pituitary weight in ovariectomized vehicle controls and in the bisphenol A 11 and 78 mg/kg bw/day dose groups compared to sham controls. Pituitary weight did not differ from sham controls after 128 mg/kg bw/day bisphenol A and was greater than in sham controls after 250 mg/kg bw/day bisphenol A. Basal prolactin levels did not differ between the sham and ovariectomized vehicle controls. Serum prolactin levels were increased in the 128 and 250 mg/kg bw/day bisphenol A groups compared to the ovariectomized vehicle controls. Ovariectomy reduced the numbers of prolactin-positive cells in the pituitary. The number of prolactin positive cells in the pituitary was increased by 64% in the 250 mg/kg bw/day group compared to the ovariectomized controls. The study authors concluded that the reproductive tract and neuroendocrine axis of Wistar rats can respond to bisphenol A.

**Strengths/Weaknesses:** This study represents a comprehensive neuroendocrine assessment across multiple doses. Weaknesses are the absence of a positive control to demonstrate maximal response in endpoints examined, high dose levels required to induce response, and the sc route of administration.

**Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate but of limited utility for the evaluation process.

**Funabashi et al. (483)**, supported by Yokoyama City University, examined the effects of bisphenol A exposure on expression of progesterone receptor mRNA in the brain of ovariectomized rats. The effects of butylbenzyl phthalate were also examined but will not be discussed. **[No information was provided on feed, caging, or bedding materials.]** Wistar rats were ovariectomized at 7–8 weeks of age. Ten days following ovariectomy, 6 rats/group were sc injected with sesame oil vehicle, 10 mg bisphenol A **[purity not reported]**, or 10 µg 17β-estradiol. Rats were killed 24 hours later and the preoptic area, medial basal

## 4.0 Reproductive Toxicity Data

1 hypothalamus, and anterior pituitary were removed. Expression of mRNAs for progesterone receptor,  
2 preproenkephalin, and neurotensin were assessed by Northern blot. Data were analyzed by ANOVA  
3 followed by Fisher protected least significant difference test. Exposure to bisphenol A resulted in  
4 increased expression of progesterone receptor mRNA in the preoptic area and anterior pituitary.  
5 Bisphenol A did not affect expression of mRNA for neurotensin in the preoptic area or preproenkephalin  
6 in medial basal hypothalamus. 17 $\beta$ -Estradiol increased expression of mRNA for progesterone receptor in  
7 the preoptic area, medial basal hypothalamus, and anterior pituitary and increased preproenkephalin  
8 mRNA expression in medial basal hypothalamus. The study authors concluded that bisphenol A increases  
9 expression of progesterone receptor mRNA in the preoptic area of adult ovariectomized rats.

10  
11 **Strengths/Weaknesses:** Strengths are the use of a positive control and the biological plausibility of the  
12 model. Weaknesses include subcutaneous administration of a single high dose level. .

13  
14 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate for inclusion but of limited  
15 utility.

16  
17 **Yamasaki et al. (158)** conducted a 28-day exposure study that provided some information on the  
18 reproductive organs of male and female rats. **[Complete details of this study are included in Section 2.**  
19 **Results for females are discussed in this section, and results for males are discussed in Section**  
20 **4.2.2.1.]** CD rats were fed a commercial diet (MF Oriental Yeast Co.) and housed in stainless steel wire  
21 mesh cages. Ten 7-week-old rats/sex/group were gavaged with bisphenol A **[98% purity]** at 0 (olive oil  
22 vehicle), 40, 200, or 1000 mg/kg bw/day for 28 days. Due to the death of 1 animal exhibiting clinical  
23 signs in the 1000 mg/kg bw/day group, the high dose was reduced to 600 mg/kg bw/day on the 8<sup>th</sup> day of  
24 the study. In an additional study, rats were exposed to ethinyl estradiol at 0, 10, 50, or 200  $\mu$ g/kg bw/day  
25 for 28 days. There were no treatment-related alterations in blood levels of thyroid hormones, FSH, LH,  
26 17 $\beta$ -estradiol, prolactin, or testosterone. The numbers of females with diestrus lasting 4 or more days was  
27 increased in the high-dose group. Relative weights of ovary and uterus were unaffected. No gross or  
28 histopathological alterations were reported for reproductive organs. The study authors concluded that  
29 change in estrous cyclicity was the only useful endpoint for evaluating the endocrine-mediated effects of  
30 bisphenol A. In comparison, females from the mid- and/or high-dose ethinyl estradiol group experienced  
31 alterations in estrous cyclicity, decreased ovarian weight, increased uterine weight, and histopathological  
32 changes in the ovary, uterus, and vagina.

33  
34 **Strengths/Weaknesses:** This study was well-conducted, used an appropriate route of administration, a  
35 positive control group, adequate sample sizes, a range of doses, and evaluations of both sexes.  
36 Weaknesses include failure to define the criteria for an abnormal estrous cycle, female necropsy at a point  
37 unrelated to stage of estrous.

38  
39 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
40 evaluation process.

41  
42 **Spencer et al. (484)**, supported by NIH, evaluated the uterine response to bisphenol A before and after  
43 deciduoma formation in pseudopregnant Sprague Dawley rats. **[Cage and bedding materials and feed**  
44 **were not indicated.]** Adult females underwent mechanical cervical stimulation to induce  
45 pseudopregnancy **[pseudopregnancy day not indicated]**. On pseudopregnancy day 4, deciduoma  
46 formation was induced under ether anesthesia by antimesometrial uterine epithelial trauma, applied  
47 through a laparotomy under ether anesthesia. Rats were treated with sc bisphenol A **[97% purity]** 0 or  
48 200 mg/kg bw in alcohol/saline on pseudopregnancy days 1–4 and killed on pseudopregnancy day 5, or  
49 treated on pseudopregnancy days 5–8 and killed on pseudopregnancy day 9. Uteri and pseudopregnancy  
50 day 9 endometria were harvested. Uteri were weighed and homogenized for measurement of protein and  
51 DNA content. Inducible nitric oxide synthase activity, decidual prolactin-related protein mRNA, ER

#### 4.0 Reproductive Toxicity Data

1 mRNA, and cytosolic ER binding sites were measured in uteri and/or endometria. Blood was obtained for  
 2 determination of serum 17 $\beta$ -estradiol and progesterone. [**n = 5 was indicated for some of the data**  
 3 **presentations.**] Results are summarized in [Table 87](#). The authors called attention to the difference in  
 4 bisphenol A effect depending on whether exposure was prior to or after deciduoma induction. They  
 5 concluded that there was a decrease in proliferation when bisphenol A was given during deciduoma  
 6 induction, with a decrease in decidual proteins, in spite of a lack of differential effect on *ER* mRNA or  
 7 cytosolic ER binding sites. The authors also concluded that bisphenol A activity appeared to be  
 8 antagonized by progesterone [**although they probably meant that bisphenol A antagonized the action**  
 9 **of progesterone**].

10  
 11 **Table 87. Bisphenol A Effects on Pseudopregnant Rats**

Endpoint	Treatment period, pseudopregnancy day	
	1–4	5–8
Uterus		
Wet weight	↑1.4-fold	↓63%
Protein content	↑1.4-fold	↓64%
DNA content	↔	↓53%
Decidual prolactin-related protein mRNA <sup>a</sup>	↔	↓44%
<i>ER</i> mRNA <sup>a</sup>	↓29%	↓50%
Cytosolic ER-binding sites	↓57%	↓37%
Nitric oxide synthase activity <sup>a</sup>	↔	↓50%
Pseudopregnancy day 9 endometrium		
Decidual prolactin-related protein mRNA <sup>a</sup>	Not applicable	↓48%
<i>ER</i> mRNA <sup>a</sup>	Not applicable	↓43%
Nitric oxide synthase activity <sup>a</sup>	Not applicable	↓40%
Serum		
17 $\beta$ -Estradiol	↔	↔
Progesterone	↔	↓49%

↑,↓,↔ Statistically significant increase, decrease, or no change compared to vehicle control.

<sup>a</sup>Estimated from a study graph by CERHR.

From Spencer et al. (484).

12  
 13 **Strengths/Weaknesses:** These data are intriguing, but the functional consequences of bisphenol A  
 14 administration on decidual formation were not assessed and the sc route of administration and the use of a  
 15 single high dose are a weakness.

16  
 17 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate but of limited utility to the  
 18 evaluation process.

19  
 20 **Funabashi et al. (485)**, supported by Yokohama City University, examined the effects of bisphenol A  
 21 exposure on sexual behavior and progesterone receptor expression in adult rats. Wistar rats were  
 22 ovariectomized at 7–8 weeks of age. [**No information was provided on feed, caging, or bedding**  
 23 **materials.**] In two sets of experiments, an immunohistochemistry technique was used to measure  
 24 expression of progesterone receptor in the preoptic area and ventromedial hypothalamus following  
 25 bisphenol A exposure. In the first experiment, 3–5 rats/group were sc injected with sesame oil vehicle, 10  
 26 mg bisphenol A (~40 mg/kg bw) [**purity not reported**], or 10  $\mu$ g 17 $\beta$ -estradiol (~40  $\mu$ g/kg bw) 2 weeks  
 27 following ovariectomy. In the second experiment, ovariectomized rats (3–4/group) were sc injected with  
 28 bisphenol A at 0.001, 0.010, 0.1, or 1 mg (~0.004, 0.040, 0.4, or 4 mg/kg bw). Rats were killed the day  
 29 following dosing, and brains were removed and fixed in 2% paraformaldehyde. Statistical analyses  
 30 included ANOVA followed by Scheffé post hoc test and Kruskal-Wallis test. Sexual behavior was

#### 4.0 Reproductive Toxicity Data

1 examined in a third experiment. Ovariectomized rats were sc injected with sesame oil vehicle, 10 mg  
2 bisphenol A, or 10 µg 17β-estradiol. The next day, rats were injected with 1 mg progesterone or vehicle  
3 to generate 4 treatment groups: sesame oil + progesterone (n = 5), bisphenol A + sesame oil (n = 5),  
4 bisphenol A + progesterone (n = 8), or estradiol + progesterone (n = 6). Examination of behavior with a  
5 sexually receptive male was conducted 5–7 hours following progesterone or vehicle injection. Statistical  
6 analyses included ANOVA followed by Scheffé post hoc test.

7  
8 In the first experiment, injection of rats with 10 mg bisphenol A increased progesterone-positive cells in  
9 both the preoptic area and ventromedial hypothalamus. The dose-response experiment demonstrated that  
10 dose-related increases in progesterone-positive cells in both brain regions occurred following exposure to  
11 ≥0.1 mg bisphenol A. In sexual behavior testing, treatment with bisphenol A had no effect on lordosis  
12 quotient. Rejection quotient was significantly higher in rats exposed to 10 mg bisphenol A and primed  
13 with 1 mg progesterone than in the vehicle control rats primed with progesterone. Treatment with 17®-  
14 estradiol resulted in increased numbers of progesterone positive cells in the preoptic area and ventral  
15 medial hypothalamus and increased lordosis quotient. The study authors concluded that the findings  
16 suggest that bisphenol A influences sexual behavior by altering the progesterone receptor system in the  
17 hypothalamus.

18  
19 **Strengths/Weaknesses:** This study appears to have been relatively well conducted with the incorporation  
20 of a positive control group and examination of anatomical and functional endpoints. The number of  
21 animals per group is sufficient given the nature of this study design. However, the route of administration  
22 was sc.

23  
24 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate for the evaluation process  
25 but of limited utility due to the route of administration.

26  
27 **Funabashi et al. (486)**, supported by Yokohama City University and the Japanese Ministry of Education,  
28 Culture, Sports, Science, and Technology, examined the effects of bisphenol A exposure on expression of  
29 progesterone receptor mRNA in brain of adult ovariectomized rats. *p*-Nonylphenol and 4-tert-octyl  
30 phenol were also examined, but will not be discussed. **[No information was provided on feed, housing,  
31 or bedding materials.]** Wistar rats were ovariectomized at 7 weeks of age, and experiments were  
32 conducted 10 days following ovariectomy. In the first experiment, 6 rats/group were sc injected with  
33 sesame oil vehicle or 10 mg bisphenol A (~40 mg/kg bw) **[purity not reported]**. Rats were killed 24  
34 hours following injection, and frontal, parietal, and temporal cortex were removed. In a second  
35 experiment, frontal, temporal, and occipital cortex were collected from rats at 0, 6, 12, or 24 hours  
36 following injection with 10 mg bisphenol A; 5–6 rats were killed and examined at each time point. In  
37 both experiments, progesterone receptor mRNA expression was determined by Northern Blot in each area  
38 of the cortex. Data were analyzed by ANOVA followed by Fisher protected least significant difference  
39 post hoc test. At 24 hours following bisphenol A exposure, expression of progesterone receptor mRNA  
40 was increased in the frontal cortex and decreased in the temporal cortex. In the time-course experiments,  
41 expression of progesterone receptor mRNA was increased in the frontal cortex and decreased in the  
42 temporal cortex from 6 to 24 hours following exposure. Bisphenol A had no effect on expression of  
43 progesterone receptor mRNA in the parietal or occipital cortex. The study authors concluded that  
44 bisphenol A can alter the neocortical function through the progesterone receptor in adult rats, but the  
45 physiological significance of the effect is not known.

46  
47 **Strengths/Weaknesses:** This study links relatively high single-dose (10 mg) sc bisphenol A  
48 administration to the induction of progesterone receptor mRNA, an estrogenic response. Weaknesses is  
49 the absence of a positive control to demonstrate maximal response in estrogen-mediated increases in  
50 progesterone mRNA and the failure to examine any physiological or functional endpoints. It was also not  
51 determined if increases in mRNA were associated with increases in progesterone receptor protein. There

## 4.0 Reproductive Toxicity Data

1 was only one dose level administered at a single time point. The sc route of dose administration is a  
2 weakness.

3  
4 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate for inclusion but of limited  
5 utility.

6  
7 **Della Seta et al. (477)**, supported by a grant from MURST, Italy, examined the effects of bisphenol A  
8 exposure on maternal behavior in rats. **[No information was provided in the manuscript on the type of**  
9 **chow, bedding, and caging used. The Expert Panel has been informed that Harlan Teklad 2018**  
10 **chow, Lignocel bedding, and polysulfone cages were used (F. Faraboli et al., personal**  
11 **communication, March 1, 2007).]** Female Sprague Dawley rats were trained to ingest peanut oil from a  
12 micropipette. At 14 weeks of age, female rats were mated for 48 hours. On the day following mating,  
13 females were randomly assigned to groups administered peanut oil (n=23) or 0.040 mg/kg bw/day  
14 bisphenol A **[purity not indicated in the manuscript; ≥95% according to the authors (F. Faraboli et**  
15 **al., personal communication, March 1, 2007)]** (n=17) through a micropipette. Dosing was continued  
16 through the gestation and lactation periods. Two days following delivery, litters were culled to 4 male and  
17 4 female pups and were cross-fostered within treatment groups. Pups were weighed on days 2, 7, and 21  
18 following birth. Maternal behavior was tested at 3 and 4 days and at 8 and 9 days following delivery. In  
19 30-minute test sessions, frequency, duration, and latency of behaviors such as retrieving pups, licking  
20 pups, postures, and nest building were evaluated with pups of the same sex. Behavior with pups of the  
21 opposite sex was evaluated on the second day of the test period, and the order of testing with male and  
22 female pups was reversed during each testing period (days 3–4 and 8–9). Data were analyzed by general  
23 linear model, Duncan multiple range test, and/or Mann-Whitney *U* test. The numbers of females giving  
24 birth were 9 of 17 in the bisphenol A group and 18 of 23 in the control group. Nine dams in the control  
25 group and 7 in the bisphenol A group were evaluated for maternal behavior. The only significant effect  
26 reported for bisphenol A was reduced duration of licking-grooming pups, which occurred with both sexes  
27 of pups during both observation periods **[~25–50 % decrease as estimated from a graph]**. Effects  
28 reported to be marginally significant were decreased frequencies of licking-grooming of pups ( $P < 0.09$ ),  
29 anogenital licking of pups ( $P < 0.08$ ), and arched back posture ( $P < 0.07$ ). The study authors concluded  
30 that maternal behavior in rats is influenced by prolonged exposure to low bisphenol A doses during  
31 pregnancy and lactation.

32  
33 This behavioral study suggested that a low, oral dose of bisphenol A (0.040 mg/kg bw/day) affects  
34 pregnancy and maternal behavior.

35  
36 **Strengths/Weaknesses:** Weaknesses include the use of a single dose level and an unusually low  
37 pregnancy rate in the controls (18/23) as well as the authors emphasis upon marginally significant  
38 bisphenol A effects

39  
40 **Utility (adequacy) of the Evaluation Process:** This study is adequate and of high utility for the  
41 evaluation process.

### 42 43 4.2.1.2 Mouse

44  
45 **Park et al. (487)**, support not indicated, examined the effects of bisphenol A exposure on the  
46 reproductive and hematological systems of male and female mice. **[Results for females are discussed**  
47 **here, and results for males are discussed in Section 4.2.2.2.]** Adult ICR mice were fed mouse  
48 formulation feed (Cheil Feed). **[No information was provided about caging or bedding materials.]**  
49 Fifteen mice/sex/group were ip injected with bisphenol A **[purity unknown]** in an ethanol/corn oil  
50 vehicle at 0.05, 0.5, or 5.0 mg/kg bw on 5 occasions (every 3 days over a 14-day period). One control  
51 group received no treatment and a second control group was ip injected with corn oil. Females were

#### 4.0 Reproductive Toxicity Data

1 examined 7 days following administration. Reproductive organs were weighed and fixed in Bouin  
2 solution, and histopathological examination was conducted. Hematological and clinical chemistry  
3 endpoints were also assessed. Data were analyzed by least significant difference test.  
4

5 Exposure to bisphenol A had no effect on body weight. Significant decreases were observed for right  
6 ovary weight in the mid- and high-dose group and left ovary weight in the mid-dose group [**25–27%  
7 lower**]. No treatment effects were observed for uterine or ovarian histology. There were no effects of  
8 bisphenol A treatment on hematological endpoints in females. Blood urea nitrogen levels were  
9 significantly decreased [**by 28–32%**] in females of all dose groups. The study authors did not report  
10 conclusions regarding study findings.

11  
12 **Strengths/Weaknesses:** The study design regarding frequency and route of administration and the lack of  
13 an appropriate positive control are weaknesses.

14  
15 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate though of limited utility for  
16 the evaluation process.

17  
18 **Berger et al. (409)**, supported by the Natural Sciences and Engineering Research Council of Canada,  
19 examined the effect of bisphenol A exposure on blastocyst implantation in mice. CF-1 mice were housed  
20 in polypropylene cages and were fed Harlan Teklad 22/5 rodent chow, which was stated to contain soy.  
21 [**No information was provided about bedding materials.**] On GD 1–4 or 5 [**described as GD 1–5 in  
22 methods section and GD 1–4 in study figures and tables**] (GD 0 = day of vaginal plug), 8–9  
23 mice/group were sc injected with peanut oil vehicle or bisphenol A (97% purity) at 10.125  
24 mg/animal/day. [**Assuming that the mice weighed 0.02 kg at the start of gestation (115), CERHR  
25 estimated bisphenol A intake at 500 mg/kg bw/day.**] Mice were killed on GD 6 for an examination of  
26 implantation sites. Data were analyzed by chi-squared test or 2 sample *t*-test. The number of implantation  
27 sites was significantly reduced in the treated animals (mean of ~2.5 compared to ~15 in controls).  
28 Implantation sites were observed in 8 of 8 control females at a range of 12–17/female. Six of 9 females in  
29 the bisphenol group had no implantation sites. The study authors concluded that pregnancy disruption  
30 occurred during the period of implantation.

31  
32 **Strengths/Weaknesses:** Weaknesses include lack of experimental details for examining the uteri, use of a  
33 single high dose, number of corpora lutea were not recorded.

34  
35 **Utility (Adequacy) for CERHR Evaluation Process:** Due to the absence of key information and faulty  
36 methodology, this study is inadequate for evaluation process.

37  
38 **Al-Hiyasat et al. (488)**, supported by Jordan University of Science and Technology, examined the effect  
39 of bisphenol A and dental composite leachate on fertility of female mice. In this study, Swiss mice were  
40 fed a standard laboratory feed containing soy protein. [**No information was provided on caging and  
41 bedding materials.**] At 60 days of age, 11 mice/group were gavaged with distilled water or composite  
42 leachate for 28 days. Components of the composite leachate were identified by HPLC and included tri-  
43 (ethylene glycol)-dimethacrylate (5945 mg/L), bisphenol A glycerolate dimethacrylate (2097 mg/L), and  
44 bisphenol A (78 mg/L). [**Based on the reported volume of administration of 0.2 mL and a body  
45 weight of 34.4 g, CERHR estimated bisphenol A intake from leachate at 0.45 mg/kg bw/day.**] Additional 60-day-old mice (n = 15/group) were gavaged with bisphenol A (97% purity), at doses of 0  
46 (ethanol/distilled water vehicle), 0.005, 0.025, or 0.1 mg/kg bw/day for 28 days. Five mice/group in the  
47 bisphenol A study were killed at the end of the dosing period for measurement of body, uterus, and ovary  
48 weights. All mice in the leachate study and 10 mice/group in the bisphenol A study were mated to  
49 untreated males (2 females to 1 male) for 10 days. One week following the end of the mating period, the  
50 mice were killed and examined for pregnancy, implantations, viable fetuses, and resorptions. Body,  
51

#### 4.0 Reproductive Toxicity Data

1 ovary, and uterus weights were measured in mice from the leachate study. Data were analyzed by Student  
2 *t*-test or Fisher exact test.

3  
4 Effects in the leachate group included increased relative (to body weight) ovarian weight and decreased  
5 percentages of pregnant mice. In mice exposed to bisphenol A, body weights were decreased at all dose  
6 levels. Effects observed in mice exposed to the mid and high dose of bisphenol A included increased  
7 uterine weight, increased percentages of resorptions/implantations, and increased percentages of mice  
8 with resorptions. Ovarian weight was increased in mice of the high-dose bisphenol A group. **[Although  
9 the effects were not statistically significant, the percentages of pregnant females were 90, 77.7, 80,  
10 and 60% pregnant mice in the control and each respective dose group.]** In both the composite  
11 leachate and bisphenol A groups, there were no statistically significant effects on implantations or viable  
12 fetuses. The study authors concluded that bisphenol A and components leached from dental composite  
13 have adverse effect on fertility and the reproductive system of mice.

14  
15 **Strengths/Weaknesses:** With only 5-10/group, this study was underpowered for determination of  
16 potential bisphenol A-related effects on fertility and other endpoints. Confirmation of mating was not  
17 performed (cohabitation was for 10 days; if the mice mated on day 10, the necropsy would have been  
18 performed on GD 7. Mean body weight and reproductive organ weights of bisphenol A-treated animals  
19 were only collected from 5 mice/dose level. Moreover, the normal body weight range for 10-week-old  
20 female Swiss mice is 28–35 g. Given that there are only 5 mice/group, it is hard to draw any meaningful  
21 conclusions from these data.

22  
23 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation based  
24 on small sample size.

25  
26 **Matsumoto et al. (430)**, support not indicated, examined the effect of maternal bisphenol A exposure on  
27 growth of offspring in mice; this paper was discussed in Section 3.2.7. Because the results of this study  
28 bear on lactation competence in treated dams, the study will also be considered here. Mice were fed  
29 standard rodent chow (CE-2, Japan Clea). **[No information was provided on caging and bedding  
30 materials.]** Mice of the ddY strain were exposed to bisphenol A ( $\geq 97\%$  purity) through feed at 0 or 1%  
31 from GD 14 through PND 7. The study authors stated that the bisphenol A dose was equivalent to 1000  
32 mg/kg bw/day. **[The numbers of dams treated was not indicated. Day of vaginal plug and day of  
33 birth were not defined.]** Mice delivered pups on PND 21. Body weight of pups were monitored during  
34 the postnatal period in 31 pups from the control group and 61–89 pups from the bisphenol A group.  
35 Serum prolactin levels were measured by RIA in 3 dams/group 4 days following delivery. Pups were  
36 killed on PND 7, and stomach weight was measured. Data were analyzed by Student *t*-test.

37  
38 No differences were reported for live pups at birth. During the postnatal period, body weights of pups in  
39 the bisphenol A group were significantly lower **[by ~40%]** than control group pups. No deaths were  
40 reported for pups in the control group, but 30% of pups in the bisphenol A group died before PND 7. On  
41 PND 1, milk could be seen in stomachs of pups from the control group, but not the bisphenol A group.  
42 **[The number of pups evaluated for milk in stomach was not reported].** On PND 7, stomach weight  
43 was significantly lower **[by 40%]** in pups from the bisphenol A compared to control group. Serum  
44 prolactin level was significantly reduced **[by 46%]** in dams from the bisphenol A group. The authors  
45 concluded that administration of a high bisphenol A dose to mice resulted in suppressed postnatal growth  
46 of offspring which probably resulted from an insufficient supply of milk, which might have been due to  
47 decreased prolactin secretion.

48  
49 **Strengths/Weaknesses:** This study was conducted at a single high dose that likely induced maternal  
50 toxicity (which was not assessed); therefore, it is difficult to delineate if the findings in the mouse pups  
51 are the result of potential bisphenol A-related effects of maternal toxicity or an effect on the pup.

## 4.0 Reproductive Toxicity Data

1 **Utility (Adequacy) for CERHR Evaluation Process:** Given the likely confounding effects of maternal  
2 toxicity, this study is considered inadequate and of no utility.

### 3 4 4.2.1.3 Other mammals

5 **Nieminen et al. (489)**, support not indicated, examined the effects of bisphenol A exposure on hormone  
6 levels in the European polecat (*Mustela putorius*). Five animals/group/sex [**age not reported**] were  
7 administered bisphenol A [**purity not reported**] in feed at concentrations providing doses of 0, 10, 50, or  
8 250 mg/kg bw/day for 2 weeks. Body weight and length were measured during the study. Animals were  
9 killed at the end of the exposure period, with sampling conducted in random double-blinded order. Liver  
10 and kidney were weighed. Blood samples were obtained for measurement of hormone levels by RIA.  
11 Microsomal enzyme activities were determined. Statistical analyses included ANOVA, post hoc Duncan  
12 test, Student *t*-test, Spearman correlation coefficient, Kolmogorov-Smirnov test, and/or Levene test.

13  
14 There were no clinical signs of toxicity and no effects on body weight or body mass index following  
15 bisphenol A exposure. Absolute and relative liver weight were significantly increased in females of the  
16 high-dose group. Plasma cortisol levels were significantly reduced in females of the mid-dose group.  
17 Bisphenol A exposure had no significant effects on plasma levels of testosterone, estradiol, FSH, or  
18 thyroid hormones. Glutathione-*S*-transferase (GST) activity was significantly increased in females of the  
19 high-dose group. UDPGT activity was significantly higher in females of the mid- and high-dose group  
20 and males of the high dose group. There was no effect on 7-ethoxyresorufin O-deethylase (EROD)  
21 activity. The study authors concluded that the endocrine effects in this study were not as remarkable as  
22 the effects on liver enzymes.

23  
24 **Strengths/Weaknesses:** A strength of this study is the use of a non-rodent species and multiple doses.  
25 Weaknesses include small sample size and absence of reproductive endpoints.

26  
27 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate and not useful due to  
28 small sample size and absence of reproductive endpoints.

29  
30 **Nieminen et al. (490)**, support not indicated, examined the effects of bisphenol A exposure on endocrine  
31 endpoints in field voles (*Microtus agrestis*). Animals were housed in plastic cages with wood shavings  
32 and fed R36 diet (Lactamin, Sweden). Sexually mature field voles were randomly assigned to groups that  
33 received bisphenol A [**purity not reported**] in propylene glycol by sc injection for 4 days. Doses of  
34 bisphenol A (numbers of females in each group ) were 0 (n = 5), 10 (n = 7), 50 (n = 5), and 250 (n = 8)  
35 mg/kg bw/day. Animals were killed the day following the last dose. Body and liver weights were  
36 measured. Blood was drawn for measurement of sex steroids, thyroxine, and weight-regulating hormone  
37 levels in plasma using RIA or immunoradiometry methods. The activities of EROD, UDPGT, and GST  
38 were measured in hepatic and renal microsomes using appropriate substrates. Statistical analyses included  
39 ANOVA, post hoc Duncan test, Student *t*-test, Kolmogorov-Smirnov test, Levene test, Mann-Whitney *U*  
40 test, chi-squared test, and Spearman correlation. [**Results for males are discussed in Section 4.2.2.3.**]

41  
42 Mortality was significantly increased by bisphenol A treatment, with incidences of 18, 36, and 20% in the  
43 low- to high-dose groups. No mortality was observed in the control group. Bisphenol A treatment did not  
44 significantly affect body or liver weight. Plasma testosterone levels increased with dose, and statistical  
45 significance was attained in high-dose females compared to control females. 17 $\beta$ -Estradiol levels  
46 decreased with dose in females. Pooled (male + female) LH levels were not significantly altered by  
47 treatment. Liver EROD activity [**apparently combined for males and females**] was significantly  
48 decreased at the mid and high dose, and liver GST activities [**not clear if for males or females or both**]  
49 was significantly decreased at the highest dose level. There were no other significant effects on  
50 microsomal enzymes examined. The study authors concluded that wild mammals such as field voles  
51 could be more susceptible to bisphenol A-induced toxicity than laboratory rodents.

## 4.0 Reproductive Toxicity Data

1 **Strengths/Weaknesses:** A strength is the use of another species. The small number of voles/dose level,  
2 the subcutaneous route of administration, and questionable statistical procedures are weaknesses.

3  
4 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation  
5 process.

6  
7 **Razzoli et al. (478)**, supported by the Ministry of University Education and Research and the University  
8 of Parma, examined the effects of bisphenol A on sociosexual and exploratory behavior in female  
9 Mongolian gerbils, a monogamous species. Animals were fed Mil Morini Rodent Chow (Reggio Emilia,  
10 Italy) and housed in Plexiglass cages with wood shaving/cotton nesting material. At 11–12 weeks of age,  
11 female gerbils were trained to drink corn oil from a syringe, and 1 week later, they were paired with a  
12 male. From the 1<sup>st</sup> through the 21<sup>st</sup> day of cohabitation, 12 females/group were fed 0 (corn oil vehicle),  
13 0.002, or 0.020 mg/kg bw/day bisphenol A [**purity not indicated**] from a syringe. A group of 12 females  
14 received ethinyl estradiol, the positive control, 0.04 µg/kg bw/day during the same time period. During  
15 the cohabitation period, social behavior (e.g., agonism, social investigation, huddling, and nest sharing)  
16 was observed and body weights of females were measured. A free exploratory test, which measured the  
17 amount of time females spent in an area of a cage with home nesting material compared to the time spent  
18 in an unfamiliar area of a cage, was conducted following the 21-day cohabitation period. Exploratory  
19 behavior was evaluated by an observer blinded to treatment groups. Statistical analyses included ANOVA  
20 and Duncan test for multiple comparisons.

21  
22 Bisphenol A treatment did not affect body weight. Social sniffing was significantly increased [**by 60%**]  
23 in the low-dose bisphenol A group. Significant effects [**percent changes compared to control**] observed  
24 in the exploratory test were decreased time in the unfamiliar area at the low [**60%**] and high [**44%**] dose,  
25 fewer transitions to the unfamiliar area at the low [**60%**] and high [**50%**] dose, fewer transitions to the  
26 home cage at the high dose [**29%**], and less time in the unfamiliar area at the low dose [**46%**]. Similar  
27 results for both social sniffing and exploratory behavior were observed in the positive control group.  
28 According to the study authors, this study demonstrated that chronic exposure of adult female gerbils to  
29 environmentally relevant doses of bisphenol A during the hormonally sensitive period of cohabitation  
30 resulted in subtly altered social and exploratory behavior.

31  
32 **Strengths/Weaknesses:** This study examined behavioral endpoints in gerbils, and included a positive  
33 control (ethinyl estradiol) and 2 doses of bisphenol A. It appears to be well conducted using oral dosing,  
34 respectable sample size (given study complexity), and use of a positive control. Weaknesses include  
35 failure to account for temporally repeated measures in statistical analyses.

36  
37 **Utility (adequacy) for CERHR Evaluation Process:** This study is adequate for inclusion but of limited  
38 utility for the evaluation process.

### 39 40 4.2.1.4 Invertebrates

41 Although studies in invertebrates may be important for understanding mechanisms of action and  
42 environmental impact, the Panel views these studies as not useful for the evaluation process.

43  
44 **Oehlmann et al. (491)**, supported by the Berlin Federal Environmental Agency, reported the effects of  
45 bisphenol A on reproductive organs in the freshwater ramshorn snail (*Marisa cornuarietis*) and the  
46 marine dog whelk (*Nucella lapillus*). In the first experiment, adult ramshorn snails were exposed for 5  
47 months to bisphenol A in ethanol at 0, 1, 5, 25, or 100 µg/L. Thirty snails/group were removed every  
48 month for evaluation of reproductive organs. [**Culture ware type not indicated. The purity of**  
49 **bisphenol A and its stability during the exposure period were not reported. The snails removed for**  
50 **evaluation were adults; this species requires 8 months to attain sexual maturity. Octylphenol was**  
51 **also evaluated, but is not discussed here.**] In the second experiment, ramshorn snails were exposed to

#### 4.0 Reproductive Toxicity Data

1 bisphenol A in ethanol at 0, 1, or 100 µg/L for 1 year. Thirty F<sub>1</sub> snails per time point were removed for  
2 evaluation at 6, 8, and 12 months. In the third experiment, dog whelk were exposed to bisphenol A in  
3 glacial acetic acid at 0, 1, 25, or 100 µg/L for 3 months. Thirty specimens were removed for evaluation  
4 each month. Evaluations included measurements of sex organs and the identification of sperm or oocytes  
5 in the genital tract. Statistical analyses included ANCOVA followed by Tukey or Student-Newman-Keuls  
6 test, Kruskal-Wallis test, chi-squared test, and Weir test.

7  
8 Adult ramshorn snails were reported to show increases in volume of the capsule and albumen glands  
9 (portions of the oviduct). **[Apparently, the increase in volume was based on appearance rather than**  
10 **measurements. The measured lengths of the sex organs were not affected by treatment.]** Occasional  
11 specimens that had been exposed to bisphenol A showed rupture of the oviduct with protrusion of the egg  
12 mass. Enumeration of spawning masses and eggs showed statistically significant time-dependent  
13 increases in all bisphenol A groups. Histologic examination of the gonads did not suggest abnormalities  
14 of spermatogenesis or oogenesis. The F<sub>1</sub> snails also demonstrated a statistically significant increase in  
15 spawning mass and oocyte production at the 100 µg/L bisphenol A concentration, and some specimens  
16 showed rupture of the oviduct at 12 months of age in both bisphenol A groups. An increase in imposex  
17 **[the presence of vas deferens tissue]** was noted significantly more often in snails exposed to bisphenol  
18 A 100 µg/L than controls. Adult dog whelk demonstrated a significant increase in the length and weight  
19 of the sex glands and an increase in number of females with oocytes in the oviduct. The authors  
20 concluded that invertebrates are sensitive to bisphenol A toxicity at environmentally relevant  
21 concentrations.

22  
23 **Strengths/Weaknesses:** The study appears to be well conducted and suggests that bisphenol A has  
24 stimulatory (17β-estradiol-like) effects on the spawning masses and eggs of snails. These changes  
25 occurred in the absence of a histological correlate. The potential stability/biotransformation was discussed  
26 in the introduction but not determined during the exposure period.

27  
28 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not considered useful for the  
29 evaluation process.

30  
31 **Forbes et al. (492)**, supported by the Bisphenol A Global Industry Group, evaluated the effects of  
32 bisphenol A on reproduction in the freshwater ramshorn snail (*Marisa cornuarietis*). Bisphenol A **[purity**  
33 **not indicated]** concentrations in test water were 0, 0.10, 1.0, 16, 160, and 640 µg/L. Concentrations were  
34 periodically checked. Thirty breeding pairs per treatment level were observed for a 12-week period. The  
35 number of egg masses and number of eggs/egg mass were recorded. Hatchability was evaluated using 5  
36 consecutive egg masses collected from 5 females/replicate (75 egg masses/treatment). Juvenile growth  
37 rates were calculated for a subset of the offspring. Nested ANOVAs were used for data analysis. All  
38 snails survived. There were no significant treatment-related differences in adult egg production,  
39 hatchability, or juvenile growth rate. Interindividual variability in these parameters was prominent, and  
40 the authors concluded that a large number of replicates would be necessary using this animal model to  
41 detect reproductive effects.

42  
43 **Strengths/Weaknesses:** This study examined dose response over a 12-week exposure of freshwater  
44 snails to bisphenol A with egg masses and number of eggs/egg mass as endpoints. Although no treatment-  
45 related effects were observed, interindividual variability was high.

46  
47 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not considered useful for the  
48 evaluation process

49  
50 **Schirling et al. (493)**, supported by the county of Baden-Württemberg, examined the effects of bisphenol  
51 A on embryo development in the apple snail, *Marisa cornuarietis*. Stocks of 150 adult snails were

## 4.0 Reproductive Toxicity Data

1 maintained in a glass aquarium containing tap water and sea salt, exposed to a 12/12 hour light/dark  
2 cycle, and fed fish flake food, carrots, and cucumbers. Fifteen to twenty eggs/exposure group were placed  
3 in a glass Petri dish with bisphenol A [purity not indicated] 50 or 100 µg/L [11.4 or 22.8 mM], ethinyl  
4 estradiol 10 µg/L, DMSO 0.005% (solvent for ethinyl estradiol), or water (solvent for bisphenol A). From  
5 embryo visibility (~3.5 days after egg laying) to ~day 14, eggs were evaluated daily for formation of eyes,  
6 tentacles, heart rate, and hatching. Statistical analyses were performed using Student *t*-test or Kruskal-  
7 Wallis test.

8  
9 There were no differences in formation of eyes and tentacles between treatments groups Heart rate was  
10 significantly decreased on day 9 for bisphenol A 100 µg/liter compared to the water control group with  
11 description of “a similar trend” in hatching. [The data figure does not show a statistically significant  
12 effect of bisphenol A treatment on hatching.] There was a significantly higher hatching weight in the  
13 100 µg/L bisphenol A group compared to the water control group. Ethinyl estradiol treatment  
14 significantly decreased embryo heart rate compared to the water control group but not compared to the  
15 DMSO control. No statistically significant effects of ethinyl estradiol on time to hatch or hatching weight  
16 were demonstrated. The authors concluded that bisphenol A and ethinyl estradiol had similar effects on  
17 snail development.

18  
19 **Strengths/Weaknesses:** Weaknesses include the lack of evaluation of the achieved concentration and  
20 stability of bisphenol A in water and the comparison of ethinyl estradiol to the water control instead of the  
21 DMSO control. The authors’ conclusions are weakened by the lack of statistical significance of most of  
22 their findings.

23  
24 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not considered useful for the  
25 evaluation process

### 26 27 4.2.1.5 *In vitro*

28 Although *in vitro* studies may be important for understanding mechanisms of action and cellular and  
29 subcellular events, the Panel views these studies as not useful for the evaluation process.

30  
31 **Xu et al. (494)**, supported by the Japan Society for the Promotion of Science, examined the effects of  
32 bisphenol A exposure on mouse ovarian granulosa cells in a series of experiments. Ovarian granulosa  
33 cells were obtained from 4-week-old B6C3F<sub>1</sub> mice. Following incubation of cells with 0 or 100 fM [23  
34 pg/L] to 100 µM [23 mg/L] bisphenol A [purity not indicated] in ethanol vehicle for 72 hours, the  
35 CellTiter 96 assay was used to evaluate cell viability, and the TUNEL assay and 4',6-diamidino-2-  
36 phenylindole staining were used to evaluate apoptosis. In cells that were incubated in 100 µM [23 mg/L]  
37 bisphenol A for 24, 48, or 72 hours, the TUNEL method was used to evaluate apoptosis and a flow  
38 cytometry technique was used to assess apoptosis and the cell cycle. Bcl2 and Bax protein expression was  
39 examined by Western blot, and mRNA expression was assessed by RT-PCR in cells that were exposed to  
40 100 µM [23 mg/L] bisphenol A for 72 hours. Experiments were repeated a minimum of 3 times.  
41 Statistical analyses included ANOVA followed by Fisher protected least significant difference test.  
42 [Statistical significance was not clearly indicated for some endpoints.]

43  
44 A dose-related reduction in cell viability was observed at bisphenol A concentrations ≥100 pM [23 ng/L].  
45 Examination of cells by the TUNEL method indicated a concentration-related increase in apoptosis at  
46 bisphenol A concentrations ≥100 pM [23 ng/L]. Features noted in apoptotic cells included cellular  
47 shrinkage, membrane blebbing, and nuclear condensation. Apoptotic cells, as determined by TUNEL and  
48 the presence of sub-G<sub>1</sub> cells were increased in a time-related manner following incubation with 100 µM  
49 [23 mg/L] bisphenol A from 24 to 72 hours. An increase in G<sub>2</sub>-M arrest was also observed and reached a  
50 maximum value following a 48-hour incubation of cells with 100 µM [23 mg/L] bisphenol A (18 vs. 12%  
51 in controls). Expression of Bax protein was increased and Bcl2 protein was decreased following

#### 4.0 Reproductive Toxicity Data

1 incubation with 100  $\mu\text{M}$  [23 mg/L] bisphenol A for 72 hours. Similar expression patterns were observed  
2 for *Bax* and *Bcl2* mRNA expression [data were not shown by study authors]. The study authors  
3 concluded that bisphenol A at doses of 100 pM [23 ng/L] and higher, presumably relevant to  
4 environmental concentrations, decreases viability and increases apoptosis in granulosa cells. The study  
5 authors postulated that apoptosis may have been induced by decreases in the anti-apoptotic protein Bcl2  
6 and increases in the pro-apoptotic protein Bax.

7  
8 **Strengths/Weaknesses:** Because this study used in vitro study PMSG-stimulated murine cells,  
9 metabolism is likely to have been minimal (if present at all) and the in vitro dosimetry of bisphenol A is  
10 difficult to extrapolate to in vivo dosimetry. Bisphenol A is known to induce reactive oxygen species,  
11 which may influence the tetrazolium salt-based assay. Moreover, based on the data presented the  
12 mechanism by which bisphenol A may be inducing cell cytotoxicity/apoptosis is likely not “endocrine  
13 disruptor” mediated.

14  
15 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not considered useful for the  
16 evaluation process

17  
18 **Mlynarciková et al. (495)**, supported by the European Union, examined the effects of bisphenol A  
19 exposure on hormone production by porcine ovarian granulosa cells. Granulosa cell cultures were  
20 prepared from porcine ovaries collected from a slaughter house. The cells were incubated for 72 hours in  
21 media containing bisphenol A [purity not indicated] at  $10^{-8}$  to  $10^{-4}$  M [2.3  $\mu\text{g/L}$  to 23 mg/L] or the  
22 DMSO vehicle, with or without addition of 1  $\mu\text{g/mL}$  FSH or LH. Following the incubation period, media  
23 were collected for measurement of progesterone and  $17\beta$ -estradiol concentrations by RIA. Experiments  
24 were replicated 5–8 times. Data were analyzed by ANOVA and Bonferroni post test. Significant changes  
25 in progesterone production, included an increase at  $10^{-5}$  M [2.3 mg/L] and decrease at  $10^{-4}$  M [23 mg/L]  
26 bisphenol A. Bisphenol A significantly increased FSH-stimulated progesterone synthesis at  $10^{-6}$  M [0.23  
27 mg/L] and inhibited FSH-stimulated progesterone production at  $10^{-4}$  M [23 mg/L]. LH-induced  
28 progesterone production was inhibited by  $10^{-4}$  [23 mg/L] bisphenol A. FSH-induced  $17\beta$ -estradiol  
29 production was also inhibited by bisphenol A at all concentrations tested, but statistical significance was  
30 only attained at doses  $\geq 10^{-6}$  M [0.23 mg/L]. Bisphenol A dimethylacrylate was also tested, and most  
31 results were similar to those observed with bisphenol A. The study authors concluded that ovarian  
32 steroidogenesis might be a target of bisphenol A toxicity.

33  
34 **Strengths/Weaknesses:** Potential estrogenic effects were observed at  $10^{-5}$  M bisphenol A. Decreases in  
35 responses observed at the  $10^{-4}$  M concentration are likely due to nonspecific cytotoxicity. Bisphenol A-  
36 mediated responses in progesterone endpoints appeared to reach a near maximum at the lowest dose level  
37 examined. There was no mention of whether phenol red-free media were used or whether fetal bovine  
38 serum was charcoal-stripped. The serum likely contained steroids, which would have been potential  
39 confounding factors. Also, it appears that cell viability was not examined after the incubation period.  
40 With exception of the highest dose level, there was no dose response (inconsistent trends); the statistical  
41 flags are potentially due to random chance. Since this was an in vitro study, the potential effects of  
42 metabolism could not be assessed.

43  
44 **Utility (Adequacy) for CERHR Evaluation Process:** Due the weaknesses and limitation in the  
45 experimental design, this study is considered inadequate.

46  
47 **Mohri and Yoshida (496)**, supported by the Japanese Ministry of Education, Science, Sports, and  
48 Culture, examined the effects of bisphenol A and  $17\beta$ -estradiol exposure on calcium oscillations in  
49 immature mouse oocytes. Immature oocytes with intact germinal vesicles were obtained from 8–12-week-  
50 old CD-1/ICR mice and incubated in bisphenol A [purity not indicated] in a DMSO vehicle at

## 4.0 Reproductive Toxicity Data

1 concentrations of 0 or 10 nM [2.3 µg/L] to 100 µM [23 mg/L] for 60 minutes. Calcium oscillations were  
2 measured using a Fura-2 dye and image analyzer. Data were analyzed by Student *t*-test. At 100 µM [23  
3 mg/L] bisphenol A, the duration of calcium oscillations was significantly shortened and the oscillations  
4 became irregular. The same findings were observed following exposure to 17β-estradiol at concentrations  
5 that were 10,000-fold lower than that of bisphenol A, producing the same effect. The study authors stated  
6 that estrogens may affect the oocyte by regulating calcium oscillations and that bisphenol A could affect  
7 oocyte maturation.

8  
9 **Strengths/Weaknesses:** This study appears to have been well conducted; however, because this study  
10 used an in vitro system, metabolism could not be assessed. It is unclear if calcium oscillations play a role  
11 in oocyte maturation in other species, including humans.

12  
13 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not considered useful for the  
14 evaluation process,

### 15 16 4.2.2 Male

17 Studies on the androgenicity of bisphenol A, including Hershberger assays, are discussed in Section 2.2.3.

#### 18 19 4.2.2.1 Rat

20 **Yamasaki et al. (158)**, support not indicated, conducted a 28-day exposure study that provided some  
21 information on the reproductive organs of male and female rats. [Complete details of this study are  
22 included in Section 2. Results for males are discussed in this section, and results for females are  
23 discussed in Section 4.2.1.1.] CD rats were fed a commercial diet (MF Oriental Yeast Co.) and housed in  
24 stainless steel wire mesh cages. Ten 7-week-old rats/sex/group were gavaged with bisphenol A [98%  
25 purity] at 0 (olive oil vehicle), 40, 200, or 1000 mg/kg bw/day for 28 days. Due to the death of 1 animal  
26 exhibiting clinical signs in the 1000 mg/kg bw/day group, the high dose was reduced to 600 mg/kg  
27 bw/day on the eighth day of the study. In an additional study, rats were exposed to ethinyl estradiol at 0,  
28 10, 50, or 200 µg/kg bw/day for 28 days. There were no treatment-related abnormalities in sperm or  
29 alterations in blood levels of thyroid hormones, FSH, LH, 17β-estradiol, prolactin, or testosterone.  
30 Changes in relative reproductive organ weights [assumed to be relative to body weight] included a 28%  
31 decrease in relative ventral prostate weight and 21% increase in relative testis weight in the high-dose  
32 group. No gross or histopathological alterations were reported for reproductive organs. The study authors  
33 concluded that change in estrous cyclicity was the only useful endpoint for evaluating the endocrine-  
34 mediated effects of bisphenol A. In comparison, male rats exposed to the mid and/or high doses of ethinyl  
35 estradiol experienced decreased prostate, seminal vesicle, and pituitary weights; increased testis weight;  
36 and histopathological alterations in prostate, seminal vesicle, mammary gland, and testis.

37  
38 **Strengths/Weaknesses:** This study was well-conducted, used an appropriate route of administration, a  
39 positive control group, adequate sample sizes, a range of doses, and evaluations of both sexes. A  
40 weaknesses include an insufficient duration of exposure to examine the full spermatogenic cycle.

41  
42 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
43 evaluation process.

44  
45 **Takahashi and Oishi (497)**, support not indicated, examined the effects of bisphenol A exposure on  
46 testis of rats. F344 rats were fed standard, soy-containing diet (CE-2, Clea Japan, Inc. Tokyo) and housed  
47 in stainless steel suspended cages. Four-week-old male rats (n = 8/group) were administered bisphenol A  
48 (99.0% purity) through diet at concentrations of 0, 0.25, 0.5, or 1.0% for 44 days. The study authors  
49 estimated bisphenol A intake at 235, 466, and 950 mg/kg bw/day. The stability of bisphenol A in the diet  
50 was verified. Food intake was measured, and animals were weighed and observed daily for clinical signs.  
51 Rats were killed when mean body weight of controls reached ~200 g. Testosterone levels were measured

#### 4.0 Reproductive Toxicity Data

1 in serum using an ELISA method. Preputial gland, testes, epididymides, prostate, seminal vesicles,  
 2 kidneys, and liver were weighed. The testis was fixed in buffered 6% formaldehyde and examined  
 3 histologically. Statistical analyses included Bartlett test, ANOVA, Dunnet or Scheffé parametric test,  
 4 Kruskal-Wallis test, Dunnet non-parametric test, Wilcoxon rank sum test, chi-squared test, Mantel-  
 5 Haenzel test, and Fisher exact test.

6  
 7 Statistically significant findings are summarized in [Table 88](#). Body weight gain and terminal body  
 8 weights were reduced in males of the mid- and high-dose groups. Food intake was said to be slightly  
 9 decreased according to dose. Absolute organ weight effects included decreased weight of preputial glands  
 10 at all doses; liver in the mid and high dose group; and seminal vesicles with coagulation glands, dorsal  
 11 and lateral prostate, and hypophysis at the high dose. **[The Expert Panel assumes that by coagulation**  
 12 **gland, the authors mean the anterior prostate or coagulating gland.]** Significant organ weight effects  
 13 relative to body weights are summarized in [Table 88](#). Changes in relative organ weights included  
 14 decreased preputial gland weight and increased kidney weights at all doses, decreased liver weight at the  
 15 mid and high dose, and decreased dorsal and lateral prostate weight at the high dose. Testicular lesions  
 16 observed with bisphenol A treatment included seminiferous tubule degeneration at the mid and high dose,  
 17 disorganized spermatids at all dose levels, and differences in percentages of seminiferous tubules in  
 18 spermatogenic stages at all dose levels. Although it does not appear that statistical significance was  
 19 attained, dose-related increases in arrested spermatogenesis and disappearance of elongated spermatids  
 20 were also reported. There were no significant effects on serum testosterone concentrations. The study  
 21 authors concluded that bisphenol A was toxic to the testis and accessory sex organs of F344 rats at a  
 22 minimum toxic dose of 235 mg/kg bw/day.

23  
 24 **Table 88. Effects Observed in Male Rats Exposed to Bisphenol A Through Diet**

Endpoint	Dose, % in diet [mg/kg bw/day]						
	0.25	0.5	1.0	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
Terminal body weight	↔	↓13%	↓18%	0.55 [522]	0.42 [399]	0.41 [389]	0.30 [285]
Relative weight							
Dorsal and lateral prostate	↔	↔	↓32%	0.29 [276]	0.22 [209]	0.52 [494]	0.36 [342]
Preputial gland <sup>a</sup>	↓22%	↓26%	↓25%	0.13 [124]	0.09 [86]	0.18 [171]	0.12 [114]
Liver	↔	↓10%	↓14%	0.69 [656]	0.56 [532]	0.30 [285]	0.23 [218]
Kidney	↑8%	↑8%	↑12%	0.99 [940]	0.69 [656]	0.50 [475]	0.34 [323]
No. rats with							
Seminiferous tubule degeneration <sup>b</sup>	↔	↑ to 6/8	↑ to 5/8				
Disorganization of stage I-VI spermatids (+ severity) <sup>b</sup>	↑ to 4 of 8	↔	↔				
Disorganization of stage I-VI spermatids (2+ severity) <sup>b</sup>	↔	↔	↑ to 6 of 8	0.36 [342]	0.22 [209]		
% Seminiferous tubules in stages							
I-VI	↓59%	↓70%	↓53%				
IX-XI	↑3.4-fold	↑5.2-fold	↑4-fold				
XII-XIV	↑3.2-fold	↑3.6-fold	↑3-fold				

↑, ↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effect compared to controls.

<sup>a</sup>Benchmark doses were estimated using a polynomial model.

<sup>b</sup>Control value = 0 of 8. From Takahashi and Oishi (497).

25  
 26 Findings suggest a hormonal effect on hormone-dependent reproductive tissues at all doses examined.  
 27 The lowest dose level, 0.25% in diet, exhibited histopathological changes in the testes, most strikingly  
 28 described as a large alteration in the relative frequency of the different stages of the seminiferous

#### 4.0 Reproductive Toxicity Data

1 epithelium. Due to techniques used for fixation and embedding of the testes, the histopathological  
2 analyses may be of limited value.

3  
4 **Strengths/Weaknesses:** This paper reports a relatively well conducted study with a relevant route of  
5 administration. General toxicity was demonstrated. Formalin produces excessive shrinkage of testes when  
6 followed by paraffin embedding and is inappropriate especially when staging will be conducted.

7  
8 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
9 evaluation process.

10  
11 **Sakaue et al. (498)**, supported by the Japanese Science and Technology Agency, examined the effect of  
12 bisphenol A exposure on spermatogenesis in the adult rat. Animals were fed CE-2 chow (CLEA Japan)  
13 and housed in stainless steel wire caging. Thirteen-week old male Sprague Dawley rats (5/group) were  
14 gavaged for 6 days with the ethanol/corn oil vehicle or bisphenol A (99.6% purity) at doses 0.020, 0.200,  
15 2, 20, or 200 mg/kg bw/day. The high dose was based upon a preliminary experiment that demonstrated  
16 reduced daily sperm production in a Holtzman rat gavaged with 200 mg/kg bw/day bisphenol A for 6  
17 days. In this study, rats were killed 2 days following dosing (at 14 weeks of age) or at 18 weeks of age.  
18 Testes were weighed. Sperm endpoints were measured from one testis. Histopathological examinations  
19 were conducted on the other testis after fixation in Bouin fluid, paraffin embedding, and staining with  
20 hematoxylin and eosin. Statistical analyses included Student *t*-test, ANOVA, and Fisher protected least  
21 significant difference test.

22  
23 There were no changes in daily sperm production/g testis at 14 compared to 18 weeks of age. **[No data  
24 were shown for 14-week-old rats, and results of bisphenol A treatment were not discussed.]**  
25 Bisphenol A did not significantly affect body or testis weight at 18 weeks of age. In the 18-week-old rats,  
26 daily sperm production and daily sperm production/g tissue were significantly reduced **[by ~25%]** in all  
27 bisphenol A treatment groups. The study authors noted the lack of a dose-response relationship and that  
28 daily sperm production in treated groups at 18 weeks of age was comparable to that of 14-week-old  
29 controls. Histopathological evaluations of testis revealed no evidence of atrophy or disrupted  
30 spermatogenesis in the seminiferous tubules. **[Data were not shown by study authors.]**

31  
32 To obtain more dose-response information, Sakaue et al. (498) repeated the study in 8 rats/group dosed  
33 **[assumed by gavage as in the first study]** with 0.000002, 0.00002, 0.0002, 0.002, 0.020, 0.200, or 2  
34 mg/kg bw/day bisphenol A. **[It is assumed that ages of rats, treatment period, and observation  
35 periods were the same as in the first study.]** Body and testis weights were not affected by bisphenol A  
36 treatment at week 18. At week 18, significant decreases in daily sperm production and daily sperm  
37 production/g tissue were observed at 0.020, 0.200, and 2 mg/kg bw/day. **[The decrease compared to  
38 control was estimated from a graph. For daily sperm production, the decreases were ~30% at 0.020  
39 mg/kg bw/day, ~34% at 0.200 mg/kg bw/day, and ~32% at 2 mg/kg bw/day. For daily sperm  
40 production/g tissue, the decreases were ~24% at 0.020 mg/kg bw/day, ~32% at 0.200 mg/kg bw/day,  
41 and ~28% at 2 mg/kg bw/day.]**

42  
43 In a third experiment, rats were given a single oral dose of 0.020 mg/kg bw bisphenol A. Six hours later,  
44 the rats were killed, the right testis was homogenized, and the cytosol was examined for protein  
45 expression using two-dimensional polyacrylamide gel electrophoresis. Changes in intensity and mobility  
46 were noted for 3 unidentified proteins. The study authors concluded that the dose-response curve for  
47 bisphenol A affects on spermatogenesis in the adult rat was monotonic rather than having an inverted U-  
48 shape.

49  
50 **Strengths/Weaknesses:** This study used a relevant route of administration and multiple doses. A  
51 weakness is the brief exposure period. Variability in control daily sperm production between the first and

#### 4.0 Reproductive Toxicity Data

1 second study is disturbing; given the small sample (5 or 8/group), this variability severely decreases  
2 confidence in the data. No histopathologic correlate was presented.

3  
4 **Utility (adequacy) for CERHR Evaluation Process:** This study is adequate but of limited utility due to  
5 small sample and variable control values between experiments.

6  
7 **Ashby et al. (499)**, support not indicated, examined the effects of bisphenol A exposure on sperm  
8 production in rats. The study attempted to replicate earlier findings from Sakaue et al. (498). Five  
9 independent experiments were conducted, and the conditions for each experiment are summarized in  
10 [Table 89](#). Some of the experiments used the same conditions as the Sakaue et al. (498) study, including  
11 stainless steel cages with no bedding, CE2 diet (CLEA, Tokyo, Japan), and glass water bottles. In the first  
12 4 studies, 10–20 adult (~13-week-old) Sprague Dawley rats/group were gavaged with bisphenol A (99%  
13 purity) at 0 (ethanol/corn oil vehicle), 0.020, 2, or 200 mg/kg bw/day for 6 days. Concentrations of dosing  
14 solutions were verified. In the fifth study, rats fed different diets were gavaged with vehicle for 6 days.  
15 Rats were fed 1 of 3 diets as indicated in [Table 89](#). Phytoestrogen aglycone content of the feed was  
16 measured. Respective concentrations of daidzein, genistein, and coumestrol in each feed were reported at  
17 94, 62, and 0.6 µg/g diet for Rat and Mouse No. 3 (RM3; Special Diet Services Ltd.); 40, 23, and 0.1 µg/g  
18 diet for 5002 (Purina Mills); and 157, 106, and 2.2 µg/g CE2 diet. Ten rats were used in each group,  
19 except in third and fourth studies, where 20 control rats were split into 2 groups prior to dosing. Rats were  
20 administered drinking water through an automatic system in the first study and via glass bottles in the  
21 other studies. In the first study, rats were housed 3/cage at the start of the study and 2/cage later in the  
22 study. In the other 4 studies, rats were housed 2/cage. Rats were weighed during the study. Animals were  
23 killed at 18 weeks of age, 5 weeks after the start of dosing. Liver, kidney, and reproductive organs were  
24 weighed, and sperm counts were obtained. In the first 4 studies, data were analyzed by ANOVA,  
25 ANCOVA for organ and body weights, and Dunnett test. Results from all 4 studies were also analyzed by  
26 ANOVA in an attempt to increase study power. Data from the fifth study were analyzed by Fisher least  
27 significant difference test.

28  
29 In the four studies that compared the effects of bisphenol A exposure to a vehicle control group, there  
30 were no significant effects of bisphenol A exposure on sperm count, daily sperm production, or weights  
31 of body, liver, kidney, testis, prostate, epididymis, or seminal vesicle. One animal exposed to 200 mg/kg  
32 bw/day bisphenol A in the third study was reported to have unexpectedly small testes and epididymides,  
33 but the study authors indicated that inclusion of this animal in later statistical analyses had no effect on  
34 outcome. One animal in the 200 mg/kg bw/day group in the fourth study had a small testis. No significant  
35 effects were observed when data from the first 4 experiments were pooled and analyzed. The study  
36 authors noted that some endpoints were variable from one experiment to the other. It was noted that  
37 prostate weights were 10% lower in animals from Experiment 1 than from Experiments 2–4. Sperm  
38 counts and daily sperm production were reportedly different in control animals from Experiment 1  
39 compared to Experiment 2. It was noted that rats were fed different diets in Experiment 1 (RM3) and  
40 Experiment 2 (5002), and a study to examine the effects of feed was conducted. In the study examining  
41 effects in rats fed different diets but exposed to vehicle, no effects of diet on daily sperm production were  
42 observed. The only significant effect reported was a 9% lower weight of right epididymis in rats fed CE2  
43 compared to RM3 or 5002 feed. The study authors stated that the effect was likely spurious due to lack of  
44 effect on other endpoints, no effect on left or total epididymis weight, and lack of the effect in the first 4  
45 experiments. The study authors concluded that there was no evidence in their study that bisphenol A  
46 affected reproductive organ weights or daily sperm production. Lack of bisphenol A-induced effect on  
47 daily sperm production was in contrast to observations of the Sakaue et al. (498) study, which reported a  
48 decrease in this endpoint. Subtle genetic differences in the rats were suggested as a possible reason for  
49 differences in results between the 2 studies.

## 4.0 Reproductive Toxicity Data

1 **Table 89. Conditions Used in Experiments to Study Bisphenol A Effects on Sperm Production in**  
 2 **Rats**

Experiment	Bisphenol A doses, mg/kg bw/day	No. rats/group	Diet/water	Caging
1	0, 0.020, 2, or 200	10	RM3/Automatic system	Stainless steel, unspecified bedding
2	0, 0.020, 2, or 200	10	5002/Glass bottles	Stainless steel, no bedding
3	0, 0.020, 2, or 200	10/bisphenol A group; 20 in control group	5002/ Glass bottles	Stainless steel, no bedding
4	0, 0.020, 2, or 200	10/bisphenol A group; 20 in control group	CE2/ Glass bottles	Stainless steel, no bedding
5	0	10	RM3, 5002 or CE2/not specified	Not specified

From Ashby et al. (499).

3  
 4 Given the robustness and comprehensiveness of this study, it is highly useful. It strongly suggests that the  
 5 NOAEL for potential bisphenol A-mediated effects on the adult rat reproductive system exceeds 200  
 6 mg/kg/day. Absence of confirmation of the work of Sakaue et al. (498) led to an extensive study of the  
 7 potential variables (e.g. diet, housing, etc.) that might account for the discrepancies. These data suggests  
 8 that subtle changes in study endpoints, especially daily sperm production and organ weights, may occur  
 9 by random chance or genetic differences in the respective lab's supplier of rats may play a role. These  
 10 data also strongly suggest bisphenol A administered orally has no effect on sperm production albeit  
 11 following only 6 days of administration.

12  
 13 **Strengths/Weaknesses:** This paper reports a well conducted, comprehensive assessment of the potential  
 14 effects of bisphenol A delivered by 6 daily doses on daily sperm production. The 6 day treatment period  
 15 is a (understandable) weakness.

16  
 17 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and useful for the  
 18 evaluation process.

19  
 20 **Tohei et al. (500)**, supported in part by the Japan Society for the Promotion of Science, examined the  
 21 effects of bisphenol A exposure on testicular function of Wistar-Imamichi rats. **[No information was**  
 22 **provided about composition of chow, bedding, or caging.]** In a series of studies, rats were dosed with  
 23 bisphenol A **[purity not indicated]** in sesame oil by sc injection for 2 weeks. Bisphenol A doses were 0.1  
 24 or 1 mg/day **[~0.3 or 3 mg/kg bw/day based on the reported body weights of 300–350 g]**. The dose of  
 25 1 mg/day bisphenol A was stated to be similar to the highest exposures reported in humans, which were  
 26 based on saliva levels measured in patients receiving composite dental sealants. Doses and exposure  
 27 duration were based on results of preliminary studies. Five or 6 animals/dose group were used in each  
 28 experiment. Statistical analyses included ANOVA, Fisher protected least significant difference test, and  
 29 Mann-Whitney *U* test.

30  
 31 In the first study conducted to examine testicular and pituitary function, LH, FSH, prolactin, testosterone,  
 32 and inhibin were measured in plasma, pituitary, and/or testis by RIA in rats sc dosed with 1 mg/day  
 33 bisphenol A for 2 weeks. Statistically significant effects **[percent differences compared to controls, as**  
 34 **estimated from a graph]** included increases in plasma levels of LH **[150%]** and prolactin **[1067%]** and  
 35 decreases in levels of plasma testosterone **[29%]** and testicular inhibin **[36%]**.

#### 4.0 Reproductive Toxicity Data

1 In a second experiment to examine testicular response, rats were sc dosed with 0.1 or 1 mg/day bisphenol  
2 A for 2 weeks. The rats then received 10 IU hCG through an atrial cannula. Blood samples were drawn  
3 for measurement of progesterone and testosterone levels before and at various time intervals between 30  
4 and 180 minutes following the hCG challenge. Plasma progesterone and testosterone levels were increased  
5 following the hCG challenge in control rats. In the bisphenol A-treated rats, only a slight increase in  
6 progesterone levels occurred 30 minutes following challenge, and plasma progesterone levels were  
7 significantly lower compared to the control group at 60–150 minutes following challenge. There was an  
8 increase in plasma testosterone level following challenge of the bisphenol A group, but values were  
9 significantly lower than control values at 90–120 minutes following the challenge.

10  
11 In a third experiment examining pituitary response, adult male rats were castrated 5 days before bisphenol  
12 A treatment. Castrated rats were sc injected with 1 mg/day bisphenol A and 75 µg/day testosterone  
13 propionate for 2 weeks. The rats then received 250 ng gonadotropin-releasing hormone by sc injection.  
14 Plasma LH was measured before and at various time intervals between 0.25 and 4 hours following the  
15 gonadotropin-releasing hormone challenge. No statistically significant effects were observed.

16  
17 In a fourth study, males were dosed with 1 mg/day bisphenol A for 2 weeks and then paired with females  
18 in proestrus. Sexual function was evaluated by scoring mounts, intromissions, and ejaculations. No  
19 significant effects were observed for sexual function. Based on the findings reported in all studies, the  
20 study authors concluded that “The testis is probably a more sensitive site for [bisphenol A] action than the  
21 hypothalamus-pituitary axis.”

22  
23 **Strengths/Weaknesses:** RIAs appear to have been competently conducted. sc is not a relevant route of  
24 exposure, and the sample size was limited. Blood collection via decapitation is not appropriate, because  
25 decapitation stress affects plasma prolactin and LH secretion. No mention is made of the order of killing.  
26 If controls were killed first and the guillotine was not cleaned between uses (and animals were not in  
27 separate rooms), there may be serious confounding of the data. Because rat plasma testosterone levels are  
28 normally highly variable, the low degree of variability in this study, given the small sample size, is  
29 remarkable ( $\sim \pm 0.12$  ng/mL). No functional consequence of the alterations in hormone levels were  
30 described. Weaknesses include use of two doses delivered subcutaneously, critically small sample sizes,  
31 use of an inappropriate method of plasma collection, the stressful nature of cannula insertion just one day  
32 prior to measurement, and inappropriate statistical analyses that did not account for temporally repeated  
33 measures.

34  
35 **Utility (adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation  
36 process.

37  
38 **Kim et al. (154)**, supported by the Korean Ministry of Health and Social Welfare, examined the effects of  
39 bisphenol A exposure on the male reproductive system. A translation of the study was provided by the  
40 American Plastics Council. Four-week-old male F344 rats (7/group) were given bisphenol A in drinking  
41 water at 0 (ethanol vehicle), 0.1, 1, 10, or 100 ppm for 13 weeks. According to the study authors, these  
42 values were equivalent to 0.011, 0.116, 1.094, and 11.846 mg/kg bw/day. **[No information was provided**  
43 **about bisphenol A purity, or feed, caging, or bedding materials.]** Body weight and food and water  
44 consumption were measured during the study. Urine was collected for 24 hours following completion of  
45 dosing, and then animals were killed. Blood was collected. Organs, including those of the male  
46 reproductive system, were weighed. Parts of organs were preserved in formalin and examined  
47 histologically. Testes and epididymides were preserved in liquid nitrogen to obtain sperm counts and for  
48 measurement of levels of testicular enzymes. Data were analyzed by ANOVA.

49  
50 Bisphenol A treatment had no significant effect on body weight or food or water intake. There were no  
51 effects on absolute or relative weights of the testis, epididymis, prostate, seminal vesicle, liver, kidney,

## 4.0 Reproductive Toxicity Data

1 heart, lung, spleen, or brain. Daily sperm production and number of sperm heads were unaffected by  
 2 bisphenol A treatment. No significant effects were observed for activities of testicular  $\gamma$ -glutamyl  
 3 transpeptidase, sorbitol dehydrogenase, acid phosphatase, or  $\beta$ -glucuronidase. No histopathological  
 4 alterations were reported for the testis, epididymis, seminal vesicle, prostate, spleen, or brain. Bisphenol  
 5 A levels in urine are reported in Section 2. The study authors concluded that sperm density and the male  
 6 reproductive system do not appear to be affected in F344 rats exposed to bisphenol A.

7  
 8 **Strengths/Weaknesses:** Strengths include a wide range of doses, use of an appropriate route of exposure,  
 9 and the use of Fischer 344 rats. Weaknesses include marginal sample size and the absence of information  
 10 about certain study design features.

11  
 12 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility in the  
 13 evaluation process.

14  
 15 **Chitra et al. (501)**, supported by the Lady Tata Memorial Trust, Indian Council of Medical Research, and  
 16 the Population Council, examined the effects of bisphenol A on the reproductive system of male rats.  
 17 Animals were given “standard commercial laboratory chow.” **[Bedding and caging materials were not**  
 18 **reported.]** Six 45-day-old male Wistar rats/group were orally dosed **[gavage assumed]** with bisphenol A  
 19 (97% purity) in olive oil at 0, 0.0002, 0.002, and 0.020 mg/kg bw/day for 45 days. Rats were killed 24  
 20 hours following the last treatment. Testes, epididymides, seminal vesicles, and ventral prostate were  
 21 weighed. Epididymal sperm counts and motility were assessed. Antioxidant enzyme activities were  
 22 measured in sperm. Statistical analyses included ANOVA followed by Student *t*-test. Significant effects  
 23 on organ weights and sperm endpoints are summarized in Table 90. Bisphenol A treatment did not affect  
 24 body weight. Absolute and relative (to body weight) weights of testis and epididymis and were reduced,  
 25 and absolute and relative ventral prostate weights were increased at all dose levels. Effects on relative  
 26 organ weights are summarized in Table 90. Sperm motility was decreased at all dose levels, and sperm  
 27 counts were reduced at the mid and high dose. There were dose-related decreases in activity of superoxide  
 28 dismutase, catalase, glutathione reductase, and glutathione peroxidase in sperm at all dose levels.  
 29 Hydrogen peroxide generation and lipid peroxidation in sperm increased dose-dependently at all dose  
 30 levels. The study authors concluded that adverse effects of bisphenol A on the male reproductive system  
 31 may be due to oxidative stress.

32  
 33 **Table 90. Reproductive Effects in Male Rats Orally Dosed with Bisphenol A**

Endpoint	Dose, mg/kg bw/day				BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
	0.0002	0.002	0.020					
Relative organ weight								
Testis	↓5%	↓6%	↓7%	0.056	0.021	0.014	0.0087	
Epididymis	↓13%	↓17%	↓26%	0.011	0.0082	0.0069	0.0050	
Ventral prostate	↑13%	↑34%	↑29%	0.014	0.0083	0.015	0.0089	
Epididymal sperm motility <sup>a</sup>	↓23%	↓37%	↓41%					
Epididymal sperm count	↔	↓18%	↓27%					

↑,↓ Statistically significant increase, decrease; ↔ no statistically significant effect.

<sup>a</sup>Values estimated from a graph by CERHR; data estimated from graphs were not modeled.

From Chitra et al. (501).

34  
 35 Although these studies have a limited number of animals per group, they appear to be relatively well  
 36 conducted, and there are apparently consistent dose-dependent changes in testis and epididymis weights  
 37 and sperm parameters. The epididymal (portion not mentioned) sperm numbers measured in this study are  
 38 consistent with the daily sperm production measured by Sakaue et al. (498). A potential significant  
 39 concern in this study is the use of olive oil as the vehicle. The stability/reactivity of bisphenol A was not

#### 4.0 Reproductive Toxicity Data

1 determined and it is possible that bisphenol A interacted with olive oil, resulting in the observed findings.  
2 This study provides suggestive data that bisphenol A induces oxidative stress in epididymal sperm and  
3 alters testis and epididymis weights at low doses.

4  
5 **Strengths/Weaknesses:** Strengths include the use of oral and low multiple doses and appropriate  
6 measures. A weakness includes the marginal sample size.

7  
8 **Utility (adequacy) of CERHR Evaluation Process:** This study is adequate for inclusion but of limited  
9 utility based on small group size.

10  
11 **Chitra et al. (502)**, supported by the Population Council, New York, examined the effects of bisphenol A  
12 and vitamin C exposure on epididymis and sperm counts in rats. Wistar rats (45-days old) were fed  
13 standard commercial laboratory chow and housed in plastic cages. **[No information was provided about**  
14 **bedding.]** Four rats/group were orally dosed with bisphenol A (97% purity) at 0 (olive oil vehicle),  
15 0.0002, 0.002, or 0.020 mg/kg bw/day for 60 days. Additional rats received the same bisphenol A doses  
16 in conjunction with 40 mg vitamin C. **[The specific method of oral dosing was not stated. A vehicle**  
17 **control group administered vitamin C was not included.]** Rats were killed 24 hours following the last  
18 dose. Epididymides were fixed in Bouin solution and examined histologically. Sperm were counted and  
19 examined for viability and motility. Levels of antioxidant enzymes were measured in sperm and  
20 epididymis. Data were analyzed by ANOVA followed by Student *t*-test.

21  
22 There was no effect on sperm viability, but significant dose-related reductions were observed in sperm  
23 motility and count in all dose groups. **[In the low- to high-dose group, sperm motility was reduced to**  
24 **~70, 60, and 55% of control levels. Sperm counts in the low to high dose group were ~12, 30, and**  
25 **40% lower than control values.]** Complete degeneration of epithelia of caput, corpus, and cauda  
26 epididymis was reported at all dose levels. **[It was not clear if the effect occurred in every rat of each**  
27 **dose group.]** Significant dose-related decreases in glutathione peroxidase and superoxide dismutase  
28 activity and increased lipid peroxidation were observed in sperm and epididymis of rats from each  
29 bisphenol A treatment group. No changes in sperm motility, sperm count, antioxidant enzyme activity, or  
30 lipid peroxidation were observed when bisphenol A was administered with vitamin C. The study authors  
31 concluded that bisphenol A induced oxidative stress and degeneration of epididymal epithelium, and  
32 vitamin C protected against those effects.

33  
34 **Strengths/Weaknesses:** A critical weakness is the use of only 4 animals per dose group.

35  
36 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for inclusion due to  
37 concerns with group size.

38  
39 **Saito et al. (503)**, support not indicated, examined the effects of bisphenol A exposure on sex hormone  
40 levels in male rats. Wistar rats were fed MF feed (Oriental Yeast Co.). **[No information was provided**  
41 **about bedding and caging materials.]** Eight or 9 rats/group were sc injected with bisphenol A **[purity**  
42 **not reported]** at 0 (corn oil vehicle), 0.005, or 5 mg every 2 days from 3 to 11 weeks of age. **[Based on a**  
43 **graph showing body weights of ~50 g at the beginning of treatment and ~300 g at the end of**  
44 **treatment, the bisphenol A doses would have been 0.1 and 100 mg/kg bw at the beginning of the**  
45 **treatment period and 0.017 and 17 mg/kg bw at the end of the treatment period.]** Additional groups  
46 of 8–9 rats were injected with 5 µg/day 17β-estradiol or diethylstilbestrol. Rats were killed at 13 weeks of  
47 age, 2 weeks following the last treatment. Body, testes, and other reproductive organs were weighed.  
48 Levels of 17β-estradiol and testosterone were measured in plasma by RIA. Data were analyzed by  
49 Student *t*-test and Dunnett test. No clinical signs of toxicity or changes in behavior were observed.  
50 Exposure to bisphenol A did not affect body weight gain or absolute or relative testis weight. No effects  
51 were observed for weights of prostate, preputial gland, or epididymis. **[Data were not shown by study**

#### 4.0 Reproductive Toxicity Data

1 **authors.]** Plasma testosterone levels were significantly reduced in the low bisphenol A group [**by ~1.5**  
2 **fold]** and plasma estradiol levels were significantly increased in the high bisphenol A dose group [**by ~8-**  
3 **fold]**. Effects observed with 17 $\beta$ -estradiol and diethylstilbestrol exposure included decreased body weight  
4 gain, reduced absolute and relative testis weight, decreased plasma testosterone levels, and increased  
5 plasma 17 $\beta$ -estradiol levels. The study authors concluded that bisphenol A disturbed sex steroid  
6 production in male rats.

7  
8 Single point testosterone measurements are normally highly variable; the apparent significant decrease in  
9 testosterone observed in this study may be spurious and due to the small group size, an unusual low  
10 variability in testosterone, and the use of the Student *t*-test, an inappropriate statistical test for this  
11 analysis. There is some concern with the dynamic range of the 17 $\beta$ -estradiol RIA as 17 $\beta$ -estradiol is  
12 normally measured in pg/mL.

13  
14 **Strengths/Weaknesses:** Weaknesses include the sc route of exposure, the use of an inappropriate method  
15 of anesthesia when measuring hormone levels, inadequate sample sizes for highly variable testosterone  
16 endpoint, and inappropriate statistical tests on hormone data.

17  
18 **Utility (Adequacy) for CERHR Evaluation Process:** Based on experimental design concerns, this study  
19 is inadequate for the evaluation.

20  
21 **Takahashi and Oishi (504)**, support not indicated, examined species, strain, and route differences in  
22 reproductive systems of male rodents exposed to bisphenol A. The studies in rats are discussed in this  
23 section, and the studies in mice are discussed in Section 4.2.2.2. Animals were housed in stainless steel  
24 suspended cages or “chip-bedded” plastic cages. [**No information was provided about the type of chow**  
25 **used.]** Animals used in this study were 4 weeks old at the start of dosing. In the dietary portion of the  
26 study, male Wistar rats or Holtzman SD rats were given feed containing 0 or 0.25% bisphenol A (>99.0%  
27 purity) for 2 months. There were 8 animals in each dose group. The 0.25% dose group was reported to  
28 produce minimal testicular effects in a previous study. Mean bisphenol A intakes were estimated by study  
29 authors at ~200 mg/kg bw/day in rats. In parenteral exposure studies, 4-week-old male Wistar rats were sc  
30 dosed with bisphenol A in propylene glycol at 0 or 200 mg/kg bw on 4 days/week for 1 month.  
31 Additional male Wistar rats were given ip injections of bisphenol A in propylene glycol at 0, 2, or 20  
32 mg/kg bw 4 days/week for 1 month. An ip dose of 200 mg/kg bw was originally administered but resulted  
33 in death. There were 5–6 animals/group in the parenteral exposure studies. In both the dietary and  
34 parenteral exposure studies, animals were observed daily for clinical signs, and body weight and food  
35 intake were measured. Animals were killed at the end of the dosing period. Liver, kidney, and  
36 reproductive organs were weighed. Testes were fixed in formaldehyde solution and examined  
37 histologically. The study authors noted that the appropriate fixative for the testis is Bouin solution but that  
38 obvious and severe injuries could be detected with the method used in the present study. Testosterone was  
39 measured in serum by ELISA. Daily sperm production and efficiency and epididymal sperm reserves  
40 were evaluated. Statistical analyses included *F* test, Student *t*-test, Aspin-Welch test, Bartlett test,  
41 ANOVA, Dunnett test, Kruskal-Wallis test, Dunnett non-parametric test, Wilcoxon rank-sum test, chi-  
42 squared test, Mantel-Haenzel test, and Fisher exact test.

43  
44 In rats exposed through diet, there was no effect on body weight or absolute organ weight. Relative liver  
45 weight was significantly increased in Wistar rats exposed to bisphenol A. [**Data were not shown by**  
46 **study authors.]** The study authors indicated that they forgot to weigh seminal vesicles and prostate  
47 glands. No effects were reported for reproductive organ histopathology, daily sperm production or  
48 efficiency of production, epididymal sperm reserves, or serum testosterone levels in rats exposed to  
49 bisphenol A through diet. [**Data were not shown by study authors.]**

50

## 4.0 Reproductive Toxicity Data

1 In the portion of the study where rats were administered 200 mg/kg bw bisphenol A, stiffness was  
 2 observed at the injection site. Terminal body weight was lower **[by 20%]** in treated rats. Treatment  
 3 resulted in **[~20%]** decreases in absolute liver, kidney, preputial gland, and testis weight and **[~40–80%]**  
 4 decreases in epididymis, seminal vesicle, and prostate weight. The study authors also reported decreases  
 5 in relative weights of epididymis, seminal vesicle and coagulation gland, and prostate. **[Data were not  
 6 shown. The Expert Panel assumes that by coagulation gland, the authors mean the anterior  
 7 prostate or coagulating gland.]** No histopathological alterations were observed in the seminiferous  
 8 tubules of control animals. In the bisphenol A group, histopathological observations (incidence) in  
 9 seminiferous tubules included focal atrophy (60%), exfoliation (60%), detachment (20%), missing stage  
 10 VII/VIII spermatids (40%), retention of stage IX/XI spermatids (60%), and loss of basement membrane  
 11 (20%). Bisphenol A treatment reduced daily sperm production **[by ~25%, as estimated from a graph  
 12 for total production but not per g testis.]** Reserves in head and body of the epididymis and the cauda  
 13 epididymis were also reduced/g of tissue in bisphenol A-treated rats **[by ~43 % in the head and body of  
 14 epididymis and 63% in the cauda epididymis, as estimated from a graph]**. There was no significant  
 15 effect on serum testosterone level.

16  
 17 Effects in rats administered bisphenol A by ip injection are summarized in [Table 91](#). At 20 mg/kg bw,  
 18 terminal body weight and prostate, liver, and kidney weight were reduced. Serum testosterone levels were  
 19 also reduced in rats from the 20 mg/kg bw/day group. There were no effects on testicular histopathology  
 20 or sperm endpoints. **[Data were not shown by study authors.]** Enlarged ileum was observed at necropsy  
 21 in the 20 mg/kg bw group and histopathological examination revealed mucosal degeneration and  
 22 hyperplastic duodenum, jejunum, ileum, and cecum. The study authors concluded that bisphenol A is  
 23 more toxic through sc and ip exposure routes than by oral exposure in the diet.

24  
 25 **Table 91. Effects in Rats Given Bisphenol A by IP Injection**

Endpoint	Dose, mg/kg bw					
	2	20	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>SD</sub>
Weight						
Terminal body	↔	↓12%	19	12	17	5
Ventral prostate	↔	↓29%	7	5	9	6
Liver	↔	↓18%	14	8	12	6
Kidney	↔	↓12%	20	11	19	6
Serum testosterone	↔	↓69%	3	2	16	9

↑,↓ Statistically significant increase, decrease; ↔ no statistically significant effect.  
 From Takahashi and Oishi (504).

26  
 27 This paper reports a comprehensive study comparing 2 mouse and 2 rat strains using minimal numbers of  
 28 animals per group. The data suggest that systemic exposure is necessary for bisphenol A estrogenic  
 29 activity to be exhibited and strongly indicate that route of administration (oral vs. ip) is an important  
 30 consideration. A minimal exposure range; the study did not explore low doses.

31  
 32 Due to differences in strain sensitivities, a NOAEL was not established. Nevertheless, it is likely to be  
 33 near 0.25% in the diet.

34  
 35 **Strengths/Weaknesses:** Strengths include multiple routes of exposure, use of two strains of mice and  
 36 rats, and a comparison of the oral, ip, and subcutaneous routes. Weaknesses include use of single high  
 37 doses administered for different durations across groups using minimal sample sizes

38  
 39 **Utility (adequacy) of CERHR Evaluation Process:** This study is adequate but of limited utility.

40

#### 4.0 Reproductive Toxicity Data

1 **Herath et al. (505)**, supported by Japan Society for Promotion of Science and the Japanese Ministry of  
2 Education, Culture, Sports, Science, and Technology, examined the effects of bisphenol A exposure on  
3 reproductive hormones and sperm endpoints in male rats. Octylphenol was also examined in this study,  
4 but results will not be discussed. Wistar-Imamichi rats were fed a soy-containing commercial feed (Nosan  
5 Corporation, Japan) and housed in metal cages. Rats were randomly assigned to groups and beginning at  
6 50 days of age, 10–11 rats/group were sc injected with bisphenol A ( $\geq 95\%$  purity) at 0 (DMSO vehicle)  
7 or 3 mg/kg bw/day for 5 weeks. Rats were weighed during the study. LH, testosterone, and progesterone  
8 concentration were measured in blood after 2 weeks of treatment and on the following day, 1 hour after a  
9 challenge with gonadotropin-releasing hormone. Rats were killed after 5 weeks of treatment. Blood was  
10 obtained for measurement by RIA of LH, progesterone, testosterone, immunoreactive inhibin, and insulin  
11 growth factor 1 levels. The testis, seminal vesicle, epididymis, and prostate were weighed, and sperm  
12 counts and motility were determined. A total of 5–11 rats/group were examined for each endpoint.  
13 Statistical analyses included ANOVA and Duncan Multiple Range test.

14  
15 No statistically significant effects on baseline LH, testosterone, or progesterone levels were observed  
16 following 2 weeks of bisphenol A treatment. Following injection with gonadotropin-releasing hormone,  
17 LH levels were significantly increased in the bisphenol A group and progesterone levels were  
18 significantly increased in the vehicle control group. In the bisphenol A group compared to the control  
19 group, incremental increases following injection with gonadotropin-releasing hormone were smaller for  
20 testosterone [**~410 vs. 875%**] and progesterone [**~75 vs. 510%**]; statistical significance was reported for  
21 the progesterone effect. Following 5 weeks of bisphenol A treatment, significant effects on plasma  
22 hormone levels compared to controls included decreased testosterone [**by ~55%**] and increased insulin-  
23 like growth factor 1 [**by ~20%**]. Ventral prostate weight was significantly higher [**by ~29%**] in the  
24 bisphenol A versus control group, but there were no effects on testis, seminal vesicle, or right epididymis  
25 weight. [**Relative reproductive organ weights were not reported.**] Epididymal sperm counts were  
26 significantly reduced [**by ~10%**] in the bisphenol A group, but there was no significant effect on sperm  
27 motility. The study authors concluded that bisphenol A exposure can affect basal and gonadotropin-  
28 releasing hormone-stimulated LH production and reduced daily sperm production in rats.

29  
30 **Strengths/Weaknesses:** This study appears to have been relatively well conducted. A major weakness of  
31 this paper is the inconsistency in the hormone data (control data after 2 weeks were dramatically different  
32 than after 5 weeks even though both are from sexually mature rats). The subcutaneous route of  
33 administration with the use of DMSO as vehicle are weaknesses.

34  
35 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate and not useful for the  
36 evaluation process primarily due to the significant inconsistencies in the hormone data from control  
37 animals.

38  
39 **Toyama et al. (506)**, supported in part by the Japanese Ministry of Environment and Ministry of  
40 Education, Science, Sports, and Culture, examined the effects of bisphenol A exposure on the  
41 reproductive system of male rats and mice. [**No information was provided about feed, caging, or**  
42 **bedding materials. The mouse portion of the study is discussed in Section 4.2.2.2.**] Adult male Wistar  
43 rats (n = 12/group) were sc injected with bisphenol A [**purity not indicated**] at 0.020 or 0.200 mg/kg  
44 bw/day for 6 consecutive days. Three control animals were sc injected with the DMSO/olive oil vehicle  
45 for 6 days. Ten animals/bisphenol A group and 2 controls were killed the day following treatment and  
46 perfused with glutaraldehyde. Testes were weighed and examined by light and electron microscopy.  
47 Epididymis, preputial gland, ventral prostate, and seminal vesicle with coagulating glands were also  
48 weighed. The remaining animals, 2 in each bisphenol A group and 1 in the control group, were held an  
49 additional 2 months and then subjected to fertility tests. In fertility testing, each male was mated to 2  
50 untreated females. One of the 2 mated females was kept until parturition. [**The males were apparently**

## 4.0 Reproductive Toxicity Data

1 **killed for an examination of reproductive organs following fertility testing.]** Results were  
2 qualitatively reported, and statistical analyses were not conducted.

3  
4 The description of the results was limited primarily to rats in the 0.020 mg/kg bw/day group. Body and  
5 male accessory reproductive organ weights were not affected by bisphenol A treatment. **[Data were not  
6 shown by study authors.]** In the bisphenol A group, examination by light microscopy revealed  
7 exfoliation of round spermatids, deformed heads of mature spermatids, and multinucleated giant cells in  
8 seminiferous epithelium. Testicular effects observed by electron microscopy included abnormal  
9 acrosomal caps and invagination and/or vacuole formation in nuclei of spermatids beyond step 1.  
10 Ectoplasmic specialization around Sertoli cells was also affected by bisphenol A treatment. No  
11 histological or ultrastructural abnormalities were observed in the testis 2 months following exposure.  
12 Sexual behavior was observed to be normal in treated males. Females delivered normal pups and litter  
13 sizes were similar between groups. The study authors concluded that bisphenol A exposure did not affect  
14 fertility in rats and that adverse effects were transient.

15  
16 **Strengths/Weaknesses:** Definite conclusions cannot be drawn from such a limited data set; the fertility  
17 assessment was not meaningful due to the sample size (2/group). The background incidence of the  
18 electron microscope findings was not discussed. Another weakness is the subcutaneous route with DMSO  
19 as a vehicle.

20  
21 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate and not useful in the  
22 evaluation.

### 23 24 4.2.2.2 Mouse

25 **Takao et al. (507)**, support not indicated, examined the effects of bisphenol A exposure on the  
26 reproductive system of mice. Five-week-old male C57BL/6 mice were exposed to bisphenol A **[purity  
27 not indicated]** in drinking water at 0 (0.005% ethanol in water vehicle), 0.0005, or 0.050 g/L for 4 or 8  
28 weeks. **[Based on daily water intakes and body weights reported in the study, bisphenol A intake  
29 was estimated by CERHR at 0.14 and 13 mg/kg bw/day.]** To maintain bisphenol A at a stable  
30 concentration, drinking water was changed twice a week, but the stability of bisphenol A was not verified.  
31 Mice were killed, and both testes and spleen were removed and weighed. One testis was processed for  
32 histopathological evaluation. Plasma testosterone, corticosterone, and LH levels were measured in 7  
33 mice/group using RIA or enzyme immunoassay. **[No information was provided on the purity of  
34 bisphenol A, time between last dose and sacrifice, or the type of chow, caging, or bedding materials  
35 used. Very few details were provided on the methods, including histopathological evaluation.]**  
36 Statistical analyses included ANOVA followed by Fisher protected least significant difference test.

37  
38 Water intake was significantly reduced **[by 8%]** in the high-dose group exposed for 4 weeks. There were  
39 no effects on body weight or absolute or relative (to body weight) testis or spleen weight. Plasma  
40 testosterone levels were reduced **[by 87–89%]** in the high-dose group, but statistical significance was  
41 attained only in the group exposed for 8 weeks. No statistically significant changes were reported for  
42 plasma corticosterone or LH levels. The number of multinucleated cells in the seminiferous tubules was  
43 increased in high-dose mice treated for 8 weeks. The study authors concluded that exposure to bisphenol  
44 A around the peripubertal period may disrupt the reproductive tracts of male mice.

45  
46 **Strengths/Weaknesses:** This study lacks important experimental details on methodology, including  
47 numbers of treated animals. Although it appears that bisphenol A in the drinking water results in a dose-  
48 related decrease in plasma testosterone, this endpoint is highly variable because testosterone is secreted in  
49 a pulsatile manner, and controls for the week 4 and 8 varied by ~30%.

#### 4.0 Reproductive Toxicity Data

**Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate and not useful for the evaluation process due to the paucity of important experimental details and the variability of the testosterone data.

**Al-Hiyasat (508)**, supported by the Deanship of Scientific Research at Jordan University of Science and Technology, examined the effect of bisphenol A exposure on fertility of male mice. **[No information was provided about composition of chow, bedding, or caging.]** Ten 60-day-old male Swiss mice/group were gavaged with the ethanol/distilled water vehicle or bisphenol A (97% purity) for 30 days. **[The study listed the bisphenol A doses as 5, 25, and 100 ng/kg bw. An erratum was later released that indicated the correct units were µg/kg bw (0.005, 0.025, and 0.1 mg/kg bw/day).]** Following the dosing period, each male was mated for 10 days with 2 untreated female mice, who were placed inside the cage of the male during the same time period. The males were then killed for an evaluation of testes, seminal vesicles, and preputial gland weights. Sperm counts and daily sperm production were determined. Mated females were killed 10 days later to determine numbers of pregnancies, implantation sites, viable fetuses, total resorptions, and females with resorptions. **[There was no indication that mating was confirmed by checking for sperm in the vagina.]** Data were analyzed by Student *t*-test or Fisher exact test.

Results that obtained statistical significance are summarized in [Table 92](#). Body weights were lower in all dose groups compared to controls. There were no evident dose-response relationships for organ weights. Absolute testis weight was decreased at the low dose, and absolute seminal vesicle weight was reduced at the mid and high dose. Effects on relative organ weights are summarized in [Table 92](#). Decreases in testicular sperm counts and daily sperm production were observed at the mid and high dose. Total sperm counts in the epididymis were decreased at all dose levels, and sperm counts/mg epididymis were decreased at the mid and high dose. The total number of resorptions and females with resorptions were increased at all dose levels. The percentage of pregnant females was reduced at the mid and high dose. The study authors concluded that bisphenol A could adversely affect fertility and reproduction of adult male mice.

**Table 92. Effects Observed Following Gavage of Male Mice with Bisphenol A and Mating with Untreated Females**

Endpoint	Dose, mg/kg bw/day						
	0.005	0.025	0.1	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
Body weight	↓18%	↓21%	↓13%				
Relative weight							
Testis	↔	↑26%	↔				
Seminal vesicle	↔	↓27%	↔				
No. sperm/testis	↔	↓17%	↓29%	0.035	0.029	0.036	0.028
No. sperm/mg testis	↔	↓16%	↓37%	0.027	0.023	0.029	0.023
Daily sperm production	↔	↓17%	↓29%	0.035	0.029	0.036	0.028
Efficiency of sperm production	↔	↓16%	↓37%	0.027	0.023	0.029	0.023
No. sperm/epididymis	↓14%	↓25%	↓35%	0.033	0.026	0.040	0.030
Sperm/mg epididymis	↔	↓17%	↓31%	0.033	0.025	0.053	0.038
Percent pregnant females	↔	↓40%	↓33%				
Resorptions/implantation site (3% control rate)	13%	15%	13%				
Percent females with resorption sites	↑2.5-fold	↑3.8-fold	↑3.4-fold				

↑, ↓ Statistically significant increase, decrease, ↔ no statistically significant effect.

#### 4.0 Reproductive Toxicity Data

Endpoint	Dose, mg/kg bw/day				BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
	0.005	0.025	0.1					

From Al-Hiyasat (508).

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The number of animals per group was too small (n=10) for a definitive assessment of study endpoints. The method of randomization (or initial body weights) was not presented. There is also an absence of a dose response in several of the endpoints assessed. Given that mice usually have poor (relative to rats) fertility rates, the confidence in control data is limited. The male mice were killed shortly after the mating period, which may have influenced/confounded the number of sperm in the epididymis. Student *t*-test is an inappropriate analysis for organ weights (ANOVA with appropriate post hoc test would be appropriate). Statistical significance is suspect, and the changes in organ weights are minimal in magnitude.

**Strengths/Weaknesses:** Weaknesses include small sample sizes for endpoints, inadequate coverage of the full spermatogenesis cycle in dosing duration, measurement of sperm counts without allowing adequate time following mating, and inappropriate accounting of sire influences on resorption rates in statistical analyses. Sample sizes are small for fertility assessments.

**Utility (adequacy) of CERHR Evaluation Process:** This study is adequate for inclusion. Data on tissue weights are of limited utility for the evaluation process, however fertility data are not useful.

**Nagao et al. (428)**, support not indicated, examined the effects of bisphenol A in mice following exposure during different life stages. An initial experiment, described in more detail in Section 3.2.7, found that C57BL/6N mice were more sensitive to 17 $\beta$ -estradiol than ICR mice, and the study authors therefore used C57BL/6N mice to examine the effects of bisphenol A. Life stages examined included prenatal development, adolescence, and adulthood. The study conducted in adult mice is described here, while the studies conducted during prenatal development and adolescence are described in Section 3.2.7. C57BL/6N mice were fed PLD (phytoestrogen-low diet, Oriental Japan). They were housed in polycarbonate cages with wood bedding. Daidzein and genistein levels were analyzed in diet, tap water, and bedding and found to be below 0.5 mg/100 g. At 10 weeks of age, 20 male mice/group were gavaged with bisphenol A (99.0% purity) at 0.002, 0.020, or 0.200 mg/kg bw/day for 6 days. Twenty control males/group were given 0.5% carboxymethyl cellulose [**assumed to be the vehicle**]. Six weeks after the final dose was administered, the mice were weighed and 15 males/group were killed and necropsied. The testis, epididymis, and seminal vesicles with coagulating glands were weighed. The ventral prostate was not weighed due to difficulties in obtaining only prostate and determining the precise weight. Epididymal sperm counts were obtained. Histopathological examinations were conducted for organs fixed in Bouin solution. Data were analyzed by Bartlett test to determine homogeneity of variance, followed by ANOVA when homogeneity of variance was confirmed or Kruskal-Wallis analysis of ranks when variance was not homogenous. Dunnett test was used for multiple comparisons.

In the bisphenol A group, there were no significant differences in body weight gain or terminal body weights. [**Data were not shown.**] There were no significant differences in absolute or relative (to body weight) weights of the testis, epididymis, or seminal vesicles. There were no significant effects on sperm count. No histopathological alterations in reproductive organs were reported. The study authors concluded that low-dose bisphenol A exposure of mice did not reduce sperm density.

**Strengths/Weaknesses:** This study was well conducted and adds to the understanding of the potential effects of low doses of bisphenol A administered by a relevant route of exposure. Strengths are an appropriate number of mice per group, the use of response to 17 $\beta$ -estradiol in 2 strains of mice to identify the most sensitive strain, and the presentation of sperm data in light of historical control data.

#### 4.0 Reproductive Toxicity Data

1 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
2 evaluation process.

3  
4 **Peknicová et al. (509)**, supported by the Czech Republic and EU, examined the effects of bisphenol A  
5 exposure on mouse sperm. CD-1 mice were given ST1 feed (Velaz a.s., Prague). Three generations of  
6 mice were exposed to bisphenol A [**purity not indicated**] through drinking water at doses of 0.000002  
7 and 0.000020 mg “/animal’s weight/day.” It was stated that there were 6 pairs of mice in the control  
8 group. Litter size was evaluated in 3 generations; 1 litter was examined in the first and second generation  
9 and 2 litters were examined in the third generation. In each generation, samples of sperm were collected  
10 from all males and a histopathological investigation of testes was conducted in  $\geq 3$  males/group. Sperm  
11 acrosomal status was assessed using an immunohistochemical and Western blot method. Statistical  
12 analyses included ANOVA and Newman–Keuls test. [**Very few experimental details were provided.**  
13 **No information was provided on bedding and caging materials, bisphenol A purity, the numbers of**  
14 **mice in each treatment group, treatment of the control group, ages of mice during treatment,**  
15 **durations of treatment, sample sizes and litter representation for sperm effects, and mating**  
16 **procedures. It was not clear if female rats were also treated.**] Litter sizes were significantly reduced in  
17 the first and second generation of mice treated with the low dose (5–6.7 pups/litter vs. 11.5–12 pups/litter  
18 in controls). There were no effects of bisphenol A treatment on testes weight. [**Data were not shown by**  
19 **authors.**] Pathological changes observed in testes from the low-dose group included damaged  
20 seminiferous tubule and reduced spermatogenesis. Acrosome integrity, evaluated as percent cells binding  
21 monoclonal antibodies to acrosin and intra-acrosomal proteins, was significantly reduced in all 3  
22 generations of the low-dose group (48.5–57.7 compared to 93.3–95% integrity in controls) and the third  
23 generation of the high-dose group (62.5 compared to 93.3% integrity in controls). [**While the text of the**  
24 **study stated that acrosomal integrity was significantly affected only in the third generation of the**  
25 **high-dose group, the caption for Figure 7 of the study stated that both the second and third**  
26 **generations were significantly affected. Based on findings reported in the figure, it appears that the**  
27 **description in the text is correct.**] The study authors concluded that bisphenol A exposure negatively  
28 impacts fertility, spermatogenesis, and sperm quality in mice.

29  
30 **Strengths/Weaknesses:** Although potentially interesting findings are presented, the study lacks many  
31 important details and sample sizes are critically inadequate.

32  
33 **Utility (Adequacy) for CERHR Evaluation Process:** Due to study design concerns, this study is  
34 inadequate and has no utility for the evaluation.

35  
36 **Takahashi and Oishi (504)**, support not indicated, examined species, strain, and route differences in  
37 reproductive systems of male rodents exposed to bisphenol A. Studies on mice are discussed here, and  
38 studies on rats are discussed in Section 4.2.2.1. Animals were housed in stainless steel suspended cages or  
39 “chip-bedded” plastic cages. [**No information was provided about the type of chow used.**] Animals  
40 used in this study were 4 weeks old at the start of dosing. In the dietary portion of the study, CD-1 (ICR)  
41 mice and C57BL/6CrSlc mice were given feed containing 0 or 0.25% bisphenol A (>99.0% purity) for 2  
42 months. There were 8 animals in each dose group. The 0.25% dose was reported to produce minimal  
43 testicular effects in a previous study. Mean bisphenol A intakes were estimated by study authors at ~400  
44 mg/kg bw/day in mice. The parenteral exposure studies were performed only in rats. Animals were  
45 observed daily for clinical signs, and body weight and food intake were measured. Animals were killed at  
46 the end of the dosing period. Liver, kidney, and reproductive organs were weighed. Testes were fixed in  
47 formaldehyde solution and examined histologically. The study authors noted that the appropriate fixative  
48 for the testis is Bouin solution, but that obvious and severe injuries could be detected with the method  
49 used in the present study. Testosterone was measured in serum by ELISA. Daily sperm production and  
50 efficiency and epididymal sperm reserves were evaluated. Statistical analyses included *F* test, Student *t*-

## 4.0 Reproductive Toxicity Data

1 test, Aspin-Welch test, Bartlett test, ANOVA, Dunnett test, Kruskal-Wallis test, Dunnett non-parametric  
2 test, Wilcoxon rank-sum test, chi-squared test, Mantel-Haenzel test, and Fisher exact test.

3  
4 There were no significant effects on organ or body weights in C57BL/6CrSlc mice exposed through diet.  
5 In CD-1 (ICR) mice exposed through diet, there were increases in absolute testis [16%], liver [12%], and  
6 kidney [20%] weights and a decrease in absolute epididymis [12%] weight. The study authors reported  
7 that relative testis weight was not significantly affected, but when the value from 1 mouse with a high  
8 relative testis weight was deleted, the effect attained statistical significance. **[Data were not shown by  
9 study authors.]** No effects were reported for testis histopathology, daily sperm production or efficiency  
10 of production, epididymal sperm reserves, or serum testosterone levels in mice exposed to bisphenol A  
11 through diet. **[Data were not shown by study authors.]** The study authors concluded that the testicular  
12 toxicity of bisphenol A is “relatively weak,” based on the co-occurrence of liver and kidney toxicity at  
13 exposure levels causing testicular effects.

14  
15 **Strengths/Weaknesses:** A strength is the use of dietary exposure and the examination of strain  
16 differences in mice. Weaknesses include use of a single very high dose level.

17  
18 **Utility (adequacy) of CERHR Evaluation Process:** This study is adequate but of limited utility.

19  
20 **Park et al. (487)**, support not indicated, examined the effects of bisphenol A exposure on the  
21 reproductive and hematological systems of male and female mice. **[Results for males are discussed  
22 here, and results for females are discussed in Section 4.2.1.2.]** Adult ICR mice were fed mouse  
23 formulation feed (Cheil Feed). **[No information was provided about caging or bedding materials.]**  
24 Fifteen mice/sex/group were ip injected with bisphenol A **[purity not indicated]** in an ethanol/corn oil  
25 vehicle at 0.05, 0.5, or 5.0 mg/kg bw on 5 occasions (every 3 days over a 14-day period). One control  
26 group received no treatment, and a second control group was ip injected with corn oil. Males were  
27 examined 2 days following administration. Semen was collected and assessed for sperm number,  
28 viability, and motility. Reproductive organs were weighed and fixed in Bouin solution, and  
29 histopathological examination was conducted. Hematological and clinical chemistry endpoints were also  
30 assessed. Data were analyzed by least significant difference test.

31  
32 Exposure to bisphenol A had no effect on body weight or on weights of male reproductive organs  
33 including testis, epididymis, vesicular gland, or coagulating gland. Reductions in sperm concentrations  
34 **[by 18%]** and increases in sperm abnormalities **[by 28%]** were significant in the high-dose group. There  
35 were no treatment effects on testicular histology. There were no significant effects on hematological or  
36 clinical chemistry endpoints in males treated with bisphenol A. The study authors did not report  
37 conclusions regarding study findings.

38  
39 **Strengths/Weaknesses:** Weaknesses include the ip route. Frequency of administration was every 3 days  
40 and, given the half-life of the chemical, it is unlikely that sufficient blood chemical levels were sustained  
41 to induce “maximal” bisphenol A-mediated responses.

42  
43 **Utility (Adequacy) for CERHR Evaluation Process:** Given the dosing paradigm (ip injection every 3  
44 days) this study is adequate but of limited utility in the evaluation process.

45  
46 **Toyama et al. (506)**, supported in part by the Japanese Ministry of Environment and Ministry of  
47 Education, Science, Sports, and Culture, examined the effects of bisphenol A exposure on the  
48 reproductive system of male rats and mice. **[No information was provided about feed, caging, or  
49 bedding materials. The mouse study is discussed here, and the rat study is discussed in Section  
50 4.2.2.1.]** Adult male ICR mice (n = 12/group) were sc injected with bisphenol A **[purity not indicated]** at  
51 0.020 or 0.200 mg/kg bw/day for 6 consecutive days. Three control animals were sc injected with the

#### 4.0 Reproductive Toxicity Data

1 DMSO/olive oil vehicle for 6 days. Ten animals/bisphenol A group and 2 controls were killed the day  
2 following treatment and perfused with glutaraldehyde. Testes were weighed and examined by light and  
3 electron microscopy. Epididymis, preputial gland, ventral prostate, and seminal vesicle with coagulating  
4 glands were also weighed. The remaining animals, 2 males in each bisphenol A treatment group and 1  
5 control male, were held an additional 2 months and then subjected to fertility tests. In fertility testing,  
6 each male was mated to 2 untreated females. One of the 2 mated females was kept until parturition. **[The**  
7 **males were apparently killed for an examination of reproductive organs following fertility testing.]**  
8 Results were qualitatively reported, and statistical analyses were not conducted.  
9

10 The study authors noted that all effects were similar between rats and mice and between dose groups, and  
11 their description of results was primarily limited to rats in the 0.020 mg/kg bw/day group. Body and male  
12 accessory reproductive organ weights were not affected by bisphenol A treatment. **[Data were not shown**  
13 **by study authors.]** In the bisphenol A group, examination by light microscopy revealed exfoliation of  
14 round spermatids, deformed heads of mature spermatids, and multinucleated giant cells in seminiferous  
15 epithelium. Testicular effects observed by electron microscopy included abnormal acrosomal caps and  
16 invagination and/or vacuole formation in nuclei of spermatids beyond step 1. Ectoplasmic specialization  
17 around Sertoli cells was also affected by bisphenol A treatment. No histological or ultrastructural  
18 abnormalities were observed in testes 2 months following exposure. Sexual behavior was observed to be  
19 normal in treated males. Females delivered normal pups and litter sizes were similar between groups. The  
20 study authors concluded that bisphenol A exposure did not affect fertility in mice and that adverse effects  
21 were transient.  
22

23 **Strengths/Weaknesses:** It is not possible to draw definite conclusions from such a limited data set; the  
24 fertility assessment was not meaningful due to the small sample size (2/group). The background incidence  
25 of the electron microscopy findings was not discussed. An additional weakness is the subcutaneous route  
26 with the use of DMSO as vehicle.  
27

28 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate and not useful due to the  
29 limited number of animals per group.  
30

31 **Anahara et al. (510)**, supported by the Japanese Ministry of Environment and Ministry of Education,  
32 Culture, Sports, Science, and Technology, examined the effects of bisphenol A exposure on expression of  
33 cortactin protein in the mouse testis. Cortactin is an actin binding protein that makes up the apical  
34 ectoplasmic specialization between Sertoli cells and spermatids and the basal ectoplasmic specialization  
35 between Sertoli cells. Cortactin is one of several proteins that control spermatid development. Adult (12-  
36 week-old) male ICR mice (n = 5–7/group) were sc injected with corn oil vehicle, 0.0024 mg/kg bw/day  
37 bisphenol A, 2.5 µg/kg bw/day diethylstilbestrol, or 1.2 µg/kg bw/day 17β-estradiol for 5 days. **[No**  
38 **information was provided on purity of bisphenol A or the types of feed, caging, or bedding used.]**  
39 Animals were killed on the day following the last injection. Testes were homogenized and expression of  
40 cortactin protein was determined in testes from 5–7 rats/group by Western blot, immunohistochemistry,  
41 and immunoelectron microscopy techniques. Data were analyzed by *t*-test. Exposure to bisphenol A  
42 resulted in a significant decrease in testicular cortactin protein expression **[to ~60% of control levels]**.  
43 Immunohistochemical analysis revealed that cortactin staining was reduced in the apical ectoplasmic  
44 specialization but not in the basal ectoplasmic specialization. Examination by immunoelectron  
45 microscopy revealed no expression of cortactin around heads of spermatid and deformation of nuclei and  
46 acrosomes. Effects observed with 17β-estradiol and diethylstilbestrol were similar to those observed with  
47 bisphenol A, with the exception that diethylstilbestrol also reduced cortactin protein expression in the  
48 basal ectoplasmic specialization and did not result in deformation of spermatids. The authors concluded  
49 that exogenous chemicals can damage junctional proteins like cortactin and have adverse effects on  
50 Sertoli cell protein regulation.  
51

## 4.0 Reproductive Toxicity Data

1 **Strengths/Weaknesses:** The subcutaneous route of administration of a single dose was a weakness as  
2 were suboptimal sample sizes. Western blot analysis of cortactin was inappropriately presented as a  
3 function of the control value with no variability in the control sample. There were no apparent differences  
4 in levels of protein expression between various estrogenic agents/treatments. No adverse outcomes of the  
5 changes in cortactin were explored.

6  
7 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate and not useful for the  
8 evaluation process.

### 9 4.2.2.3 Other mammals

10 **Moon et al. (511)**, supported by Korea University Medical Science Research Center and the Korean  
11 Ministry of Education, examined the effects of bisphenol A exposure on penile function in rabbits. [No  
12 information was provided on feed or caging and bedding materials.] Male, 8–12 week-old New  
13 Zealand white rabbits were ip injected with corn oil vehicle or 150 mg/kg bw bisphenol A [purity not  
14 reported], every other day for 12 days to a cumulative dose of 900 mg/kg bw [75 mg/kg bw/day].  
15 Rabbits were killed at 4 weeks (n = 15/group) and 8 weeks (n=15/group) following bisphenol A  
16 treatment. In 5 rabbits/group, the penis was removed and fixed in 10% neutral buffered formalin for  
17 histological examination. In 10 rabbits/group, the corpora cavernosa were removed from the penis, and in  
18 vitro responses to norepinephrine, acetylcholine, sodium nitroprusside, and L-arginine were studied. Data  
19 were analyzed by Student *t*-test. Treatment with bisphenol A significantly suppressed contraction of  
20 corpora cavernosa in response to norepinephrine and relaxation in response to acetylcholine, sodium  
21 nitroprusside, and L-arginine at both stages of evaluation. Histopathological observations in the bisphenol  
22 A-treated rabbits but not control rabbits at both ages included intracavernosal fibrosis in conjunction with  
23 decreased sinusoidal spaces. Compared to rabbits in the control group, both age groups of rabbits exposed  
24 to bisphenol A had significantly increased trabecular smooth muscle content (73.3–83.2 versus 33.2% in  
25 controls) and a non-significant difference in thickness of tunica albuginea (0.93–1.12 mm versus 0.32–  
26 0.43 mm in controls). The study authors concluded that bisphenol A may affect erectile responses by  
27 inducing histological alterations in the penis.

28  
29  
30 **Strengths/Weaknesses:** There is no evidence that bisphenol A has any effect on the ability to attain an  
31 erection resulting in copulation in mice or rats. The lack of a plausible rationale is a weakness. This study  
32 does not have a concurrent control (e.g., 17 $\beta$ -estradiol) to ascertain if the observed effects are the result of  
33 estrogenic responses in the penis. The route of administration and use of a single dose are weaknesses.

34  
35 **Utility (Adequacy) for CERHR Evaluation Process:** Due to the weakness identified above and the  
36 nature of the endpoints examined, this study is inadequate and of no utility for human risk assessment

37  
38 **Nieminen et al. (489)**, support not indicated, examined the effects of bisphenol A [purity not indicated]  
39 exposure on hormone levels in the European polecat (*Mustela putorius*). There were no significant effects  
40 on plasma levels of testosterone, estradiol, FSH, or thyroid hormones. Details of this study are discussed  
41 in Section 4.2.1.3.

42  
43 This study provides evidence that the bisphenol A administered to polecats increases GST and UDPGT  
44 activity. Since these findings were dose-related it appears that in the polecat bisphenol A increases phase  
45 2 metabolism but has minimal effects on hormone levels. Due to the limited number of animals and the  
46 absence of a dose-response relationship, the hormonal changes in this study are difficult to interpret.

47  
48 **Strengths/Weaknesses:** Strengths include the use of a non-rodent species and multiple doses.  
49 Weaknesses include small sample sizes and the limited nature of reproductive endpoints.

## 4.0 Reproductive Toxicity Data

1 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate and not useful for the  
2 evaluation process.

3  
4 **Nieminen et al. (490)**, support not indicated, examined the effects of bisphenol A exposure on endocrine  
5 endpoints in field voles (*Microtus agrestis*). Animals were housed in plastic cages with wood shavings  
6 and fed R36 diet (Lactamin, Sweden). Sexually mature field voles were randomly assigned to groups that  
7 received bisphenol A [**purity not reported**] in propylene glycol by sc injection for 4 days. Doses of  
8 bisphenol A (numbers of males in each group ) were 0 (n = 6), 10 (n = 4), 50 (n = 6), and 250 (n = 7)  
9 mg/kg bw/day. Animals were killed the day following the last dose. Body and liver weights were  
10 measured. Blood was drawn for measurement of sex steroids, thyroxine, and weight regulating hormone  
11 levels in plasma using RIA or immunoradiometry methods. The activities of EROD, UDPGT, and GST  
12 were measured in hepatic and renal microsomes using appropriate substrates. Statistical analyses included  
13 ANOVA, post hoc Duncan test, Student *t*-test, Kolmogorov-Smirnov test, Levene test, Mann-Whitney *U*  
14 test, chi-squared test, and Spearman correlation. [**Results for females are discussed in Section 4.2.1.3.**]

15  
16 Mortality was significantly increased by bisphenol A treatment, with incidences of 18, 36, and 20% in the  
17 low-to high-dose groups. No mortality was observed in the control group. Bisphenol A treatment did not  
18 significantly affect body, liver, or testis weight. Plasma testosterone levels increased with dose, and  
19 statistical significance was attained in high-dose males and females. Pooled (male + female) LH levels  
20 were not significantly altered by treatment. Liver EROD activity [**apparently combined for males and**  
21 **females**] was significantly decreased at the mid and high dose and liver GST activities [**not clear if for**  
22 **males or females or both**] was significantly decreased at the highest dose level. There were no other  
23 significant effects on microsomal enzymes examined. The study authors concluded that wild mammals  
24 such as field voles could be more susceptible to bisphenol A-induced toxicity than laboratory rodents.

25  
26 **Strengths/Weaknesses:** Strengths include the use of a non-rodent species and multiple doses.  
27 Weaknesses include small sample sizes and limited nature of reproductive endpoints as well as the use of  
28 the subcutaneous route of administration.

29  
30 **Utility (Adequacy) for CERHR Evaluation Process:** This study is inadequate for the evaluation.

### 31 4.2.2.4 Fish and invertebrates

32  
33 Although studies in fish and invertebrates may be important for understanding mechanisms of action and  
34 environmental impact, the Panel views these studies as not useful for the evaluation process.

35  
36 **Shioda and Wakabayashi (512)**, supported by the Japanese Ministry of Education, examined the effects  
37 of bisphenol A exposure on reproductive capability of male medaka (*Oryzias latipes*). Adult male medaka  
38 were housed for 2 weeks in glass beakers containing distilled water and bisphenol A [**purity not**  
39 **indicated**] at 0, 0.3, 1, 3, or 10  $\mu\text{M}$  [**0, 0.07, 0.23, 0.69, or 2.3 mg/L**]. [**The number of male fish treated**  
40 **was not reported. Though not specifically stated, it was suggested that fish in the negative control**  
41 **group were exposed to the acetone vehicle.**] Following exposure, each male was housed with two  
42 females in beakers containing distilled water. The numbers of eggs spawned, fertilized, and hatched were  
43 determined. Statistical analyses included *F* test followed by *t*-test or Welch test. Exposure to bisphenol A  
44 10  $\mu\text{M}$  [**2.3 mg/L**] significantly reduced the number of eggs produced and hatched compared to the  
45 negative control group. Additional compounds were also examined, and it was reported that eggs and  
46 hatchings were significantly reduced following exposure to 17 $\beta$ -estradiol ( $\geq 3$  nM), but not nonylphenol  
47 or diethylhexyl phthalate. The study authors concluded that the reproductive effects induced by bisphenol  
48 A in this study occurred at a higher concentration than results observed in a yeast estrogen screen.

49  
50 **Strengths/Weaknesses:** This study appears to have been well conducted study and suggests that  
51 bisphenol A 2.3 mg/L in water decreases the number of medaka eggs produced and hatched

## 4.0 Reproductive Toxicity Data

1 **Utility (Adequacy) of CERHR Evaluation Process:** This study was not considered useful for the  
2 evaluation process.

3  
4 **Kinnberg and Toft (513)**, supported by the Danish Environmental Research Programme, examined the  
5 effects of bisphenol A exposure on the reproductive system of male guppies (*Poecilia reticulata*). Thirty  
6 sexually mature male guppies/group were exposed for up to 30 days to bisphenol A [**purity not**  
7 **indicated**] at nominal concentrations of 0 (acetone vehicle) 5, 50, 500, or 5000 µg/L. Levels of bisphenol  
8 A in water were verified. Exposure to the 5000 µg/L concentration was stopped after 21 days because of a  
9 high mortality rate. All fish in the high-dose group and 6 fish/group in the lower dose groups were killed  
10 and fixed in neutral buffered formalin. Histopathological examination was conducted in whole fish. The  
11 mortality rate in the 5000 µg/L group was 77%, but no increase in mortality was observed in the lower  
12 concentration groups. Testes of fish from the high-dose group contained spermatozeugmata (bundles of  
13 spermatozoa with heads pointing outward and tails in the center) in ducts, and the authors stated the effect  
14 indicated blocked spermatogonial mitosis. [**No information was provided on incidence or severity of**  
15 **testicular lesions, and it does not appear that statistical analyses were conducted.**] Additional  
16 compounds were also tested, and it was indicated that effects induced by flutamide, 1,1-dichloro-2,2-  
17 bis(p-chlorophenyl)ethylene, and 4-tert-octylphenol were similar to those observed with bisphenol A  
18 exposure. In contrast, exposure to 0.03 and 0.1 µg/L 17β-estradiol resulted in hypertrophy of Sertoli cells  
19 and efferent duct cells. The study authors concluded that a high bisphenol A concentration induced  
20 adverse effects on testicular structure.

21  
22 **Strengths/Weaknesses:** This study appears to have been well conducted. The metabolism of bisphenol A  
23 in fish is unknown. It appears the bisphenol A does not exhibit the typical 17β-estradiol-like effect on the  
24 testis. Findings occurred at high relative exposures. There was no apparent low-dose effect.

25  
26 **Utility (Adequacy) for CERHR Evaluation Process:** This study was not considered useful for the  
27 evaluation process.

28  
29 **Oehlmann et al. (491)**, supported by the Berlin Federal Environmental Agency, reported the effects of  
30 bisphenol A on reproductive organs in the freshwater ramshorn snail (*Marisa cornuarietis*) and the  
31 marine dog whelk (*Nucella lapillus*). Details of this study are discussed in Section 4.2.1.4, and most of  
32 the findings pertained to female snails. Adult ramshorn snails did not show abnormalities of male sexual  
33 organs or gonads after exposure to bisphenol A [**purity not indicated**] concentrations up to 100 µg/L for  
34 5 months or after exposure for the first year of life. In the dog whelk, a 1 month exposure to 1, 25, or 100  
35 µg/L bisphenol A significantly decreased the proportion of males with sperm in the seminal vesicles  
36 compared to the vehicle-exposed control. The length of the penis and prostate gland were also reduced by  
37 all concentrations of bisphenol A in this animal. The authors concluded that bisphenol A toxicity occurs  
38 in invertebrates at environmentally relevant concentrations.

39  
40 **Strengths/Weaknesses:** The study appears to have been well conducted and suggests that bisphenol A  
41 has an effect on the dog whelk. The potential stability/biotransformation was discussed in the introduction  
42 but not determined during the exposure period.

43  
44 **Utility (Adequacy) for CERHR Evaluation Process:** This study was not considered useful for the  
45 evaluation process.

### 47 4.2.2.5 *In vitro*

48 While cell culture studies can provide useful insights into cellular and subcellular mechanisms, most of  
49 these studies are considered of no utility for the evaluation process. The Akingbemi et al 2004 (350) study  
50 should nevertheless be considered for mechanistic value, and is considered adequate but of limited utility  
51 by the Panel for the evaluation process.

#### 4.0 Reproductive Toxicity Data

1 **Nikula et al. (514)**, support not indicated, examined the in vitro effects of bisphenol A on steroidogenesis  
2 in mouse Leydig tumor cell cultures. Octyl phenols were also examined in this study, but results will not  
3 be discussed. In the first experiment, cells were incubated for 48 hours in media containing bisphenol A  
4 **[purity not indicated]** at 0 (ethanol vehicle) or  $10^{-7}$ – $10^{-4}$  M **[0.023–23 µg/L]** or estradiol at  $10^{-8}$  M  
5 **[culture ware type not indicated]**. Production of cyclic adenosine monophosphate (cAMP) and  
6 progesterone was measured following the incubation period and at 1 and 3 hours following a challenge  
7 with 10 ng/mL hCG. In additional experiments, the cells were exposed to bisphenol A at 0 or  $10^{-6}$  M  
8 **[0.23 µg/L]** or 17β-estradiol or diethylstilbestrol at  $10^{-8}$  M. Production of cAMP and progesterone was  
9 measured following the incubation period and at 1 and/or 3 hours following challenge with hCG,  
10 forskolin, cholera toxin, or 8-bromo-cAMP. An additional study measured binding of  $^{125}$ I-hCG to the LH  
11 receptor following a 48-hour exposure to bisphenol A at 0 or  $10^{-6}$  M **[0.23 µg/L]**. Each experiment  
12 contained 5–8 replicates, and results from 3 independent experiments were pooled. Data were analyzed by  
13 ANOVA followed by Fisher test.

14  
15 Bisphenol A had no effect on basal cAMP or progesterone production. At 3 hours following the hCG  
16 challenge, the increase in cAMP production was attenuated following previous exposure to bisphenol A at  
17 concentrations  $\geq 10^{-7}$  M **[0.023 µg/L]** and increase in progesterone production was reduced at bisphenol A  
18 concentrations  $\geq 10^{-6}$  M **[0.23 µg/L]**. At 3 hours following challenge,  $10^{-6}$  M **[0.23 µg/L]** bisphenol A  
19 decreased hCG-induced cAMP production but had no effect on forskolin- or cholera toxin-induced cAMP  
20 production. Following 3-hour challenges, hCG-induced progesterone production was reduced following  
21 exposure to  $10^{-6}$  M **[0.23 µg/L]** bisphenol A, but there were no effects on forskolin-, cholera toxin-, or 8-  
22 bromo-cAMP-induced progesterone production. Generally, 17β-estradiol and diethylstilbestrol attenuated  
23 hCG-, forskolin, and 8-bromo-cAMP-induced progesterone production. Bisphenol A exposure had no  
24 effect on binding of  $^{125}$ I-hCG to the LH receptor. The study authors concluded that bisphenol A appears to  
25 inhibit cAMP formation and steroidogenesis in rat Leydig tumor cells by preventing coupling between the  
26 LH receptor and adenylate cyclase. Because no inhibition of cAMP production was observed following  
27 incubation of cells with 17β-estradiol, the study authors concluded that the effects of bisphenol A may not  
28 be estrogen related.

29  
30 **Strengths/Weaknesses:** This appears to be a well conducted in vitro study. Stimulation occurred in the  
31 absence of steroid-rich fetal bovine serum. There was no mention of whether phenol red-free media were  
32 used. Cell viability does not appear to have been determined. Because this study used an in vitro system,  
33 the effects of metabolism were limited. Nonetheless, this study provides compelling evidence that the  
34 actions of bisphenol A may be non-estrogen mediated.

35  
36 **Utility (Adequacy) for CERHR Evaluation Process:** This study was not considered useful for the  
37 evaluation process.

38  
39 **Murono et al. (515)**, from the Centers for Disease Control and Prevention, examined the effects of  
40 bisphenol A exposure on steroidogenesis in cultured rat Leydig cells. Leydig cell cultures were prepared  
41 from testes of 55–65-day-old Sprague Dawley rats (n = 8–10). Cells were incubated in 0 or 1–1000 nM  
42 **[0.23–230 µg/L]** bisphenol A **[purity not indicated]** in DMSO vehicle, with and without 10 IU/mL  
43 hCG for 24 hours **[culture ware not indicated]**. Following the incubation period, testosterone level was  
44 measured by RIA and  $^{125}$ I-hCG binding to LH receptors was assessed. Media containing  
45 hydroxycholesterol was then added to the cultures, and testosterone production following a 4-hour  
46 incubation period was measured. The effects of 17β-estradiol and 4-*tert*-octylphenol were also examined,  
47 but will not be discussed. Cell viability was evaluated by trypan blue exclusion and found to be  
48 unaffected at the bisphenol A concentrations used in this study. Three experiments with 4  
49 samples/experiment were conducted. Data were analyzed by ANOVA and Student-Newman-Keuls test.  
50 Bisphenol A had no effect on basal or hCG-induced testosterone production or hCG binding to LH  
51 receptors. **[Data were not shown by study authors.]** Conversion of hydroxycholesterol to testosterone

#### 4.0 Reproductive Toxicity Data

1 was also unaffected by exposure of Leydig cells to bisphenol A. No effect on testosterone production was  
2 observed following exposure of cells to 17 $\beta$ -estradiol. The study authors noted the similarity of effect  
3 between bisphenol A and 17 $\beta$ -estradiol, which differed from the modest effects observed with 4-*tert*-  
4 octylphenol exposure.

5  
6 **Strengths/Weaknesses:** This study appears to have been well conducted. Phenol red-free media were  
7 used and cell viability after treatment was assessed. There was likely limited metabolism of bisphenol A,  
8 and the activity of metabolites cannot be assessed.

9  
10 **Utility (Adequacy) for CERHR Evaluation Process:** This study was not considered useful for the  
11 evaluation process.

12  
13 **Akingbemi et al. (350)**, supported by NIEHS, US EPA, NICHHD, and NIH, conducted in vitro studies to  
14 examine the effects of bisphenol A exposure on Leydig cell cultures. In vivo studies were also conducted  
15 and are described in Section 3 because exposures were commenced in immature animals. In a series of  
16 studies, testosterone production by Leydig cells was assessed following incubation of cells with various  
17 doses of bisphenol A or bisphenol A in combination with other compounds. Leydig cells were obtained  
18 from 90-day-old rats. In a dose-response study, testosterone and 17 $\beta$ -estradiol levels were measured in  
19 Leydig cells that were incubated with bisphenol A [**purity not indicated**] at 0 (ethanol vehicle), 0.01, 0.1,  
20 1, 10, 100, or 1000 nM [**0, 0.0023, 0.023, 0.23, 2.3, 23, and 230  $\mu$ g/L**] bisphenol A for 18 hours [**culture**  
21 **were not indicated**]. To determine if bisphenol A induces estrogenic effects on Leydig cells, testosterone  
22 production was also measured in cells incubated with diethylstilbestrol or 2,2-bis(*p*-hydroxyphenyl)-  
23 1,1,1-trichloroethane, a metabolite of methoxychlor, at the same concentrations as bisphenol A. In  
24 mechanistic studies, Leydig cells were incubated with 0.01 nM [**0.0023  $\mu$ g/L**] bisphenol A, with and  
25 without the addition of LH or the antiestrogenic compound ICI 182,780. Endpoints assessed included  
26 testosterone and 17 $\beta$ -estradiol production and expression of mRNA for steroidogenic metabolizing  
27 enzymes, ER, and steroidogenic acute regulatory protein, a substance that transports the cholesterol used  
28 in testosterone synthesis. Levels of hormones in media were measured using RIA methods, and mRNA  
29 expression was evaluated using RT-PCR techniques. Statistical analyses included ANOVA and the  
30 Duncan multiple range test.

31  
32 In the concentration-response study, production of testosterone by Leydig cells was decreased following  
33 exposure to bisphenol A at 0.01 nM [**0.0023  $\mu$ g/L**] but not at higher doses. Diethylstilbestrol reduced  
34 testosterone production at all dose levels, and 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane reduced  
35 testosterone production at concentrations  $\geq$ 100 nM. Some statistically significant effects were observed in  
36 the mechanistic studies in which cells were exposed to 0.01 nM bisphenol A. In one study, LH-stimulated  
37 but not basal testosterone production was reduced by bisphenol A exposure. A second study demonstrated  
38 a decrease in basal testosterone production following bisphenol A exposure, but no decrease in  
39 testosterone level was observed following incubation of cells with bisphenol A in combination with ICI  
40 182,270. 17 $\beta$ -Estradiol production was decreased in cells exposed to bisphenol A. Changes in mRNA  
41 expression following bisphenol A exposure included reduced expression of mRNA for the steroidogenic  
42 enzymes P45017 $\beta$ -hydroxylase and aromatase. ER $\beta$  was not detected in Leydig cells, and expression of  
43 ER $\beta$  mRNA was not affected. The study authors concluded that environmentally relevant concentrations  
44 of bisphenol A act directly on Leydig cells to inhibit steroidogenesis, presumably via the ER.

45  
46 **Strengths/Weaknesses:** This study appears to have been very well-conducted. The study used a wide  
47 dose range and showed decreased testosterone production in in vitro Leydig cell cultures at low (0.1 nM)  
48 but not at higher concentrations. The response of multiple endpoints provides compelling evidence of a  
49 biological effect at 0.01 nM. An explanation for the selective effect of bisphenol A at this single low  
50 concentration (0.1 nM) was not provided, nor was the dose range of this effect explored.

## 4.0 Reproductive Toxicity Data

1 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate but of limited utility for  
2 the evaluation process.

3  
4 **Song et al. (516)**, supported by the Hormone Research Center and the Korean Andrological Society,  
5 examined the role of bisphenol A in inducing expression of orphan nuclear receptor *Nur77*, a receptor that  
6 plays an important role in the regulation of LH-induced steroidogenesis in Leydig cells. Methods used in  
7 this study are described in conjunction with the results. **[It does not appear that statistical analyses**  
8 **were conducted in this study.]** Following treatment of the mouse Leydig cell line K28 with bisphenol A  
9 **[purity not indicated]** at  $\geq 0.01 \mu\text{M}$ , expression of *Nur77* mRNA was increased in a dose-related  
10 manner, with saturation of expression observed at  $1 \mu\text{M}$  **[0.23 mg/L] [culture ware not indicated]**. In a  
11 time-response study with  $1 \mu\text{M}$  **[0.23 mg/L]** bisphenol A, maximal expression of *Nur77* mRNA was  
12 observed at 30 minutes following treatment, basal levels of expression were observed from 2 to 12 hours  
13 following treatment, and expression was again increased at 24 hours following treatment. When K28 cells  
14 were pretreated with the protein kinase inhibitor H89 or the mitogen-activated protein kinase (MAPK)  
15 inhibitor PD98059, induction of *Nur77* mRNA by bisphenol A was reduced by 40–45%. Induction of *c-*  
16 *fos* and *c-jun* mRNA occurred concurrently with induction of *Nur77* mRNA. Bisphenol A-induced  
17 increases in *Nur77* promoter activity were greater following transfection of cells with *Nur77* promoter  
18 reporter and *c-jun* but not with *c-fos*. Possible activation of MAPK by bisphenol A was examined using  
19 an immunoblot method with an antibody specific for phosphorylated MAPK. Phosphorylation of MAPK  
20 reached a maximum level at 10 minutes following bisphenol A treatment. No changes in bisphenol A-  
21 induced induction of *Nur77* were observed following pretreatment with a protein kinase C inhibitor or  
22 P13K inhibitor. The study authors stated that together these results suggest possible involvement of the  
23 protein kinase A and MAPK pathways in bisphenol A-induced induction of *Nur77*.

24  
25 In K28 cells transfected with *Nur77* promoter or monomer binding site-luciferase reporters, gene  
26 promoter activities and transactivation were increased following treatment with  $\geq 0.1 \mu\text{M}$  **[0.023 mg/L]**  
27 bisphenol A, thus suggesting similar responses between promoter activity and mRNA induction. In a  
28 yeast assay, bisphenol A had no effect on interactions between *Nur77* and its corepressor, silencing  
29 mediator of retinoid and thyroid receptor.

30  
31 Exposure of K28 cells to  $1 \mu\text{M}$  **[0.23 mg/L]** bisphenol A resulted in increased progesterone production,  
32 which was inhibited 25% by the overexpression of dominant negative *Nur77*, which reduces the  
33 transactivation activity of *Nur77*. Expression of mRNA for steroidogenic enzymes was investigated and it  
34 was found that bisphenol A treatment increased expression of steroidogenic acute regulatory mRNA,  
35 cholesterol side-chain cleavage enzyme, and  $3\beta$ -hydroxysteroid dehydrogenase. Effects of bisphenol A on  
36 expression of mRNA for *Nur77* and steroidogenesis enzymes was tested in prepubertal mice (18 days  
37 old). Injection of 5 mice/group with 125 mg/kg bw/day bisphenol A resulted in increased expression of  
38 *Nur77* mRNA and testosterone levels in mouse testis from 1–6 hours following exposure. **[Very few**  
39 **details were provided for the in vivo experiment.]** The study authors concluded that the results of these  
40 studies indicate that bisphenol A induces *Nur77* gene expression and alters steroidogenesis in Leydig  
41 cells, indicating a possible novel mechanism of toxicity.

42  
43 **Strengths/Weaknesses:** This study appears to have been well conducted and links in vitro bisphenol A  
44 administration to dose-related (classic, not inverted) activation of *Nur77* and subsequent downstream  
45 signal transducing proteins. Various confirmatory experiments supported this relationship. These data  
46 strongly suggest that bisphenol A ( $>0.1 \mu\text{M}$ ) activates *Nur77*. The toxicological implications of these  
47 findings were not addressed.

48  
49 **Utility (Adequacy) for CERHR Evaluation Process:** This study was not considered useful for the  
50 evaluation process.

51

## 4.0 Reproductive Toxicity Data

1 **Hughes et al. (517)**, supported by the Medical Research Council, the British Heart Fund, and the  
2 European Chemical Industry Council, examined the effects of bisphenol A on rat testicular calcium  
3 pumps. Other phenolic compounds were examined, some in greater detail than bisphenol A, but this  
4 discussion is limited to bisphenol A. Studies were conducted to determine the effects of bisphenol A  
5 exposure on calcium ATPase pump activity, calcium uptake in testicular microsomes, calcium levels in  
6 the TM4 Sertoli cell line, and TM4 cell viability [culture ware not indicated]. In the cell-viability study,  
7 cells were exposed to bisphenol A [purity not indicated] at 0, 100, 300, or 600  $\mu\text{M}$  [0, 23, 68, or 137  
8 mg/L] for 16 hours. In each study, 2–12 samples/group were analyzed. [For most studies, very few  
9 details were provided about procedures such as exposure concentrations used and time that cells  
10 were incubated. There was no discussion of statistical procedures, and it was not clear if statistical  
11 analyses were conducted for some endpoints.]

12  
13 Bisphenol A inhibited calcium ATPase activity in rat testis microsomes. Mean  $\pm$  SEM median inhibitory  
14 concentration ( $\text{IC}_{50}$ ) values were reported at  $0.40 \pm 0.15 \mu\text{M}$  [91  $\pm$  34  $\mu\text{g/L}$ ] for inhibition of calcium  
15 ATPase activity and  $2.5 \pm 1.0 \mu\text{M}$  [571  $\pm$  228  $\mu\text{g/L}$ ] for calcium uptake. Exposure to 200  $\mu\text{M}$  [47 mg/L]  
16 bisphenol A increased intracellular calcium levels in TM4 cells. A viability study was conducted to  
17 determine if increased intracellular calcium levels resulted in cell death. Bisphenol A exposure resulted in  
18 reduced TM4 cell viability (percent viability compared to control cells was 93, 64, and 17% at  
19 concentrations of 100, 300, and 600  $\mu\text{M}$ ). The study authors concluded that these results provide evidence  
20 that environmental estrogens may induce toxicity in male reproductive development by disrupting  
21 calcium homeostasis.

22  
23 **Strengths/Weaknesses:** This interesting mechanistic study examined the role of bisphenol A in  
24 modulating intracellular calcium levels. It is difficult to interpret the relationship between microsomal and  
25 intact cell effects of bisphenol A given the large difference in concentrations needed to produce an effect.  
26 Moreover, it is not clear if bisphenol A caused cytotoxicity by a calcium-dependent or non-calcium-  
27 mediated process.

28  
29 **Utility (Adequacy) for CERHR Evaluation Process:** This study was not considered useful for the  
30 evaluation process.

31  
32 **Tabuchi et al. (518)**, supported by the Japanese Ministry of Education, Culture, Sports, Science, and  
33 Technology and Takeda Science Foundation, examined the effects of bisphenol A exposure on viability  
34 and gene expression in TTE3 cells, a mouse Sertoli cell line. The cells were incubated for 24 hours in  
35 media containing 0 or 24–400  $\mu\text{M}$  [5.5–91 mg/L] bisphenol A (99.7% purity) in a DMSO vehicle  
36 [culture ware not indicated]. Cell viability was determined, and gene expression changes were  
37 examined using microarray and PCR techniques. Data were analyzed by Dunnett multiple comparison test  
38 or Student *t*-test. Compared to values in control cells, bisphenol A exposure reduced cell viability by 25%  
39 at 100  $\mu\text{M}$  [23 mg/L], 33% at 200  $\mu\text{M}$  [46 mg/L], and 96% at 400  $\mu\text{M}$  [91 mg/L]. Based on the results of  
40 the cell-viability studies, a bisphenol A concentration of 200  $\mu\text{M}$  [46 mg/L] was selected for the gene  
41 expression studies. Of 1081 genes examined by microarray, mRNA was downregulated in 3 cases and  
42 upregulated in 10 cases. Six genes were selected for evaluation of mRNA expression by PCR, and of  
43 those genes, 1 was downregulated (*ER $\alpha$* ) and 5 were upregulated (*iNOS*, *chop-10*, *odc*, *BipGRP78*, and  
44 *osip*). The study authors concluded that microarray analysis is a useful tool for investigating molecular  
45 mechanisms of bisphenol A-induced toxicity in testicular cells.

46  
47 **Strengths/Weaknesses:** This interesting mechanistic study appears to have been well conducted, but it is  
48 unclear from the data if bisphenol A-related changes in *chop-10* are a primary (or secondary) effect or are  
49 the result of cytotoxicity.

50  
51 **Utility (Adequacy) for CERHR Evaluation Process:** This study is not useful in the evaluation.

#### 4.0 Reproductive Toxicity Data

1 **Tabuchi and Kondo (519)**, supported by Japanese Ministry of Education, Culture, Sports, Science, and  
2 Technology, Takeda Science Foundation, and Toyama Daiichi Bank Foundation, conducted a series of  
3 experiments to examine the effects of in vitro bisphenol A exposure on gene expression in mouse Sertoli  
4 cells. The experiments used TTE3 cells, an immortalized Sertoli cell line established from transgenic  
5 mice expressing temperature-sensitive simian virus large T-antigen. Cells were exposed to bisphenol A  
6 (99.7% purity) in a DMSO vehicle [**culture ware not discussed**]. The majority of experiments were  
7 repeated 2–4 times, and data were analyzed by Student *t*-test. [**Statistical significance was not reported**  
8 **in the results section of the study.**] Prior to conducting gene expression studies, cells were exposed to  
9 25–400  $\mu\text{M}$  [**5.7–91 mg/L**] bisphenol A for 3–24 hours, and viability was determined using a tetrazolium  
10 compound. Cell viability was reduced at bisphenol A concentrations  $\geq 200 \mu\text{M}$  [**46 mg/L**], and reductions  
11 in viability were increased with longer durations of exposure. Intracellular calcium levels were measured  
12 using a fluorescence imaging technique over a 15-minute period in cells exposed to 0–400  $\mu\text{M}$  [**0–91**  
13 **mg/L**] bisphenol A, and a dose-related increase in calcium influx was observed at  $\geq 100 \mu\text{M}$  [**23 mg/L**].  
14 Based on results for cell viability and calcium influx studies, a concentration of 200  $\mu\text{M}$  [**46 mg/L**] was  
15 selected for the gene-expression experiments.

16  
17 Using a PCR technique, it was determined that expression of mRNA for transferrin was decreased and  
18 glucose-regulated protein mRNA was increased by bisphenol A exposure of up to 24 hours. Observations  
19 of increased intracellular calcium concentration and upregulated glucose-regulated protein mRNA  
20 expression led the study authors to conclude that bisphenol A stresses the endoplasmic reticulum. Gene  
21 expression was analyzed by a cDNA microarray technique after exposure for 3, 6, 12, and 24 hours, and it  
22 was determined that 31 of the 865 genes examined were upregulated by exposure to bisphenol A; no  
23 downregulation of genes was observed. The greatest change in gene expression was observed for *chop-*  
24 *10*, a stress-response gene. Upregulation of 4 genes, *c-myc*, *fra-2*, *odc*, and *chop-10*, were confirmed by  
25 quantitative PCR. *Chop-10* was determined to be the most responsive gene. To determine if *chop-10* was  
26 required for development of endoplasmic reticulum stress and cell injury, a stably transfected cell line  
27 expressing *chop-10* antisense RNA (*chopR14*) was developed. Mock cells were used as negative controls  
28 in studies where cells were exposed to 200  $\mu\text{M}$  [**46 mg/L**] bisphenol A for up to 24 hours. Production of  
29 chop-10 protein, as determined by Western blot analysis, was reduced in the *chopR14* cells compared to  
30 the mock cells following exposure to bisphenol A. In contrast to the mock cells, no reductions in cell  
31 viability or transferrin mRNA expression were observed in the *chopR14* cells following bisphenol A  
32 exposure. There were no changes in glucose-regulated protein mRNA expression in *chopR14* versus  
33 mock cells. The study authors postulated that bisphenol A may disrupt the male reproductive system by  
34 altering calcium homeostasis in Sertoli cell endoplasmic reticulum without interacting with the ER and  
35 that genes such as *chop-10* may be involved in the process.

36  
37 **Strengths/Weaknesses:** This mechanistic study appears to have been well conducted, but it is unclear  
38 from the data if bisphenol A-related changes in *chop-10* are a primary (or secondary) effect or are the  
39 result of cytotoxicity. Calcium levels were also affected and collectively these changes may be the result  
40 of apoptosis initiated by some other mechanism.

41  
42 **Utility (Adequacy) for the CERHR Evaluation Process:** This study was not considered useful for the  
43 evaluation process.

44  
45 **Tabuchi et al. (520)**, supported in part by the Japanese Ministry of Education, Culture, Sports, Science,  
46 and Technology, examined the effects of bisphenol A on gene expression in mouse Sertoli cell cultures.  
47 TTE3 cells were incubated in media containing bisphenol A [**purity not reported**] at 0 (DMSO vehicle)  
48 or 200  $\mu\text{M}$  [**46 mg/L**] for up to 12 hours [**culture ware type not discussed**]. Cells were examined for  
49 viability using dye exclusion assays and for apoptosis by formation of DNA ladders. RNA was extracted  
50 from cells, and gene expression was determined by PCR and microarray analyses. Data were analyzed by  
51 Student *t*-test. Cell viability was decreased in a time-related manner between 3 and 12 hours of bisphenol

## 4.0 Reproductive Toxicity Data

1 A exposure, but there was no evidence of apoptosis. PCR analysis indicated that bisphenol A exposure  
2 significantly and time-dependently increased mRNA transcripts for 2 endoplasmic reticulum stress  
3 markers, *hspa5* and *ddit3*. Microarray analysis demonstrated that 661 sets of genes were downregulated  
4 and 604 sets of genes were upregulated more than 2-fold following bisphenol A exposure. Pathway  
5 analysis of decreased gene clusters revealed 2 significant genetic networks associated with the cell cycle  
6 or cell growth and proliferation. In increased gene clusters, two genetic networks were associated with  
7 cell death, DNA replication, recombination and repair, or injuries and abnormalities. The study authors  
8 concluded that the genes, genetic clusters, and genetic networks identified in this study are likely involved  
9 in Sertoli cell injury following bisphenol A exposure.

10  
11 **Strengths/Weaknesses:** State-of-the-art technology was used in this study to examine gene expression  
12 changes after in vitro bisphenol A exposure of a Sertoli cell line. Only one dose level was examined. The  
13 use of hormone rich fetal bovine serum in the media may be a confounder. The absence of DNA  
14 laddering is not conclusive evidence of the absence of apoptosis (e.g., adherent cells undergoing apoptosis  
15 often are released into the culture media). Moreover, it is not surprising that given this “high” bisphenol A  
16 concentration, “novel” and likely non-specific gene changes were noted.

17  
18 **Utility (Adequacy) for CERHR Evaluation Process:** This study was not considered useful for the  
19 evaluation process.

### 20 21 4.2.3 Male and female

#### 22 23 4.2.3.1 Rat

24 Two unpublished studies performed by the International Research and Development Corporation for  
25 General Electric (335, 336) provided some information on reproductive toxicity in rats orally exposed to  
26 bisphenol A. The studies are described in detail in Section 3.2.3.1. There was no effect on fertility in male  
27 and female rats given feed containing up to 9000 ppm bisphenol A (~650 mg/kg bw/day in males and 950  
28 mg/kg bw/day in females) for an unspecified period prior to mating (335). A second study reported no  
29 effects on estrus cyclicity or gestation length [**data not shown by study authors**] or male or female  
30 fertility in rats given feed containing bisphenol A at up to 1000 ppm (~60 mg/kg bw/day in males and 100  
31 mg/kg bw/day in females) for ~70 days before mating (336).

32  
33 **Emm et al. (337)**, supported by the Japanese Ministry of Health and Welfare, conducted a multigeneration  
34 reproductive toxicity study of bisphenol A in CD rats. Animals were housed in suspended stainless steel  
35 cages at the beginning of the study. From GD 17, wood chips were used as bedding. Rats were fed CRF-1  
36 chow (Oriental Yeast Co). In the study that was conducted according to GLP, F<sub>0</sub> male rats and female rats  
37 with 4–5-day estrous cycles were randomly assigned to groups of 25/sex. Five-week-old males and 10-  
38 week-old females were gavaged with 0 (distilled water vehicle), 0.0002, 0.002, 0.020, or 0.200 mg/kg  
39 bw/day bisphenol A (99.9% purity). Males were dosed for 10 weeks prior to mating and during the  
40 mating period, which lasted up to 2 weeks. Females were dosed from 2 weeks prior to mating, and during  
41 the mating, gestation, and lactation periods. Doses were based on results of studies by Nagel et al. (275)  
42 and vom Saal et al. (392). Stability and concentration of dosing solutions were verified. Dams delivered  
43 and nursed their pups. At weaning on PND 22 (day of birth defined as PND 0), 1 or 2 F<sub>1</sub>  
44 weanlings/litter/sex (25/sex/group) were selected to continue in the study. Dosing of F<sub>1</sub> animals began on  
45 PND 23 and continued for 10 weeks prior to mating and through the mating period, which lasted up to 3  
46 weeks. Dosing was continued through the gestation and lactation periods. Twenty-five F<sub>2</sub>  
47 weanlings/sex/group were selected on PND 22. Beginning on PND 22, male F<sub>2</sub> rats were dosed for 4  
48 weeks and females were dosed for 11 weeks prior to being killed.

49  
50 Endpoints examined in adult rats included clinical signs, body weight, and food intake. Fertility,  
51 population, and gestational indices were examined in mating rats. Vaginal smears were evaluated for two

#### 4.0 Reproductive Toxicity Data

1 weeks prior to mating in F<sub>0</sub> and F<sub>1</sub> females and at 9–11 weeks of age in F<sub>2</sub> females. Dams were killed and  
2 necropsied following weaning of their pups, and uterine implantation sites were examined. Males were  
3 killed following mating. Organs were weighed and histopathology examinations were conducted in  
4 control and high-dose animals. Sperm endpoints were measured in F<sub>0</sub> and F<sub>1</sub> adult males. Serum hormone  
5 levels were measured in 6 adult F<sub>0</sub> and F<sub>1</sub> males and proestrous females. At birth, pups were counted,  
6 sexed, and examined for viability and external malformations. On PND 4, litters were culled to 4 male  
7 and 4 female pups. At weaning, 1 male and female F<sub>1</sub> and F<sub>2</sub> weanling was killed for organ weight  
8 measurement; histopathology exams were conducted in seminal vesicles and coagulating glands of F<sub>2</sub>  
9 weanlings. Survival and growth were monitored during the postnatal period. Pups were examined for  
10 developmental landmarks and attainment of vaginal opening or preputial separation. Anogenital distance  
11 in pups was examined at numerous time points during the lactation period and through adulthood.  
12 Behavioral testing was conducted at 5–7 weeks of age. The litter was considered the experimental unit in  
13 data obtained prior to weaning. Statistical analyses included Bartlett test for homogeneity of variance,  
14 ANOVA, and/or Dunnett multiple comparison, Kruskal-Wallis, Mann-Whitney *U*, chi-squared, or Fisher  
15 exact tests.

16  
17 In F<sub>0</sub> and F<sub>1</sub> adult animals, there were no treatment-related effects on clinical signs, body weight gain, or  
18 death. The only significant reproductive effects reported in adult animals were non-dose-related decreases  
19 in percentages of females with normal estrous cycles (76 versus 96% in controls) and reduced gestation  
20 duration (by 0.5 days) in the F<sub>1</sub> group treated with 0.020 mg/kg bw/day. Bisphenol A did not significantly  
21 affect the precoital interval, copulation index, fertility index, gestation index, number of implantations, or  
22 delivery index. There were no adverse effects on sperm endpoints such as count, motility, or morphology  
23 in F<sub>0</sub> or F<sub>1</sub> males. A significant decrease in abnormal and tailless sperm was observed in F<sub>1</sub> males of the  
24 0.020 mg/kg bw/day group. There was no evidence of histopathological effects in reproductive organs of  
25 F<sub>0</sub> animals that did not copulate or had totally resorbed litters or in F<sub>1</sub> animals of the high-dose group.

26 **[Data were not shown by study authors.]** In F<sub>0</sub> females, there were significant decreases in serum LH  
27 concentrations at 0.0002, 0.002, and 0.020 mg/kg bw/day and in serum triiodothyronine levels at 0.200  
28 mg/kg bw/day. **[Data were not shown by study authors.]** Organ weight changes in F<sub>1</sub> adult males  
29 included decreased absolute weights of lung at 0.0002 and 0.200 mg/kg bw/day, kidney at 0.2 mg/kg  
30 bw/day, and testis at 0.020 mg/kg bw/day. Absolute ovarian weight was decreased in females of the  
31 0.0002 mg/kg bw/day group. Seminal vesicle weight was decreased in F<sub>2</sub> males of the 0.200 mg/kg  
32 bw/day group. **[Data were not shown by study authors].**

33  
34 There were no significant effects on number of F<sub>1</sub> or F<sub>2</sub> pups delivered, sex ratio, or pup survival during  
35 the lactation period. Body weights of F<sub>1</sub> pups in the 0.020 mg/kg bw/day group were significantly lower  
36 **[by 6–7%]** on PND 14 and 21. Testicular descent was delayed by 0.7 days in F<sub>2</sub> offspring from the 0.020  
37 and 0.200 mg/kg bw/day groups. There were no significant effects on age of pinna detachment, incisor  
38 eruption, or eye opening. Some significant but non-dose-related effects on reflex development were  
39 observed. Day of mid-air righting reflex was accelerated by 1.2 days in F<sub>1</sub> males and 1.5 days in F<sub>1</sub>  
40 females of the 0.020 mg/kg bw/day group. In F<sub>2</sub> males, negative geotaxis was delayed by 0.8 days at  
41 0.0002 mg/kg bw/day, 0.5 days at 0.002 mg/kg bw/day, and 0.8 days at 0.020 mg/kg bw/day. Bisphenol  
42 A treatment did not significantly affect age of vaginal opening or preputial separation in F<sub>1</sub> or F<sub>2</sub>  
43 offspring. Some sporadic and small (within 5% of control values) changes in anogenital distance were  
44 observed in F<sub>1</sub> and F<sub>2</sub> offspring. In F<sub>1</sub> males, decreased anogenital distance was observed in the 0.0002  
45 mg/kg bw/day group on PND 57 and in the 0.020 mg/kg bw/day group on PND 106, 113, and on the day  
46 of sacrifice. In F<sub>1</sub> females, anogenital distance was decreased in the 0.200 mg/kg bw/day group on PND 4  
47 and increased in the 0.002 and 0.020 mg/kg bw/day group on PND 7. Decreases in anogenital distance of  
48 F<sub>2</sub> females were observed in the 0.020 mg/kg bw/day group on PND 64, 71, 85, 92, and on the day of  
49 sacrifice and in the 0.200 mg/kg bw/day group on PND 57, 64, and on the day of sacrifice. In F<sub>1</sub>  
50 offspring, there were no significant effects on behavior, as determined by open-field testing and  
51 performance in a T-maze. **[Data were not shown by study authors.]** There was no evidence of

#### 4.0 Reproductive Toxicity Data

1 histopathological effects in seminal vesicle or coagulating gland of F<sub>2</sub> pups from the high-dose group.  
2 **[Data were not shown by study authors.]** Organ weight changes in F<sub>1</sub> male weanlings included  
3 decreased absolute lung weight at 0.020 and 0.200 mg/kg bw/day group and decreased kidney weight at  
4 0.020 mg/kg bw/day. In male F<sub>2</sub> weanlings, significant decreases were observed in absolute and relative  
5 seminal vesicle weight and absolute thyroid weight at 0.002 mg/kg bw/day, absolute lung weight at 0.020  
6 mg/kg bw/day, and relative heart weight at 0.200 mg/kg bw/day; relative liver weight was significantly  
7 increased in F<sub>2</sub> males of the 0.002 mg/kg bw/day group. The study authors concluded that oral  
8 administration of bisphenol A at 0.0002 to 0.200 mg/kg bw/day to 2 generations of rats did not cause  
9 changes in reproduction or development.

10  
11 **[The NTP Statistics Subpanel (340) reviewed an unpublished study that appeared to be the same**  
12 **study later published as Ema et al. (337). The subpanel noted that in general they agreed with the**  
13 **statistical methodology used in the study but stated that the Dunnett test does not require**  
14 **significance of ANOVA. It was noted that the anogenital distance findings were the most difficult to**  
15 **interpret. The Subpanel noted that many of the anogenital distance effects remained statistically**  
16 **significant when analyzed by ANCOVA, a method they considered superior to adjustment by body**  
17 **weight. The NTP Subpanel agreed with the author's conclusion that effects on anogenital distance**  
18 **were not biologically significant. They noted an error in the unpublished study abstract that**  
19 **described increases in anogenital distance in F<sub>1</sub> and F<sub>2</sub> females in the 0.020 and 0.2 mg/kg bw/day**  
20 **groups when actually the effect should have been decreased anogenital distance. [It was not clear to**  
21 **CERHR if this error was carried forward to the published report.]**

22  
23 **Strengths/Weaknesses:** This well-designed comprehensive low-dose assessment of potential bisphenol  
24 A-related effects on multiple generations of rats examined a wide variety of hormonally sensitive  
25 endpoints. The study had appropriate power with an appropriate number of rats per group. Route of  
26 administration (oral) was appropriate. The concentrations of the dosing solutions were verified (both prior  
27 and after). It would have been helpful if a dose level that caused maternal toxicity was also used;  
28 however, given the objective of this study it is a minor point. This thorough multiple generation rat study  
29 is highly valuable for human risk assessment of low dose oral exposure to bisphenol A. This study  
30 indicates that the NOAEL for bisphenol A exceeds 0.2 mg/kg bw/day under the conditions of this study.

31  
32 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
33 evaluation process.

34  
35 **Tyl et al. (338, 475),** sponsored by The Society of the Plastics Industry, Inc., conducted a multigeneration  
36 study of bisphenol A in rats. In the study that was conducted according to GLP, Sprague Dawley rats  
37 were fed Purina Certified Rodent Chow® 5002. F<sub>0</sub> rats (30/sex/group) were exposed to bisphenol A  
38 (99.5% purity) in feed for 10 weeks prior to mating. **[Age at start of exposure was not reported, but**  
39 **based on information provided in the discussion, it appears that the animals were adults at the start**  
40 **of exposure.]** Vaginal smears were evaluated during the last 3 weeks of the prebreeding period. Exposure  
41 continued through a 2-week mating period. Males were exposed an additional 3 weeks following mating,  
42 and females were exposed through gestation and lactation. Concentrations of bisphenol A added to feed  
43 were 0, 0.015, 0.3, 4.5, 75, 750, or 7500 ppm. Target intakes were ~0, 0.0009, 0.018, 0.27, 4.5, 45, and  
44 450 mg/kg bw/day in males and 0.001, 0.02, 0.30, 5, 50, and 500 mg/kg bw/day in females. Actual  
45 intakes were 0.0007–0.003, 0.015–0.062, 0.22–0.73, 4.1–15.4, 37.6–167.2, and 434–1823 mg/kg bw/day.  
46 The study was designed to include low-dose exposures reported to increase prostate weights (275, 521)  
47 and maximally tolerated doses expected to result in toxicity. Concentration, stability, and homogeneity of  
48 bisphenol A in feed were verified. During the study, body weight and food intake were measured and  
49 animals were examined for clinical signs. F<sub>0</sub> males were killed and necropsied following delivery of the  
50 F<sub>1</sub> litter. Histopathological evaluation of organs was conducted in all control animals and 10  
51 animals/bisphenol A dose group. Reproductive organs were weighed and sperm endpoints were

#### 4.0 Reproductive Toxicity Data

1 evaluated. F<sub>0</sub> females were killed and necropsied following weaning of their litters. Selected organs were  
2 weighed and ovarian primordial follicles were counted.  
3

4 On PND 4, F<sub>1</sub> litters were culled to 10 pups, with equal numbers of each sex when possible. Endpoints  
5 examined in pups included growth and survival in the prenatal period and retained areolae or nipples on  
6 PND 11–13. At weaning on PND 21, 30 F<sub>1</sub> offspring/sex/group were randomly selected and exposed to  
7 bisphenol A in the diet according to the same protocol as F<sub>0</sub> rats. Those selected offspring were monitored  
8 for vaginal opening and preputial separation and later mated. Up to 3 F<sub>1</sub> weanlings/sex/litter were killed  
9 for organ weight measurement. Mating and evaluation of F<sub>1</sub> offspring were conducted according to the  
10 same procedures described for F<sub>0</sub> rats. The same procedures were repeated in F<sub>2</sub> rats and F<sub>3</sub> litters during  
11 the lactation period. Anogenital distance was measured in F<sub>2</sub> and F<sub>3</sub> rats at birth. Following weaning of F<sub>3</sub>  
12 offspring, up to 3/sex/litter were randomly selected for necropsy. Thirty/sex/dose were selected for  
13 evaluation of vaginal patency, preputial separation, and estrous cyclicity. Bisphenol A exposure was  
14 continued in those offspring until they were killed ~10 weeks following weaning. F<sub>3</sub> offspring were not  
15 mated, but necropsy evaluations were conducted as described above for previous generations.  
16

17 Statistical analyses for quantitative continuous data included Bartlett test for homogeneity of variances,  
18 ANOVA, Dunnett, linear trend, Kruskal-Wallis, or Mann-Whitney *U* tests. Frequency data were analyzed  
19 by chi-squared, Fisher exact, and Cochran-Armitage tests. Covariance and correlations analyses were also  
20 conducted.  
21

22 Treatment-related systemic findings with available quantitative information in adult rats are summarized  
23 in [Table 93](#). Body weights and body weight gain were consistently lower in F<sub>0</sub>, F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> adult rats of  
24 the 750 and 7500 ppm dose groups, including during gestation and lactation periods. Terminal body  
25 weight effects are summarized in [Table 93](#). Terminal body weight was reduced in all generations at 7500  
26 ppm and in F<sub>1</sub> females and F<sub>1</sub> and F<sub>2</sub> males at 750 ppm. There were no consistent or clearly treatment-  
27 related effects on feed intake. No treatment-related clinical signs were reported. In the 7500 ppm group,  
28 absolute weights of the liver in males and the kidney in both sexes were decreased across generations.  
29 Relative weights were either increased or did not attain statistical significance. **[According to Table 2 of  
30 the study, absolute liver weights were also decreased in males of the 750 ppm group. The study  
31 authors also mentioned reductions in weights of adrenal glands, spleen, pituitary, and brain at the  
32 high dose, but there were no data shown in the report for those endpoints.]** Other changes in non-  
33 reproductive organ weight occurred sporadically at lower dose and were not dose-related or consistent  
34 across generations. Relative organ weight changes that consistently attained statistical significance at the  
35 highest dose are summarized in [Table 93](#). Histopathological analyses revealed a higher incidence of mild  
36 renal tubular degeneration and chronic hepatic inflammation in F<sub>0</sub>, F<sub>1</sub>, and F<sub>2</sub> but not F<sub>3</sub> females of the  
37 7500 ppm group.  
38

39 Treatment-related effects on reproductive endpoints in adult animals are summarized in [Table 93](#). In  
40 evaluating organ weights, the study authors only considered organ weight effects to be biologically  
41 significant if statistically significant results were obtained in the same direction for absolute and relative  
42 weights. Therefore, the study authors concluded that the only treatment-related organ weight effects were  
43 reduced absolute and relative ovary weights. **[Numerous statistically significant effects on  
44 reproductive organ weights were reported in Table 2 of the study. Reductions in testes,  
45 epididymides, prostate, and seminal vesicle weights were observed in most generations of the 7500  
46 ppm group. When adjusted for body weight, organ weights were either increased or did not differ  
47 significantly from controls.]** Relative reproductive organ weight changes that consistently attained  
48 statistical significance at the highest dose are summarized in [Table 93](#). The authors reported no effect on  
49 mating, fertility, pregnancy, or gestational indices. **[With the exception of gestational length, data were  
50 not shown by study authors.]** Precoital interval, postimplantation loss, estrous cyclicity, and  
51 reproductive organ histopathology were also unaffected by bisphenol A treatment. In the high-dose group,

#### 4.0 Reproductive Toxicity Data

1 there was no adverse effect on paired ovarian primordial follicle counts but counts were significantly  
2 increased by 43% in the F<sub>0</sub> generation. Implantation sites were decreased in F<sub>0</sub>, F<sub>1</sub>, and F<sub>2</sub> dams of the  
3 7500 ppm group. The only significant effects on sperm endpoints were decreased epididymal sperm  
4 concentration in F<sub>1</sub> males and decreased daily sperm production in F<sub>3</sub> males of the 7500 ppm dose group.  
5 There were no effects on sperm morphology or motility. The study authors considered sperm to be  
6 unaffected by treatment.

7  
8 Treatment-related effects observed in developing rats are summarized in [Table 94](#). The number of live  
9 pups/litter was reduced in F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> litters of the 7500 ppm group. Body weights of F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub>  
10 pups of the 7500 mg/kg bw/day groups were lower during the lactation period. Some small (~5%)  
11 decreases in pup body weight during the lactation period at lower doses were apparently not considered  
12 treatment-related by study authors. Postnatal survival was unaffected by bisphenol A treatment. In male  
13 rats, there were no effects on anogenital distance or the presence of areolas or nipples. Anogenital  
14 distance was significantly increased in F<sub>2</sub> females at all doses except 75 and 7500 ppm; there was no  
15 affect on anogenital distance in F<sub>3</sub> females. The study authors did not consider anogenital distance effects  
16 to be biologically or toxicologically significant. Vaginal patency was delayed in F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> females,  
17 and the effect remained significant following adjustment for body weight. Preputial separation was  
18 delayed in F<sub>1</sub> males of the 750 and 7500 ppm groups, F<sub>2</sub> males in the 0.3, 75, 750, and 7500 ppm groups,  
19 and F<sub>3</sub> males of the 7500 ppm group. When adjusted for body weight, the effect remained significant in F<sub>1</sub>  
20 males of the 750 and 7500 ppm groups and F<sub>2</sub> and F<sub>3</sub> males of the 7500 ppm group. The study authors  
21 stated that reduced body weights were the most likely cause of puberty delay in males and females. **[In**  
22 **rats killed at weanling, absolute organ weights were said to be decreased at the high dose but**  
23 **increased when adjusted for body weight. The specific organs affected were not reported and no**  
24 **data were presented. The exception was ovarian weights, which were reported to parallel effects**  
25 **observed in adult females with decreases in both absolute and relative weight at 7500 ppm.]**

26  
27 The study authors concluded that there was no evidence of low-dose bisphenol effects (1 µg to 5 mg/kg  
28 bw/day) at any stage of the life cycle. They identified NOAELs of 75 ppm (~5 mg/kg bw/day) for adult  
29 systemic toxicity and 750 ppm (~50 mg/kg bw/day) for offspring and reproductive effects. The study  
30 authors concluded that bisphenol A should not be considered a selective reproductive toxicant.

## 4.0 Reproductive Toxicity Data

**Table 93. Treatment-related Effects in Adult Rats Fed Bisphenol A Through Diet in a Multigeneration Reproductive Toxicity Study**

Endpoint	Dose, ppm diet [mg/kg bw/day <sup>a</sup> ]										BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>		
	0.015 [0.0095]	0.3 [0.019]	4.5 [0.285]	75 [4.75]	750 [47.5]	7500 [475]										
Terminal body weight																
F <sub>0</sub> males	↔	↔	↔	↔	↔	↔	↓22%	3554	[225]	3137	[199]	3133	[198]	2701	[171]	
F <sub>1</sub> males	↔	↔	↔	↔	↔	↓6%	↓26%	2811	[178]	2548	[161]	2443	[155]	2153	[136]	
F <sub>2</sub> males <sup>b</sup>	↔	↔	↔	↔	↔	↓12%	↓29%	733	[46]	554	[35]	648	[41]	484	[31]	
F <sub>3</sub> males <sup>b</sup>	↔	↔	↔	↔	↔	↔	↓26%	1456	[92]	913	[58]	1260	[80]	786	[50]	
F <sub>0</sub> females	↔	↔	↔	↔	↔	↔	↓13%	5722	[362]	4753	[301]	4741	[300]	3876	[245]	
F <sub>1</sub> females	↔	↔	↔	↔	↔	↓6%	↓16%	4600	[291]	3950	[250]	3730	[236]	3142	[199]	
F <sub>2</sub> females <sup>b</sup>	↔	↔	↔	↔	↔	↔	↓14%	3863	[245]	1576	[100]	3115	[197]	1291	[82]	
F <sub>3</sub> females	↔	↔	↔	↔	↔	↔	↓20%	3664	[232]	3194	[202]	3456	[219]	2949	[187]	
Relative paired kidney weight																
F <sub>0</sub> males	↔	↔	↔	↔	↔	↔	↑14%	5903	[374]	4555	[288]	6536	[414]	5035	[319]	
F <sub>1</sub> males	↔	↔	↓5%	↔	↔	↔	↑10%	5729	[363]	4662	[295]	5053	[320]	4088	[259]	
F <sub>2</sub> males	↔	↔	↔	↔	↔	↑5%	↑18%	4524	[287]	3893	[247]	3471	[220]	2950	[187]	
F <sub>3</sub> males	↔	↔	↔	↔	↔	↔	↑16%	6986	[442]	4319	[274]	6720	[426]	3403	[216]	
F <sub>0</sub> females	↔	↔	↔	↔	↔	↔	↑7%	8008	[507]	7521	[476]	7712	[488]	6578	[417]	
F <sub>2</sub> females	↔	↔	↔	↔	↔	↔	↑6%	7930	[502]	7515	[476]	7621	[483]	6247	[396]	
Relative paired testis weight																
F <sub>0</sub> males	↔	↔	↔	↔	↔	↔	↑27%	2924	[185]	2567	[163]	2998	[190]	2596	[164]	
F <sub>1</sub> males	↔	↔	↔	↔	↔	↔	↑18%	3287	[208]	2763	[175]	4106	[260]	3428	[217]	
F <sub>2</sub> males	↔	↔	↔	↔	↔	↔	↑24%	3086	[195]	2874	[182]	3245	[206]	2779	[176]	
F <sub>3</sub> males	↔	↔	↔	↔	↔	↔	↑19%	4329	[274]	2593	[164]	5010	[317]	3298	[209]	
Relative paired epididymis weight																
F <sub>0</sub> males	↔	↔	↔	↔	↔	↔	↑19%	3804	[241]	3072	[195]	5044	[319]	4068	[258]	
F <sub>1</sub> males	↔	↔	↔	↔	↔	↔	↑19%	2963	[188]	2566	[163]	3255	[206]	2786	[17]	
F <sub>2</sub> males <sup>b</sup>	↔	↔	↔	↔	↔	↔	↑8%	↑24%	884	[56]	596	[38]	951	[60]	641	[41]
F <sub>3</sub> males	↔	↔	↔	↔	↔	↔	↑22%	3449	[218]	2516	[159]	4117	[261]	3095	[196]	
Relative liver weight																
F <sub>0</sub> females	↔	↔	↔	↔	↔	↔	↑11%	7663	[485]	5848	[370]	7965	[504]	7439	[471]	
F <sub>2</sub> females	↑	↔	↔	↔	↔	↔	↑19%	6912	[438]	3650	[231]	7454	[472]	5533	[350]	
Relative paired ovary weight]																
F <sub>0</sub> females	↔	↔	↔	↔	↔	↔	↓19%	4103	[260]	3149	[199]	7126	[451]	5387	[341]	
F <sub>1</sub> females	↔	↔	↔	↔	↔	↔	↓15%	5754	[364]	3964	[251]	10,237	[648]	6966	[441]	
F <sub>2</sub> females	↓15%	↔	↓15%	↓11%	↔	↔	↓24%	7053	[447]	3520	[223]	7646	[484]	6360	[403]	

#### 4.0 Reproductive Toxicity Data

Endpoint	Dose, ppm diet [mg/kg bw/day <sup>a</sup> ]						BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>				
	0.015 [0.0095]	0.3 [0.019]	4.5 [0.285]	75 [4.75]	750 [47.5]	7500 [475]								
Number with renal tubule degeneration														
F <sub>0</sub> females	0/12	0/12	0/12	0/14	0/12	4/13	6491	[411]	3848	[244]				
F <sub>1</sub> females	0/10	0/10	0/10	0/10	0/10	8/11	5498	[348]	2470	[156]				
F <sub>2</sub> females	0/11	0/10	0/12	0/11	0/12	7/13	5884	[373]	3018	[191]				
Number females with chronic liver inflammation														
F <sub>0</sub> females	0/12	1/12	0/12	0/14	1/12	3/13	4867	[308]	3214	[204]				
F <sub>1</sub> females	0/10	0/10	3/10	1/10	1/10	3/11								
F <sub>2</sub> females	1/11	0/10	2/12	2/11	2/12	5/13	3029	[192]	1856	[118]				
Number of implantation sites														
F <sub>0</sub> dams	↔	↔	↔	↔	↔	↓16%	4088	[259]	3021	[191]	8020	[508]	5832	[369]
F <sub>1</sub> dams <sup>b</sup>	↔	↔	↔	↔	↔	↓26%	6120	[388]	2383	[151]	7000	[443]	4713	[298]
F <sub>2</sub> dams	↔	↓8%	↔	↔	↔	↓18%	4917	[311]	3597	[228]	7679	[486]	5631	[357]
Epididymal sperm concentration, F <sub>1</sub>	↔	↔	↔	↔	↔	↓18%	5012	[317]	3407	[216]	11,050	[700]	7407	[469]
Daily sperm production, F <sub>3</sub>	↔	↔	↔	↔	↔	↓19%	7399	[469]	4025	[255]	8279	[524]	7596	[481]

↑, ↓ Statistically significant increase, decrease, ↔ no statistically significant effect.

<sup>a</sup>Based on target doses provided by the study authors and expressed as an average of the dose for males and females.

<sup>b</sup>Benchmark dose values were estimated using a polynomial model.

From Tyl et al. (338).

4.0 Reproductive Toxicity Data

**Table 94. Treatment-related Effects in Developing Rats in a Multigeneration Reproductive Toxicity Study of Bisphenol A**

Endpoint	Dose, ppm diet [mg/kg bw/day <sup>a</sup> ]												
	0.015 [0.0095]	0.3 [0.019]	4.5 [0.285]	75 [4.75]	750[47.5]	7500 [475]	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>			
Live pups/litter													
F <sub>1</sub>	↔	↔	↔	↔	↔	↔	↓20%	4232 [268]	3033 [192]	8823 [559]	6225 [394]		
F <sub>2</sub>	↔	↔	↔	↔	↔	↔	↓26%	6661 [422]	2405 [152]	7241 [459]	4645 [294]		
F <sub>3</sub>	↔	↓11%	↔	↔	↔	↔	↓26%	3733 [236]	2742 [174]	5943 [376]	4518 [286]		
Pup body weight													
F <sub>1</sub> , PND 4	↔	↔	↔	↔	↔	↔	↓11%	6412 [406]	4473 [283]	8860 [561]	6317 [400]		
F <sub>1</sub> , PND 7	↔	↔	↔	↔	↔	↔	↓23%	3432 [217]	2891 [183]	4179 [265]	3448 [218]		
F <sub>2</sub> , PND 7	↔	↔	↔	↔	↔	↔	↓15%	5179 [328]	4059 [257]	6023 [381]	4653 [295]		
F <sub>3</sub> , PND 7	↔	↔	↔	↔	↔	↔	↓13%	4976 [315]	3854 [244]	6474 [410]	4940 [313]		
F <sub>1</sub> , PND 14	↔	↔	↔	↔	↔	↔	↓27%	2890 [183]	2570 [163]	2789 [177]	2415 [153]		
F <sub>2</sub> , PND 14	↔	↔	↔	↔	↔	↔	↓20%	3840 [243]	3302 [209]	3579 [227]	3013 [191]		
F <sub>3</sub> , PND 14	↔	↔	↔	↔	↔	↔	↓20%	3704 [235]	3224 [204]	3323 [210]	2827 [179]		
F <sub>1</sub> , PND 21	↔ <sup>b</sup>	↔	↔	↔ <sup>b</sup>	↔ <sup>b</sup>	↔ <sup>b</sup>	↓27%	3284 [208]	2621 [166]	3523 [223]	2763 [175]		
F <sub>2</sub> , PND 21	↔ <sup>b</sup>	↔	↔	↔ <sup>b</sup>	↔ <sup>b</sup>	↔ <sup>b</sup>	↓20%	4253 [269]	3566 [226]	4219 [267]	3473 [220]		
F <sub>3</sub> , PND 21	↔ <sup>b</sup>	↔	↔	↔ <sup>b</sup>	↔ <sup>b</sup>	↔ <sup>b</sup>	↓19%	3972 [252]	3423 [217]	3575 [226]	3016 [191]		
Anogenital distance, F <sub>2</sub> females	↑3%	↑3%	↑3%	↔	↔	↔	↔						
Age of vaginal opening adjusted for body weight													
F <sub>1</sub>	↔	↔	↔	↔	↔	↔	↑3.6 days	6225 [394]	5422 [343]	3248 [206]	2786 [176]		
F <sub>2</sub>	↔	↔	↔	↔	↔	↔	↑4 days	6381 [404]	5307 [336]	4367 [277]	3600 [228]		
F <sub>3</sub>	↔	↔	↔	↔	↔	↔	↑3.2 days	7444 [471]	6325 [401]	6249 [396]	3198 [203]		
Age of preputial separation adjusted for body weight													
F <sub>1</sub>	↔	↔	↔	↔	↔	↔	↑1.7 days	↑4.9 days	7350 [466]	6485 [411]	2974 [188]	2580 [163]	
F <sub>2</sub>	↔	↔	↔	↔	↔	↔	↔	↑7.4 days	4740 [300]	4025 [255]	3809 [241]	3201 [203]	
F <sub>3</sub>	↔	↔	↔	↔	↔	↔	↔	↑4 days	8637 [547]	7466 [473]	3503 [222]	2984 [189]	

↑,↓ Statistically significant increase, decrease, ↔ no statistically significant effect.

<sup>a</sup>Based on target doses provided by the study authors and expressed as an average of the dose for males and females.

<sup>b</sup>A significant (~5%) decrease in pup body weights observed only in F<sub>1</sub> and/or F<sub>2</sub> litters was apparently not considered treatment-related by study authors. From Tyl et al. (338)3).

## 4.0 Reproductive Toxicity Data

1 [The NTP Statistics Subpanel (340) stated that the study by Tyl et al. (475) apparently lacked a check for  
2 outliers, but noted that the study was in draft form at the time of review. The NTP subpanel agreed with  
3 most author conclusions but disagreed with a conclusion that relative uterine weights were equivalent  
4 across all groups. The unnecessary use of ANOVA with Dunnett test was noted. Some possible outliers  
5 and 10-fold errors in data points that could have affected conclusions were observed. Overall, the NTP  
6 Subpanel concluded that Tyl et al. (475) study was the most comprehensive of the studies reviewed. They  
7 stated that the statistical methods were well thought out and appropriate.]  
8

9 **Strengths/Weaknesses:** This assessment of potential bisphenol A-related effects on multiple generations  
10 of rats was well-designed and comprehensive. The large number of rats/group (30), the multiple endpoints  
11 examined, and the oral route of administration (diet) are strengths. The concentration of bisphenol A in  
12 the test diet was verified, and maternal and paternal toxicity was identified. This study explored a wide  
13 dose range and demonstrates an absence of adverse effects on reproductive function at very low bisphenol  
14 A dose levels. This study is highly valuable for human risk assessment for oral exposure to bisphenol A.  
15 This study identified a NOAEL of 75 ppm (for general toxicity) and 750 ppm (for reproductive toxicity).  
16

17 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
18 evaluation process.  
19

### 4.2.3.2 Mouse

20 **NTP (522, 523)** sponsored a continuous breeding study in CD-1 mice exposed to bisphenol A through sc  
21 implants. Mice were fed Purina certified ground rodent chow (#5002) and housed in polypropylene or  
22 polycarbonate cages containing Ab-Sorb-Dri bedding. Silastic implants were used for sc dosing of mice  
23 with bisphenol A (~95% purity) in corn oil vehicle. Stability and weight of bisphenol A in pumps was  
24 verified. In the dose-range finding portion of the study (Task 1), 8 mice/sex/group (8 weeks old) received  
25 implants containing vehicle or bisphenol A. Dosages were estimated by determining the difference in  
26 bisphenol A weight at the start and end of the 14-day dosing period. It was estimated that mice received 0,  
27 6.25, 12.5, 25, 50, or 100 mg bisphenol A. Endpoints examined included body weight changes, survival,  
28 and uterine weight. Blood was collected to determine plasma bisphenol A levels. Data were analyzed by  
29 ANOVA, Duncan Multiple Range Test, chi-squared test, and Fisher exact test. The goal of Task 2 was to  
30 determine a maximum tolerated dose that produced signs of toxicity but did not reduce body weight or  
31 increase lethality by more than 10% and to identify a low dose that did not result in toxicity.  
32 Concentrations of bisphenol A in plasma were below the detection limit (3 ng/mL) in the 6.25 mg group  
33 but were reported at 7.0–7.7 µg/L in the 12.5 mg group, 8.4 µg/L in the 25 mg group, 13.1–18.5 µg/L in  
34 the 50 mg group, and 31.5–56.2 µg/L in the 100 mg group. In mice treated with bisphenol A, there were  
35 no increases in death or effects on body weight gain. The study authors noted that reproductive tract  
36 weight in the high dose group was greater [**by 52%**] than in the control group but statistical significance  
37 was not achieved because of high variability.  
38  
39

40 In the continuous breeding portion of the study (Task 2), mice were 11 weeks old at the start of dosing.  
41 Forty mice/sex/group received implants containing the vehicle and 20/sex/dose received implants  
42 containing bisphenol A at 25, 50, or 100 mg. Over a dosing period of 18 weeks, it was estimated that  
43 animals in each treatment group received 11.65, 20.05, and 38.60 mg bisphenol A. [**Assuming body**  
44 **weights of ~38 g, as indicated in the study report, doses would have been ~306, 527, and 1015 mg/kg**  
45 **bw over 18 weeks or 2.4, 4.2, and 8.1 mg/kg bw/day.**] Mice were eleven weeks old at the start of  
46 dosing, which began during a 7-day pre-mating period. The mice were then randomly paired with animals  
47 from the same dose group and housed together during a 98-day breeding period. Litters born during the  
48 breeding period were examined for viability, weighed, sexed, and discarded. Following the 98-day mating  
49 period, mice were separated for 21 days to allow for the birth of the last litter. Dosing was continued  
50 throughout the breeding and separation periods. However implants were often expelled through cutaneous  
51 lesions or the incision site. When animals expelled their implant, a new one was inserted but pregnant

## 4.0 Reproductive Toxicity Data

1 mice were allowed to complete their pregnancy before insertion of the new implant. Therefore dosing was  
2 not uniform. Endpoints examined in adult mice included body weight, number of litters/pair, and fertility.  
3 Following delivery of the final litter, parental animals were killed and animals in the 0 and 100 mg group  
4 were necropsied. Liver, brain, and reproductive organs were weighed. Data were analyzed by chi-squared  
5 test, Fisher exact test, Kruskal-Wallis test, Jonckheere test, and Mann-Whitney *U* test.

6  
7 With the exception of cutaneous lesions at the implantation site, there were no clinical signs of toxicity. In  
8 parental mice, there were no effects on body weight, mortality, fertility, or number of litters born. There  
9 were no changes in weights of organs including, liver brain, pituitary, the female reproductive tract, testis,  
10 epididymis, prostate, or seminal vesicles. Statistically significant effects observed in pups included  
11 increased numbers of live male and total pups and increased adjusted (for litter size) pup weight in the  
12 mid-dose group. Unadjusted and adjusted male and female pup weights were significantly increased at the  
13 high dose. The study authors noted that the effects observed in this study were random and most likely  
14 due to chance. They concluded that bisphenol A did not induce adverse effects on fertility in male or  
15 female mice. It was noted that further studies using a better route of exposure are needed for bisphenol A.

16  
17 **Strengths/Weaknesses:** This study appears to have been well conducted. When compared to studies that  
18 used the oral route of exposure, this study provides evidence that the manifestation of maternal toxicity is  
19 dependent on the route of administration and that route-dependent metabolism may be important for  
20 toxicity. However, the administration of bisphenol A via silastic implants makes the extrapolation for  
21 human risk assessment difficult in the absence of an improved pharmacokinetic understanding.

22  
23 **Utility (Adequacy) of CERHR Evaluation Process:** This study is adequate but of limited utility for the  
24 evaluation process.

25  
26 **NTP (523, 524)** sponsored a continuous breeding study in CD-1 mice exposed to bisphenol A (98%  
27 purity). Additional information on ovarian follicle counts in F<sub>0</sub> and F<sub>1</sub> females was published in a report  
28 by Bolon et al. (525). In this study, mice were fed NIH-07 open formula rodent chow and housed in  
29 polypropylene or polycarbonate cages containing Ab-Sorb-Dri litter. The laboratory at which the study  
30 was conducted was stated to be in full compliance with GLP regulations. In the preliminary study (Task  
31 1), 8 mice/sex/group (8 weeks old) were fed diet containing bisphenol A at 0, 0.3125, 0.625, 1.25, 2.5, or  
32 5.0% for 14 days. By assuming that a 40 g mouse ingests 7 g feed/day, the study authors estimated  
33 bisphenol intake at 0, 437.5, 875.0, 1750.0, 4375.0, 8750.0 mg/kg bw/day. The aim of the preliminary  
34 study was to determine a maximum tolerated dose that induced significant toxicity but resulted in ≥90%  
35 survival and ≤10% decrease in weight gain. Statistical analyses included ANOVA, and chi-squared test.  
36 Lethality was significantly increased in the high-dose group. Body weight gain was depressed in groups  
37 exposed to ≥1.25% bisphenol A. Clinical signs of toxicity were observed in the 2.5 and 5.0% dose groups  
38 and included dehydration, dyspnea, lethargy, tremors, ptosis, piloerection, and diarrhea.

39  
40 In the reproduction and fertility study (Task 2), 11-week-old mice were randomly assigned to treatment  
41 groups according to body weight. The mice were fed diets containing 0, 0.25, 0.5, or 1.0% bisphenol A.  
42 The NTP stated that a 40 g mouse consuming 7 g of feed/day would be exposed to bisphenol A at 437.5,  
43 875, and 1750 mg/kg bw/day. **[Based on body weight and feed intake values reported for males at ~3**  
44 **week intervals, CERHR estimated mean bisphenol A intake at ~365, 740, and 1630 mg/kg bw/day.**  
45 **Feed intakes were reported only at week 1 and 18 for females, and week 18 most likely represented**  
46 **the lactation period. For week 1, bisphenol A intake by females was estimated at 410, 890, and 1750**  
47 **mg/kg bw/day. At week 18, bisphenol A intake by females was estimated at 1090, 1785, and 3660**  
48 **mg/kg bw/day.]** There were 40 mice/sex in the vehicle control group and 20/sex in each bisphenol A  
49 group. Exposure to bisphenol A began during a 7-day pre-mating period. Following the pre-mating period,  
50 males and females from the same treatment group were randomly paired and housed together for 98 days  
51 and following the mating period, each male and female was housed separately for 21 days. Bisphenol A

#### 4.0 Reproductive Toxicity Data

1 dosing was continued throughout the mating and separation period. Concentration and stability of  
2 bisphenol A in feed were verified. During the 98-day cohabitation period, pups born were counted, sexed,  
3 and weighed. All litters excluding the last one born were killed on the day of birth so that animals could  
4 continue mating. The last litter was raised by the dam and weaned on PND 21 (day of birth not defined).  
5 Birth weight and weight gain were recorded in the last litter. Reproductive endpoints in parental rats  
6 included the number of litters born and fertility. Statistical analyses included Kruskal-Wallis ANOVA on  
7 ranks, Mann-Whitney *U* test, chi-squared test, 1-way ANOVA, arcsine square-root transformation, and  
8 Duncan multiple range test.

9  
10 In the cross-over trial (Task 3), ~20 males and females from the high-dose group were randomly paired  
11 with control mice for 7 days in order to determine the affected sex. Twenty control males and females  
12 were also paired. The animals were not exposed to bisphenol A during the 1-week mating period, but in  
13 animals from the high dose group, dosing with bisphenol A was continued for 21 days upon separation of  
14 the mating pairs. Vaginal smears were obtained from females that did not mate or did not appear to be  
15 pregnant. Fertility and offspring survival were determined. Parental mice from the control ( $n = 38/\text{sex}$ )  
16 and high-dose groups ( $n = 19/\text{sex}$ ) were necropsied within a week following completion of the cross-over  
17 trial. Body, liver, kidney, and reproductive organ weights were obtained, and sperm count, morphology,  
18 and motility were determined. Testes, ovaries, and oviducts were fixed in Bouin solution and prostate,  
19 seminal vesicles/coagulating glands, uterus, liver, and kidney were fixed in 10% neutral buffered formalin  
20 for histopathological evaluation.

21  
22 In Task 4 of the study, 20  $F_1$  mice/sex/group (at least 2/sex from 10 randomly selected litters/group) were  
23 mated within dose groups for 7 days and examined for reproductive function. Because fewer  $F_1$  mice in  
24 high-dose group were available as a result of increased mortality, only 11 mice/sex were mated. The  
25 animals continued to receive the same diet given to their parents. Vaginal smears were obtained from  
26 females that did not mate or did not appear to become pregnant. One litter/pair was examined for sex,  
27 body weight, and viability. The parental  $F_1$  animals from all dose group were killed and examined as  
28 described for Task 3 of the study.

29  
30 Treatment-related effects observed in adult rats are summarized in [Table 95](#), and effects occurring in  
31 immature rats are summarized in [Table 96](#). Bisphenol A treatment had no effect on mating or fertility  
32 index in  $F_0$  or  $F_1$  mice. Postpartum body weights were reduced in  $F_0$  dams of the high-dose group. In  $F_0$   
33 mice, the number of litters produced/pair and numbers of live  $F_1$  pups/litter were reduced at the mid- and  
34 high-dose level. A decrease in the proportion of pups born alive occurred in  $F_0$  mice of the high-dose  
35 group. No effects were observed on sex ratios of  $F_1$  or  $F_2$  pups. Weights of live  $F_1$  pups were increased at  
36 the mid and high dose. There were no significant effects when pup weights were adjusted for total  
37 numbers of live and dead pups in the litter. Therefore the NTP concluded that the increased pup weights  
38 resulted from the smaller litter size. Body weights were evaluated through PND 21 in  $F_1$  pups, and no  
39 effects were found on pup body weight gain during the lactation period. Mortality in  $F_1$  offspring during  
40 the postnatal period was increased in the high-dose group.

41  
42 The cross-over test revealed no effect on mating or fertility in either males or females exposed to  
43 bisphenol A. Postpartum body weight was not affected in the treated females. The number of live  
44 pups/litter was significantly reduced [**by 26%**] in the group containing treated males and [**by 51%**] in the  
45 group containing treated females. Live pup weight was increased in the group containing treated females,  
46 but there was no significant effect following adjustment for litter size. There were no effects on the  
47 proportion of pups born alive or on sex ratio.

48  
49 In sperm analyses conducted in high-dose  $F_0$  males and all dose groups of  $F_1$  males, sperm motility was  
50 reduced in high-dose  $F_0$  males and mid-dose  $F_1$  males. There were no effects on sperm count or  
51 morphology in either generation. Effects were observed on organ weights, which were examined in  $F_0$

#### 4.0 Reproductive Toxicity Data

1 adults of the high-dose group and F<sub>1</sub> animals from each treatment group. Effects on absolute reproductive  
 2 organ weights of F<sub>1</sub> mice included decreased right epididymis weight at all doses, decreased left  
 3 testis/epididymis weight at the mid and high dose, and decreased seminal vesicle weight at the high dose.  
 4 Significant effects on relative organ weights adjusted for body weight in F<sub>1</sub> rats included decreased right  
 5 epididymis weight at all doses, decreased seminal vesicle weight at the low and high dose, and decreased  
 6 relative left testis and epididymis weight at the mid and high dose. Reproductive organ weight effects  
 7 observed in high-dose F<sub>0</sub> males included decreased absolute and relative seminal vesicle weight. There  
 8 were no effects on prostate weight. No effects were reported for estrous cyclicity of F<sub>0</sub> females. There  
 9 were no gross or histopathological alterations in F<sub>0</sub> or F<sub>1</sub> reproductive organs including testis, epididymis,  
 10 prostate, seminal vesicles, ovary, vagina, and uterus. Effects observed in high-dose F<sub>0</sub> animals were also  
 11 summarized in a report by Morrissey et al. (526).

12  
 13 Effects were observed on non-reproductive organ weights, which were examined in F<sub>0</sub> adults of the high-  
 14 dose group and F<sub>1</sub> animals from each treatment group. In the F<sub>1</sub> mice, dose-related effects on absolute  
 15 organ weights included increased kidney/adrenal weight at all doses in both sexes and increased liver  
 16 weight in mid- and high-dose females and high-dose males. Significant effects on relative organ weight  
 17 adjusted for body weight in F<sub>1</sub> rats included increased liver and kidney/adrenal weights at all doses in  
 18 both sexes. Organ weight effects observed in high-dose F<sub>0</sub> males included increased absolute and relative  
 19 liver and kidney/adrenal weight. In F<sub>0</sub> female rats of the high-dose group, absolute and relative liver  
 20 weight and relative kidney weights were increased. Body weights of high-dose F<sub>0</sub> females were reduced  
 21 at necropsy. Histopathology was examined in F<sub>0</sub> rats of the high-dose group and F<sub>1</sub> rats from all dose  
 22 groups. Treatment-related hepatic lesions observed in both generations included multifocal necrosis,  
 23 multinucleated giant hepatocytes in males and females, and centrilobular hepatocytomegaly in males.  
 24 Multifocal mineralization of liver cells was also observed in F<sub>1</sub> females of the high-dose group. Hepatic  
 25 lesions were observed at all dose levels for F<sub>1</sub> males and in F<sub>1</sub> females of the mid- and high-dose group.  
 26 Treatment-related renal lesions were observed in both generations and described as tubular cell nuclear  
 27 variability, increased severity of spontaneous tubular interstitial lesions, cortical tubular dilatation,  
 28 mineralization of renal cells, and micro-calculi in tubular epithelium that sometimes occurred with  
 29 effaced tubular epithelium, tubular regeneration, and/or dilated tubules containing casts. **[It appears that**  
 30 **the incidence of renal lesions was increased at all doses in F<sub>1</sub> rats.]** Renal lesions were stated to  
 31 generally be more prominent in females than males. The study authors concluded that exposure of mice to  
 32 bisphenol A resulted in toxicity to the reproductive system, kidney, and liver. The possibility was noted  
 33 that some or all effects on reproductive performance may have been secondary to the generalized toxicity  
 34 of bisphenol A.

35  
 36 **Table 95. Effects Observed in Adult Mice Dosed with Bisphenol A in a Continuous Breeding Study.**

Endpoint	Dose, % in diet [mg/kg bw/day]						
	0.25 [437.5]	0.5 [875]	1.0 [1750]	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
F <sub>0</sub> males and females							
Litters/pair	↔	↓5%	↓9%	1.0 [1750]	0.74 [1295]	0.96 [1680]	0.66 [1155]
Postpartum dam weight <sup>a</sup>	↔	↔	↓6–9%	1.0 [1750]	0.83 [1452]	0.87 [1522]	0.66 [1155]
Necropsy dam weight	No data	No data	↓4%				
Percent motile sperm	No data	No data	↓39%				
Relative organ weight, males <sup>b</sup>							
Liver	No data	No data	↑29%				
Kidney/adrenal	No data	No data	↑16%				
Seminal vesicle	No data	No data	↓19%				
Relative organ weight, females <sup>b</sup>							
Liver	No data	No data	↑27%				
Kidney/adrenal	No data	No data	↑10%				
Liver lesions, males and	No data	No data	↑ <sup>d</sup>				

#### 4.0 Reproductive Toxicity Data

Endpoint	Dose, % in diet [mg/kg bw/day]						
	0.25 [437.5]	0.5 [875]	1.0 [1750]	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
females <sup>c</sup>							
Kidney lesions, males and females <sup>c</sup>	No data	No data	↑ <sup>d</sup>				
<i>F<sub>1</sub> males and females</i>							
Relative organ weight, males <sup>b</sup>							
Liver	↑7%	↑7%	↑29%	0.62 [1085]	0.42 [735]	0.59 [1032]	0.39 [682]
Kidney/adrenal <sup>e</sup>	↑16%	↑20%	↑20%	0.18 [315]	0.14 [245]	0.15 [262]	0.12 [210]
Left testis/epididymis <sup>e</sup>	↔	↓10%	↓9%	0.64 [1120]	0.32 [560]	0.53 [928]	0.27 [472]
Right testis <sup>f</sup>	↔	↓13%	↔				
Right epididymis <sup>e</sup>	↓11%	↓16%	↓18%	0.24 [420]	0.15 [262]	0.46 [805]	0.25 [438]
Seminal vesicle	↓11%	↔	↓28%	0.40 [700]	0.29 [508]	0.66 [1155]	0.47 [822]
Relative organ weight, females <sup>b</sup>							
Liver	↑6%	↑13%	↑20%	0.49 [858]	0.38 [665]	0.45 [788]	0.35 [612]
Kidney/adrenal <sup>f</sup>	↑13%	↑15%	↑13%				
Percent motile sperm <sup>f</sup>	↔	↓31%	↔				
Liver lesions, males <sup>c</sup>	↑ <sup>d</sup>	↑ <sup>d</sup>	↑ <sup>d</sup>				
Liver lesions, females <sup>c</sup>	↔	↑ <sup>d</sup>	↑ <sup>d</sup>				
Kidney lesions, males and females	↑ <sup>d</sup>	↑ <sup>d</sup>	↑ <sup>d</sup>				

↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

<sup>a</sup>Values were reported following the birth of 5 litters, the benchmark doses are for values reported following the birth of the fifth litter because the greatest magnitude of effect was observed at that time point.

<sup>b</sup>Relative organ weights were adjusted for body weight; when absolute and relative organ weights changed in the same direction, only the relative organ weights were listed in this table.

<sup>c</sup>See text for a description of the types of lesions observed

<sup>d</sup>It does not appear that statistical analyses were conducted for histopathology data, but incidence was increased compared to controls.

<sup>e</sup>Benchmark doses were estimated using a polynomial model.

<sup>f</sup>Benchmark doses were not estimated for endpoints without dose-response relationships.

From NTP (524)

1

#### 2 Table 96. Effects in Immature F<sub>1</sub> Mice in a Continuous Breeding Study with Bisphenol A

Endpoint	Dose, % in diet [mg/kg bw/day]						
	0.25 [437.5]	0.5 [875]	1.0 [1750]	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
Live pups/litter	↔	↓20%	↓48%	0.30 [525]	0.20 [350]	0.43 [752]	0.30 [525]
Proportion pups born alive	↔	↔	↓4%	3.0 [5250]	0.79 [1382]		
Live birth weight <sup>a</sup>	↔	↑5%	↑6%	0.43 [752]		0.34 [595]	
Mortality by PND 21 <sup>b</sup>	↔	↔	↑ to 37.5%	0.48 [840]	0.40 [700]		

↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

<sup>a</sup>Hill model used for benchmark dose calculations.

<sup>b</sup>Control mortality was 6.3%. Mortality was reported on a per pup basis, which limits the utility of the benchmark dose model.

From NTP (524).

3

4 This study demonstrates changes in F<sub>1</sub> male absolute reproductive weights (seminal vesicle with  
5 coagulating gland as well as epididymis; the testis and prostate appear not to have been appreciably  
6 affected). This study also suggested that reproductive toxicity and general toxicity occurred at similar  
7 dose levels. Bisphenol A-mediated general toxicity may have contributed to the observed female fertility

#### 4.0 Reproductive Toxicity Data

1 effect , because this effect was noted with dosed females cohabiting with non-dosed males. In the male,  
2 however, the effect on motility is likely bisphenol A-related, resulting in the observed fertility deficits  
3 In Task 2, a clear effect on fertility was found with a NOAEL of 0.25% bisphenol A in the diet.  
4

5 **Strengths/Weaknesses:** This comprehensive toxicology study was well-conducted. General toxicity was  
6 clearly demonstrated at all F<sub>1</sub> dose levels, and histopathological findings appear to be a sensitive indicator  
7 of effect. As a limitation of this design, because bisphenol A was in the diet, exposure to bisphenol A did  
8 not occur during cohabitation; therefore, direct exposure to bisphenol A was minimal or nonexistent  
9 during sperm maturation, capacitation and ovulation.  
10

11 **Utility (Adequacy) for CERHR Evaluation Process:** These data are adequate and of high utility for the  
12 evaluation process.  
13

14 **Tyl et al. (527)**, sponsored by the Society of the Plastics Industry, conducted a one-generation  
15 reproductive toxicity study in mice. The study was conducted to verify the findings of reduced pup  
16 numbers at birth in a continuous breeding study conducted by the NTP (524). GLP guidelines were  
17 applied in the conduct of the study. CD-1 mice were fed Purina Certified Rodent Diet Meal and housed in  
18 polycarbonate cages containing Sani-chip bedding. Mice were stratified according to body weight and  
19 randomly assigned to treatment groups. Starting at 9 weeks of age, 20 mice/sex/group were given feed  
20 containing bisphenol A (99.36% purity) 0, 5000, or 10,000 ppm. Males and females were fed the  
21 bisphenol A-containing diets during a 2-week pre-breeding period and a 1 week mating period. The day  
22 of vaginal plug detection was defined as GD 0. Exposures in females continued through the gestation  
23 period of ~19 days. The study authors reported bisphenol A intakes of 0, 840, and 1669 mg/kg bw/day in  
24 males during the prebreeding period; 0, 1055, and 1988 mg/kg bw/day in females during the prebreeding  
25 period, and 0, 870, and 1716 mg/kg bw/day in females during the gestation period. **[Intake values were  
26 obtained from the results section and study summary tables. They differed from values reported in  
27 Text Table C, which were assumed to be in error.]** Homogeneity and stability of bisphenol A in feed  
28 were verified. Parameters evaluated during the study included clinical signs, body weight, and feed  
29 intake. Reproductive endpoints evaluated included implantation loss and indices of mating, fertility,  
30 pregnancy, and gestation. F<sub>0</sub> Males were killed at the end of the breeding period; liver and kidney were  
31 weighed. At birth, pups were counted, sexed, weighed, and evaluated for viability and external alterations.  
32 F<sub>0</sub> females and F<sub>1</sub> pups were killed on the day of parturition (PND 0). Dams were assessed for clinical  
33 chemistry parameters of liver and kidney function; corpora lutea and implantation sites; uterus, ovary,  
34 kidney, and liver weight; and liver and kidney histopathology. The male, female, pregnant female, or the  
35 litter were considered statistical units. Statistical analyses included ANOVA, Levene test, GLM  
36 procedure, Dunnett test, chi-squared test, Cochran-Armitage test, and Fisher exact probability test.  
37

38 Treatment-related effects in F<sub>0</sub> animals are summarized in [Table 97](#). There were no treatment-related  
39 changes in clinical signs, body weight gain, feed intake, or food efficiency in males or in females during  
40 the prebreeding period. A transient increase in food intake occurring in females of the low-dose group on  
41 study days 0–7 did not appear to be treatment-related. Gestational body weight gain was decreased in the  
42 high dose group, beginning on GD 7 and in the low dose group beginning on GD 10. Body weights of  
43 live F<sub>0</sub> females were significantly lower in the high dose group on PND 0, but no significant differences  
44 were observed during necropsy conducted later in the day. A significant decrease in feed intake was  
45 reported for the high dose group on GD 14–17, only when the values were expressed as g/day. **[The  
46 results section indicated that food efficiency during gestation was not significantly affected, but a  
47 downward trend was observed. Table 10 of the study reported a significant decrease in food  
48 efficiency.]** Significant necropsy findings observed in males included increased absolute and relative liver  
49 weight at both doses and increased absolute paired kidney weight at the low dose. Absolute and relative  
50 liver and paired kidney weight were significantly increased in females from both dose groups.  
51 Histopathological observations in females included dose-related increases in incidence and severity of

## 4.0 Reproductive Toxicity Data

1 hepatocyte hypertrophy and increased kidney lesions (renal tubular epithelial necrosis, degeneration, and  
2 regeneration) in both dose groups. Significant clinical chemistry findings in females included increased  
3 blood urea nitrogen in the high dose group and decreased sodium, potassium, and chloride levels in the  
4 low-dose group.

5  
6 Treatment-related reproductive or developmental effects are summarized in [Table 97](#). No significant  
7 effects were observed for mating, fertility, or pregnancy indices; time to insemination; numbers of ovarian  
8 lutea or implantation sites; or implantation loss. Gestation duration was extended by ~10 hours in both  
9 dose groups; the study authors stated that the biological significance of the finding is not known. Total  
10 and live pup numbers were decreased in the high-dose group. No significant effects on pup weight were  
11 observed but a downward trend was statistically identified for female pup weight

12  
13 The study authors concluded that their study confirmed the NTP (524) finding of reduced litter size in  
14 mice fed 10,000 ppm bisphenol A in feed. The NTP finding of decreased litter size at 5000 ppm bisphenol  
15 A was not confirmed in this study, likely due, according to the authors, to the shorter exposure duration in  
16 the current study than in the NTP study. The study authors concluded that the litter size decreases in their  
17 study were likely caused by the compromised status of dams.

18  
19 **Strengths/Weaknesses:** Strengths of this report include the comprehensive design with the assessment of  
20 multiple relevant endpoints. There were adequate numbers of animals, the doses and stability of the  
21 compound were verified, and the oral route of exposure was used. Weaknesses include the limited  
22 number of doses examined and the relatively high doses studied.

23  
24 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and useful for the  
25 evaluation process.

26  
27 **Table 97. Effects Observed in Mice Fed Bisphenol A-Containing Feed for One Generation**

Endpoint	Dose, % in diet (mg/kg bw/day)					
	0.5 (840–1055) <sup>a</sup>	1 (1669–1988) <sup>a</sup>	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
F <sub>0</sub> females body weights and feed intake						
GD 17 body weight <sup>b,c</sup>	↓ 8%	↓ 11%	1292	646	742	404
PND 0 body weight <sup>c</sup>	↔	↓ 7%	2130	1675	1813	1193
GD 0–17 body weight change <sup>b,c</sup>	↓ 16%	↓ 19%	472	283	701	387
Study day 0–7 feed intake	↑ 11%	↔				
GD 14–17 feed intake (g/day)	↔	↓ 13%	1454	898	1840	1172
GD 0–17 percent food efficiency	↓ 16%	↓ 16%				
Relative (to body weights) organ weights in F <sub>0</sub> <sup>d</sup>						
Liver, male	↑ 22%	↑ 24%	706	561	705	555
Liver, female	↑ 27%	↑ 29%	615	484	746	586
Kidney, female	↑ 8%	↑ 24%	973	529	1309	863
Clinical chemistry effects in F <sub>0</sub> females, not examined in males						
Blood urea nitrogen	↔	↑ 43%	628	266		
Sodium	↓ 9%	↔				
Potassium	↓ 18%	↔				
Chloride	↓ 8%	↔				
<u>Histopathology in F<sub>0</sub> females (not examined in males)<sup>e</sup></u>						
Renal tubule epithelium degeneration (control: 0/20)	9 of 20	9 of 20				
Renal tubule epithelium necrosis (control: 0/20)	6 of 20	8 of 20	663	480		
Renal tubule regeneration (control: 12 of 20)	12 of 20	20 of 20	223	151		

#### 4.0 Reproductive Toxicity Data

Endpoint	Dose, % in diet (mg/kg bw/day)					
	0.5 (840–1055) <sup>a</sup>	1 (1669–1988) <sup>a</sup>	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
2/20)						
Centrilobular hepatocyte hypertrophy (control 0/20)	2 of 20	11 of 20	902	612		
Diffuse hepatocyte hypertrophy (control 0/20)	6 of 20	6 of 20				
Reproductive/developmental effects						
Gestational length	↑ 2%	↑ 2%				
Number of live pups	↔	↓ 15%	1116	727	1925	1189
Total number of pups	↔	↓ 15%	1116	727	1925	1189
Female pup body weight	↓0.6% <sup>f</sup>	↓4% <sup>f</sup>	2281	1728	2332	1733

↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effect;

<sup>a</sup>Bisphenol A intakes included values estimated for males and females during prebreeding or gestation; intake values for the appropriate sex were used in benchmark dose analyses; intakes during gestation were used for females.

<sup>b</sup>The effect was reported at earlier time period but is shown here only for the latest or longest time period evaluated.

<sup>c</sup>Benchmark doses were estimated using the polynomial model

<sup>d</sup>Only effects on relative organ weights were shown.

<sup>e</sup>Histopathology data were not statistically analyzed.

<sup>f</sup>By trend test

From Tyl et al. (527)

1  
2 **Tyl et al. (436)**, sponsored by the American Plastics Council, conducted a 2-generation study of  
3 bisphenol A in mice. The study was conducted according to GLP. CD-1 mice were received in two cohorts  
4 approximately 2 weeks apart and data from the 2 cohorts were combined. Mice were fed Purina Certified  
5 Ground Rodent Diet No. 5002. The supplier provided information about phytoestrogen content of feed  
6 (177–213 ppm genistein, 173–181 ppm daidzein, and 39–55 ppm glycitein). Mice were housed in  
7 polypropylene cages with Sani-Chip® bedding. Assignment of F<sub>0</sub> animals to groups involved  
8 randomization stratified by weight. F<sub>0</sub> and F<sub>1</sub> mice (28 sex/group/generation) were fed diets containing  
9 bisphenol A (99.70–99.76% purity) at 0.018, 0.18, 1.8, 30, 300, or 3500 ppm. Target intakes were 0.003,  
10 0.03, 0.3, 5, 50, or 600 mg/kg bw/day, respectively. Based on measured feed intake, the study authors  
11 estimated bisphenol A intake in males at 0.0024–0.0038, 0.024–0.037, 0.24–0.37, 3.98–6.13, 39.1–60.8,  
12 or 529–782 mg/kg bw/day. Bisphenol A intakes (in mg/kg bw/day) by females were estimated at 0.0030–  
13 0.0041, 0.030–0.042, 0.32–0.43, 5.12–7.12, 54.2–67.8, 653–910 during the pre-mating period; 0.0027–  
14 0.0029, 0.027–0.028, 0.28–0.29, 4.65–4.80, 47.0–48.6, 552–598 during the gestation period; and 0.0063–  
15 0.0087, 0.062–0.091, 0.61–0.89, 10.4–15.1, 103.2–146.4, 1264–1667 during the lactation period. In each  
16 generation, there were 2 vehicle control groups with 28 mice/sex/group. A positive control group was  
17 given feed containing 17β-estradiol at 0.5 ppm (target intake of 0.08 mg/kg bw/day). Estimated intakes  
18 for 17β-estradiol (in mg/kg bw/day) were 0.074–0.104 in males, 0.093–0.12 in females during the pre-  
19 mating period, 0.08–0.081 in females during the gestation period, and 0.160–0.25 in females during the  
20 lactation period. Dose selections were based on observations from several studies. **[The Expert Panel**  
21 **notes that a separate 2-generation study was used to characterize the dose-response relationship for**  
22 **17β-estradiol.]** Homogeneity, stability, and concentration of bisphenol A in feed were verified. Exposure  
23 of F<sub>0</sub> mice began at ~6 weeks of age. Exposure of F<sub>1</sub> animals began at weaning, although it was noted that  
24 pups began eating the dosed feed in the late lactation period. F<sub>0</sub> and F<sub>1</sub> mice were fed the bisphenol A-  
25 containing diets for a minimum of 8 weeks prior to mating and during a 2-week mating period. Exposures  
26 of males continued through the gestation period of the litters they sired. Exposures of females continued  
27 through the gestation and lactation period. During the study, adult animals were monitored for clinical  
28 signs of toxicity, body weight, and food intake.

29

## 4.0 Reproductive Toxicity Data

1 Estrous cycles were evaluated in F<sub>0</sub> and F<sub>1</sub> females during the last 3 weeks of the pre-breeding exposure  
2 period. Day of vaginal plug was defined as GD 0 and day of birth was considered PND 0. F<sub>1</sub> and F<sub>2</sub> pups  
3 were counted, sexed, weighed, and assessed for viability and physical abnormalities at birth and  
4 throughout the lactation period. Anogenital distance was measured in F<sub>1</sub> and F<sub>2</sub> pups at birth and on PND  
5 21. On PND 4, F<sub>1</sub> and F<sub>2</sub> litters were standardized to 10 pups, with equal numbers per sex when possible.  
6 Pups removed on PND 4 were killed and examined for visceral alterations, with a focus on the  
7 reproductive system. The remaining pups were maintained and weaned on PND 21. At weaning, 28 F<sub>1</sub>  
8 pups/sex/group (1 per sex per litter) were randomly selected for mating and those animals were referred to  
9 as parental mice. An additional F<sub>1</sub> male/litter was selected for a 3 month exposure (referred to as retained  
10 males). Two F<sub>1</sub> pups/sex/litter were selected for gross necropsy and organ weight measurement at  
11 weaning. Histopathological examination of reproductive organs was conducted in one PND 21  
12 pup/sex/litter. Histopathological evaluation of reproductive and systemic organs were conducted in the  
13 second F<sub>1</sub> pup from each group at weaning. All F<sub>2</sub> pups were killed at weaning and organ weights were  
14 measured. Vaginal opening and preputial separation were monitored in parental and retained F<sub>1</sub> mice.  
15 Parental F<sub>0</sub> and F<sub>1</sub> males were killed following delivery of the litters they sired. Retained F<sub>1</sub> males were  
16 killed at the same time as the parental F<sub>1</sub> males. Parental F<sub>0</sub> and F<sub>1</sub> females were killed after their pups  
17 were weaned. Organs, including those of the reproductive system, were weighed in adult F<sub>0</sub> and F<sub>1</sub>  
18 animals. Histopathological evaluations were conducted in all animals from the vehicle control group, in  
19 10 F<sub>0</sub> and F<sub>1</sub> parental animals from each treatment group, in all F<sub>1</sub> retained males, and 10 animals from  
20 the 17 $\beta$ -estradiol positive control group. Histopathological evaluation of reproductive organs was also  
21 conducted in animals with suspected reduced fertility. Testes were preserved in Bouin fixative. Daily  
22 sperm production, efficiency of daily sperm production, and epididymal sperm count, motility, and  
23 morphology, were evaluated in F<sub>0</sub> and F<sub>1</sub> males. Data from the 2 control groups were analyzed separately  
24 and then pooled for statistical analysis of treatment groups. Statistical analyses included ANOVA, Levene  
25 test, robust regression methods, Wald chi-squared test, *t*-test, Dunnett test, Fisher exact probability test,  
26 and ANCOVA.

27  
28 Treatment- or dose-related results and observations in reproductive organs of adult animals are  
29 summarized in [Table 98](#). There were no consistent effects on body weight or body weight gain in F<sub>0</sub>  
30 males. Body weight gain during lactation was increased in F<sub>0</sub> females from the 3500 ppm group. During  
31 the pre-mating period, body weights were decreased by  $\leq 10\%$  in F<sub>1</sub> parental animals from the 3500 ppm  
32 group (study days 0, 7, 49, and 56 in males and study 0 in females). In retained F<sub>1</sub> males from the 3500  
33 ppm group, body weights were decreased at most time periods between study days 7 and 84 and at  
34 necropsy. No consistent or dose-related changes in feed intake or efficiency were observed throughout the  
35 study in F<sub>0</sub> or F<sub>1</sub> animals. There were no clinical signs of toxicity or treatment-related deaths in F<sub>0</sub> or F<sub>1</sub>  
36 males or females. Increases in absolute and relative to body or brain weights of kidney and liver were  
37 consistently observed in F<sub>0</sub> and F<sub>1</sub> adults. Significant and dose-related organ weight changes relative to  
38 body weight are summarized in [Table 98](#). Other effects on organ weight (e.g., seminal vesicles,  
39 epididymides, coagulating glands, and pituitary) were not considered to be treatment-related by study  
40 authors due to factors such as lack of a dose-response relationship, no consistency between absolute and  
41 relative weights, no histopathology, or no consistency across generations. Absolute and relative prostate  
42 weights were unaffected by bisphenol A exposure. There were no treatment-related gross systemic  
43 findings in F<sub>0</sub> or F<sub>1</sub> adults. Incidence of minimal to mild hepatocyte centrilobular hypertrophy was  
44 increased in both generations at 300 and/or 3500 ppm (see [Table 98](#)). Renal nephropathy incidence was  
45 increased in F<sub>0</sub> males and in F<sub>1</sub> males and females of the 3500 ppm group. **[It did not appear that**  
46 **histopathological data were statistically analyzed.]**

47  
48 Treatment- or dose-related reproductive effects in adult animals are summarized in [Table 98](#). Bisphenol A  
49 exposure had no effect on numbers of implantation sites or resorptions or on mating, fertility, or  
50 gestational indices in F<sub>0</sub> or F<sub>1</sub> mice. Gestational length was increased in F<sub>0</sub> and F<sub>1</sub> females from the 3500  
51 ppm group; the study authors stated the effect was of unknown biological significance. Epididymal sperm

#### 4.0 Reproductive Toxicity Data

1 concentration was decreased in F<sub>0</sub> males of the 3500 ppm group but no effect was observed in F<sub>1</sub> parental  
2 or retained males. There was no effect on daily sperm production, efficiency of daily sperm production, or  
3 sperm motility or morphology in either generation. The study authors did not consider the decrease in  
4 sperm concentration in F<sub>0</sub> animals to be treatment-related based on lack of consistency between  
5 generations, no effect on any other andrological endpoint, and no effect on fertility. Estrous cyclicity and  
6 numbers of ovarian primordial follicle counts were not affected by bisphenol A exposure in F<sub>0</sub> or F<sub>1</sub>  
7 females. The only gross observation in reproductive organs was a slightly increased incidence of gross  
8 ovarian cysts in F<sub>0</sub> females from the 3500 ppm group. The incidence of paraovarian cysts was increased  
9 in F<sub>0</sub> and F<sub>1</sub> females from the 3500 ppm group. **[It did not appear that histopathological data were**  
10 **statistically analyzed.]**

11  
12 Significant findings in developing mice are summarized in [Table 99](#). Live F<sub>1</sub> and F<sub>2</sub> pups and litters at  
13 birth, sex ratio, and survival during the lactation period were not affected and there were no clinical or  
14 gross signs of toxicity in F<sub>1</sub> or F<sub>2</sub> offspring. A non-dose-related decrease in PND 21 survival index and  
15 lactational index (pups surviving on PND 21/PND 4) was described in F<sub>2</sub> pups of the 300 ppm group.  
16 **[The biological significance of the effect was not discussed by the study authors, but because the**  
17 **effect was not dose-related it is unlikely to be of biological significance.]** In F<sub>1</sub> pups from the 3500  
18 ppm group, body weights were reduced during PND 7, 14, and 21 in F<sub>1</sub> females and both sexes combined  
19 and on PND 7 and 21 in F<sub>1</sub> males. Body weight results for both sexes combined are summarized in [Table](#)  
20 [99](#). An increase in male pup body weight observed on PND 7 in the 1.8 ppm group was not considered to  
21 be treatment related by the study authors because no dose-response relationship was observed. There was  
22 no effect on anogenital distance in F<sub>1</sub> or F<sub>2</sub> males or females on PND 0. Anogenital distance was also  
23 unaffected in F<sub>2</sub> males and F<sub>1</sub> and F<sub>2</sub> females on PND 21. Anogenital distance adjusted for body weight  
24 was reduced in F<sub>1</sub> males from the 300 and 3500 ppm groups on PND 21. Based on the lack of effect on  
25 anogenital distance at birth and inconsistencies between generations, the study authors did not consider  
26 the decreases in anogenital distance in F<sub>1</sub> males to be treatment-related. An increase in anogenital distance  
27 in F<sub>2</sub> females from the 0.018 ppm group on PND 0 was not considered to be treatment related by the  
28 study authors. Preputial separation (absolute age and adjusted for body weight on day of acquisition) was  
29 delayed in parental and retained F<sub>1</sub> males of the 3500 ppm group. When adjusted for PND 30 body  
30 weight, preputial separation was delayed in retained but not parental F<sub>1</sub> males from the 3500 ppm group.  
31 Data for preputial separation adjusted for body weight on day of acquisition are shown in [Table 99](#). Body  
32 weights on day of vaginal opening were lower in F<sub>1</sub> females from the 3500 ppm group. Day of vaginal  
33 opening was accelerated in the 3500 ppm group if adjusted for PND 21 body weight, but not body weight  
34 on the day of acquisition. Due to the lack of effect when adjusted for body weight on day of acquisition,  
35 the study authors did not consider effects on vaginal opening to be treatment related.

36  
37 Shown in [Table 99](#) are significant organ weight effects relative to body weight. Dose-related organ weight  
38 changes in F<sub>1</sub> weanlings that were considered to be treatment-related by study authors included decreased  
39 absolute and relative (to body or brain weight) spleen and paired testes weights at 3500 ppm. Treatment-  
40 related absolute organ weight changes in F<sub>2</sub> weanlings included decreased weights of spleen, paired  
41 testes, and seminal vesicles with coagulating glands in the 3500 ppm group. Changes in organ weights  
42 relative to body weight in F<sub>2</sub> weanlings included decreased spleen weight in males and females and  
43 increased relative left kidney weight in 3500 ppm males. Treatment-related changes in organ weight  
44 relative to brain weight in F<sub>2</sub> weanlings were decreased spleen weight in both sexes and decreased paired  
45 testes weight at 3500 ppm and seminal vesicles with coagulating glands at 300 and 3500 ppm. Other  
46 organ weight effects (e.g., affecting epididymides, thymus, brain, ovaries, and/or uterus with cervix and  
47 vagina weights) were not considered to be dose-related due to lack of dose-response relationships or no  
48 consistent effects across generations. Included in [Table 99](#) are significant organ weight effects relative to  
49 body weight. Significant organ weight effects relative to brain weight were included in [Table 99](#) when the  
50 organ weight effect was significant only when normalized for brain weight. The study authors reported no  
51 gross findings in F<sub>1</sub> or F<sub>2</sub> weanlings. The incidence of undescended bilateral testes was increased in F<sub>1</sub>

#### 4.0 Reproductive Toxicity Data

1 and F<sub>2</sub> weanling males of the 3500 ppm group. The incidence of hepatic cytoplasm alteration (clear  
2 hepatocellular cytoplasm, slightly more basophilic cytoplasm, and/or minute vacuoles) was apparently  
3 increased in F<sub>1</sub> males from the 300 and 3500 ppm groups and F<sub>1</sub> females and F<sub>2</sub> males from the 3500 ppm  
4 group. The incidence of seminiferous tubule hypoplasia was increased in F<sub>1</sub> and F<sub>2</sub> weanlings from the  
5 3500 ppm group. **[Another histopathological finding that appeared to be possibly increased in  
6 weanlings from the 3500 ppm group was unilateral hydronephrosis in F<sub>1</sub> males. It did not appear  
7 that histopathological data were statistically analyzed.]**  
8

9 Effects of 17 $\beta$ -estradiol in males were delayed preputial separation, reduced anogenital distance at  
10 weaning but not at birth, decreased weights of testes, epididymides, and seminal vesicles with coagulating  
11 gland, and increased incidence of seminiferous tubule hypoplasia and undescended testis. Effects of 17 $\beta$ -  
12 estradiol in female mice were accelerated vaginal patency, increased uterus with cervix and vagina  
13 weight, fluid filled/enlarged uterus, enlarged/thickened vagina, increased vaginal epithelial keratinization,  
14 and prolonged gestation. Reproductive effects in the 17 $\beta$ -estradiol group included decreased fertility,  
15 increased stillbirth, reduced live pups per litter, and increased dead pups.  
16

17 The study authors identified bisphenol A NOELs of 30 ppm (~5 mg/kg bw/day) for systemic effects, 300  
18 ppm (~50 mg/kg bw/day) for developmental toxicity, and 300 ppm (~50 mg/kg bw/day) for reproductive  
19 toxicity.  
20

21 **Strengths/Weaknesses:** Strengths include the large number and range of doses examined, the rigor with  
22 which the study was performed, the large sample size in each group, the number of additional animals per  
23 litter that were retained and examined, the use of a concurrent estrogenic positive control group, and the  
24 thoroughness of the histologic evaluation.  
25

26 **Utility (Adequacy) for CERHR Evaluation Process:** This study is adequate and of high utility for the  
27 evaluation process.  
28



#### 4.0 Reproductive Toxicity Data

Endpoint	Dose, ppm diet [mg/kg bw/day based on target intakes provided by study authors]						BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
	0.018 [0.003]	0.18 [0.03]	1.8 [0.3]	30 [5]	300 [50]	3500 [600]				
F <sub>0</sub> males (12/56)	0/10	3/10	2/10	2/10	1/10	4/10	1556 [264]	750 [127]		
F <sub>1</sub> parental males (6/55)	2/10	0/10	1/10	2/10	0/10	4/10	1418 [240]	838 [142]		
F <sub>1</sub> retained males (8/50)	1/10	0/10	0/10	2/10	0/10	3/10	1991 [337]	992 [168]		
F <sub>1</sub> females (10/55)	1/10	3/10	0/10	1/10	1/11	4/11	1646 [279]	847 [144]		
Paraovarian cysts (control incidence in parentheses)										
F <sub>0</sub> (9/56)	1/11	2/12	1/11	1/12	3/14	7/17	1328 [225]	833 [141]		
F <sub>1</sub> (14/55)	1/11	1/11	1/10	2/10	2/11	7/15	1193 [202]	708 [120]		
Epididymal sperm concentration, F <sub>0</sub> <sup>a</sup>	↔	↔	↔	↔	↔	↓15%	3343 [567]	1884 [319]	3581 [607]	3241 [549]
Gestational length										
F <sub>0</sub>	↔	↔	↔	↔	↔	↑2%	21,351 [3619]	3770 [639]	6749 [1144]	3536 [599]
F <sub>1</sub>	↔	↔	↔	↔	↔	↑2%	17,820 [3020]	3784 [641]	4552 [772]	3134 [531]

↑,↓ Statistically significant increase, decrease; ↔ no statistically significant effect compared to controls

<sup>a</sup>Not considered a treatment-related effect by study authors.

## 4.0 Reproductive Toxicity Data

**Table 99. Treatment- or Dose-Related Effects in Developing Mice in a Multigeneration Reproductive Toxicity Study with Bisphenol A.**

Endpoint <sup>a</sup>	Dose, ppm diet [mg/kg bw/day based on target intakes provided by study authors]									
	0.018 [0.003]	0.18 [0.03]	1.8 [0.3]	30 [5]	300 [50]	3500 [600]	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>
Body weight										
F <sub>1</sub> , PND 7	↔	↔	↔	↔	↔	↓13%	3304 [560]	1849 [313]	3433 [582]	2403 [407]
F <sub>1</sub> , PND 14	↔	↔	↔	↔	↔	↓11%	3453 [585]	2256 [382]	3639 [617]	2988 [506]
F <sub>1</sub> , PND 21	↔	↔	↔	↔	↔	↓17%	3236 [548]	1577 [267]	3421 [580]	2342 [370]
F <sub>1</sub> male, PND 21 necropsy	↔	↔	↔	↔	↔	↓12%	3325 [564]	1845 [313]	3776 [640]	3536 [599]
F <sub>1</sub> female, PND 21 necropsy	↔	↔	↔	↔	↔	↓18%	2284 [387]	1501 [254]	4577 [776]	3529 [598]
Lactational survival indices (control index, %, in parentheses)										
F <sub>2</sub> PND 21 survival (100%) <sup>c</sup>	↔	↔	↔	↔	↓ to 86.6%	↔				
F <sub>2</sub> Lactational index (97.2%) <sup>c</sup>	↔	↔	↔	↔	↓ to 86.6%	↔				
Relative thymus to body weight, F <sub>1</sub> male, PND 21 <sup>b</sup>	↔	↔	↔	↔	↑13% <sup>b</sup>	↑10% <sup>b</sup>				
Relative spleen to body weight										
F <sub>1</sub> male, PND 21	↔	↓12%	↔	↔	↔	↓30%	3123 [529]	1074 [182]	3538 [600]	3148 [534]
F <sub>2</sub> male, PND 21	↔	↔	↔	↔	↔	↓20%	2148 [364]	1425 [242]	7013 [1189]	3560 [603]
F <sub>1</sub> female, PND 21	↔	↔	↔	↔	↔	↓23%	3168 [537]	647 [110]	4571 [775]	3677 [623]
F <sub>2</sub> female, PND 21	↔	↔	↔	↔	↔	↓21%	1787 [303]	1311 [222]	5022 [851]	3517 [596]
Relative paired testes weight to body or brain weight										
F <sub>1</sub> , PND 21 (body weight)	↔	↔	↔	↔	↔	↓8%	3578 [606]	2720 [461]	3861 [654]	3550 [602]
F <sub>2</sub> , PND 21 (brain weight)	↔	↔	↔	↔	↔	↓11%	3316 [562]	2003 [339]	5342 [905]	3571 [605]
Relative paired epididymides to body weight, F <sub>1</sub> <sup>b</sup>	↔	↑18%	↔	↔	↔	↔				
Relative brain to body weight F <sub>1</sub> female, PND 21 <sup>b</sup>	↔	↔	↔	↔	↔	↑17% <sup>b</sup>	2219 [376]	1415 [240]	3576 [606]	2825 [479]
Relative left kidney to body weight, F <sub>2</sub> male, PND 21	↔	↔	↔	↔	↔	↑6%	6664 [1129]	3540 [600]	8501 [1441]	3589 [608]
Relative seminal vesicles with coagulating gland to brain weight, F <sub>2</sub> <sup>b</sup>	↔	↔	↔	↔	↓15%	↓16%	2389 [405]	1315 [223]	11,294 [1914]	3631 [615]
Uterus with cervix and vagina weight relative to bodyweight, F <sub>2</sub> PND 21 <sup>b</sup>	↔	↔	↔	↔	↓16%	↔				
Relative paired ovary weights, F <sub>1</sub> <sup>b</sup>	↔	↔	↑	↔	↔	↔				
Hepatic cytoplasm alteration (control incidence in parentheses)										

#### 4.0 Reproductive Toxicity Data

Endpoint <sup>a</sup>	Dose, ppm diet [mg/kg bw/day based on target intakes provided by study authors]										
	0.018 [0.003]	0.18 [0.03]	1.8 [0.3]	30 [5]	300 [50]	3500 [600]	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>	
F <sub>1</sub> males (6/44)	1/26	0/17	1/22	6/24	10/20	13/20	732 [124]	546 [92.5]			
F <sub>2</sub> males (6/54)	1/25	1/25	1/25	1/24	2/20	9/23	1442 [244]	1050 [178]			
F <sub>1</sub> females (2/46)	1/27	2/21	3/24	4/26	8/16	6/22	1966 [333]	1182 [200]			
Unilateral hydronephrosis, F <sub>1</sub> males (0/44) <sup>b</sup>	0/26	0/17	0/21	0/24	0/21	3/21b					
Seminiferous tubule hypoplasia (control incidence in parentheses)											
F <sub>1</sub> (1/96)	0/54	0/37	1/45	3/51	2/45	5/43	3485 [591]	2398 [406]			
F <sub>2</sub> (5/114)	1/53	2/61	2/55	0/51	5/49	20/57	1670 [283]	1377 [233]			
Undescended testis, F <sub>1</sub> PND 21 (control 11/135)	5/79	5/54	10/70	5/78	7/50	12/600	2694 [462]	1755 [301]			
Anogenital distance adjusted for body weight F <sub>1</sub> male, PND 21 <sup>b</sup>	↔	↔	↔	↔	↓4%	↓5%	8099 [1373]	3582 [607]	10,436 [1769]	3632 [616]	
Age of preputial separation (adjusted per body weight)											
F <sub>1</sub> parental males	↔	↔	↔	↔	↔	↔	↑2 days	4450 [754]	3397 [576]	3252 [551]	2445 [414]
F <sub>1</sub> retained males	↔	↔	↔	↔	↔	↔	↑1.8 days	4288 [727]	3375 [572]	2897 [491]	2145 [364]
Body weight on day of vaginal opening in F <sub>1</sub>	↔	↔	↔	↔	↔	↔	↓22%	3076 [521]	1281 [217]	3294 [558]	1972 [334]
Age of vaginal opening adjusted for PND 21 body weight <sup>b</sup>	↔	↔	↔	↔	↔	↔	↓2.4	3501 [593]	2953 [501]	3404 [577]	2419 [410]

<sup>a</sup>Based on numbers of animals listed in data tables, it appears that statistical analyses in live animals prior to or on PND 21 considered the litter as the statistical unit, but statistical analyses conducted at PND 21 necropsy considered the individual pup as the statistical unit.

<sup>b</sup>Not considered treatment related by study authors.

<sup>c</sup>Effect was not discussed by study authors but it is unlikely related to treatment.

## 4.0 Reproductive Toxicity Data

### 4.2.3.3 Fish and invertebrates

Although studies in fish and invertebrates may be important for understanding mechanisms of action and environmental impact, the Panel views these studies as not useful for the evaluation process.

**Kwak et al. (528)**, supported by the Korean Ministry of the Environment, exposed adult male swordtail fish (*Xiphophorus helleri*) to bisphenol A 0, 0.4, 2, or 10 ppm [mg/L] for 72 hours (n = 20 fish/group). **[No information on purity or culture ware was provided.] [Nonylphenol was also studied but will not be discussed here.]** At the end of the exposure period, the fish were killed and livers were removed for measurement of vitellogenin. Testes of 10 fish/group were processed for flow cytometry by preparation of single cell suspensions stained with annexin V-fluorescein isothiocyanate and propidium iodide to detect necrosis and apoptosis. TUNEL staining was used to confirm apoptosis in testis sections. In a second experiment, juvenile male fish (30 days old) were exposed to bisphenol A in water at 0, 0.2, 2 and 20 ppb [µg/L] for 60 days, after which body length and sword length were measured. **[The sword is a portion of the caudal fin that elongates as a secondary sex characteristic.]** Statistical analysis used ANOVA followed by least significant difference test. Hepatic vitellogenin was increased by bisphenol A **[data were not shown]**. Apoptosis was increased in testes from fish exposed to bisphenol A at 10 ppm [mg/L] by TUNEL assay. **[Flow cytometry was said to be more sensitive, but data did not appear to have been statistically analyzed.]** Sword growth was decreased by bisphenol A exposure in a concentration-dependent manner, with statistically significant decreases from control at 2 and 20 ppb [µg/L]. The authors concluded that bisphenol A at 20 ppb decreases sword growth and that reproductive impairment occurs in a concentration-dependent manner.

**Strengths/Weaknesses:** This study of bisphenol A is consistent with previous reports on the effects of estrogenic compounds in fish (vitellogenin production and changes secondary sex characteristics). It is unclear exactly how these fish were maintained prior to exposure and during the long-term exposure. Bisphenol A concentrations in the test waters were not determined and only 3 concentrations of bisphenol A were used.

**Utility (Adequacy) for CERHR Evaluation Process:** Of note is the classic dose response obtained in this apparently sensitive model. Given the absence of confirmation of exposure conditions and that this is a fish species immersed in the test agent, this study is not useful in the evaluation.

**Sohoni et al. (529)**, supported by the Society of the Plastics Industry, exposed adult (122-day-old) fathead minnows (*Pimephales promelas*) to bisphenol A in water at 0, 1, 16, 160, and 640 µg/L (n = 60/group) **[No information on purity or culture ware was provided]**. Actual concentrations were 70–96% of nominal concentrations. After 42 days of exposure, 15 fish/group were killed for evaluation of somatic growth, relative gonad weight, plasma vitellogenin, and histologic assessment of the testis. Eight breeding pairs/group were segregated for continued exposure for 123 days. Eggs were removed and counted daily. On 2 occasions, eggs were continued in the same bisphenol A concentration as their parents and the percent hatching was assessed 4 days after fertilization. The remaining adult fish were killed after 71 days of exposure for evaluation of somatic growth, relative gonad weight, and histologic assessment of the gonad. Data were analyzed using 2-way ANOVA and Dunnett test or Kruskal-Wallis and Dunn multiple method test. Linear regression was used to evaluate the relationship between bisphenol A concentration and growth. There were no significant long-term effects of treatment on growth of female fish, but male fish showed an inverse relationship between bisphenol A concentration and growth with significant decrements in length and weight on pair-wise comparison at bisphenol A concentrations of 640 and 1280 µg/L. Relative gonad weight was also decreased in males and females at these bisphenol A concentrations. Plasma vitellogenin was increased in females beginning at bisphenol A concentrations of 640 µg/L and in males beginning at 160 µg/L. A delay in spermatogenesis was suggested by an increase in spermatogonia or spermatocytes and a decrease in spermatozoa in testes beginning at a bisphenol A concentration of 16 µg/L. There were no intersex gonads and no treatment-related changes in ovarian

#### 4.0 Reproductive Toxicity Data

1 histopathology. The number of eggs spawned per female was lower in the control than the treatment  
2 groups and attributed by the authors to an unexplained problem in one of the control tanks. The 1280  
3  $\mu\text{g/L}$  bisphenol A concentration resulted in failure of 7 out of 8 females to produce any eggs. Hatching  
4 was impaired in eggs exposed to bisphenol A concentrations of 640 and 1280  $\mu\text{g/L}$ . The authors noted  
5 that the bisphenol A concentrations resulting in impairment of somatic growth and reproductive success  
6 were only 7-fold lower than the 96-hour median lethal concentration, and concluded that the reproductive  
7 effects may have been the result of sublethal generalized toxicity rather than effects mediated through the  
8 endocrine axis.

9  
10 **Strengths/Weaknesses:** This study was well-conducted with multiple dose levels and concentrations in  
11 the test water were confirmed. “General toxicity” was identified and good histology was used. The  
12 conclusions regarding weak estrogenic activity were appropriate at 160  $\mu\text{g/L}$  and higher. Other effects  
13 were likely due to general toxicity. A classic dose response was noted.

14  
15 **Utility (Adequacy) for CERHR Evaluation Process:** Fish are apparently a sensitive model for  
16 assessment of responses to weak estrogenic compounds. Given that this study evaluated a fish species, it  
17 is not useful in the evaluation.

18  
19 **Kang et al. (530)**, supported by the Japanese Ministry of the Environment, exposed adult (4-month-old)  
20 breeding pairs of medaka (*Oryzias latipes*) to bisphenol A (>99% purity) in the water at 0, 1000, or 4000  
21  $\mu\text{g/L}$  for 3 weeks [**culture ware not discussed**]. Bisphenol A concentrations during the exposure period  
22 were 78–86% of nominal concentrations. Thirty-two pairs of fish had been selected for exposure during  
23 an acclimatization period based on their capacity to spawn daily, with the production of  $\geq 15$  eggs/day and  
24 90% fertility. During the exposure period, eggs were collected daily and assessed for fertility. Fertilized  
25 eggs collected on the last 3 days of the exposure period were permitted to develop in untreated water, and  
26 60 larvae/group were grown for 60 days after hatching to assess normalcy of development. The parent  
27 fish were killed at the end of the treatment period for evaluation of external sex characteristics and for  
28 histologic assessment of the gonads. Hepatic vitellogenin was also assessed. Statistical comparisons of  
29 egg number were made using ANCOVA with female body weight as a covariate. Fertility, growth  
30 endpoints, and hepatic vitellogenin data were analyzed with ANOVA or Kruskal-Wallis test with post hoc  
31 Dunnett or Mann-Whitney *U* test. There were no treatment effects on egg number, fertility, mortality,  
32 relative gonad weight, or relative liver weight in the adult fish. Ovarian tissue was found in the testis in  
33 some males in all bisphenol A-treated groups, although normal testicular tissue with apparently normal  
34 spermatogenesis was also found. Hepatic vitellogenin was increased in male fish in the high-dose group  
35 to control female levels. There were no treatment-related alterations in hepatic vitellogenin in female fish.  
36 Offspring at 60 days of age did not demonstrate treatment-related alterations in survival, growth, or  
37 secondary sex characteristics. The sex ratio was not significantly different in offspring of parents exposed  
38 to bisphenol A, although the authors noted that the low-dose group had a numerical deficit of males (41%  
39 males compared to 50% in the controls). The authors concluded that although bisphenol A increased  
40 hepatic vitellogenin in males and produced an intersex gonad, there were no adverse effects on  
41 reproductive capacity or the normalcy of offspring.

42  
43 **Strengths/Weaknesses:** This appears to have been a well conducted study. The bisphenol A findings are  
44 consistent with the work of others, using sensitive endpoints in fish such as vitellogenin production.  
45 Given the nature of the intersex gonad observation, it should be considered as adverse even though the  
46 severity was not sufficient to induce decreases in reproductive capacity under the conditions tested.

47  
48 **Utility (Adequacy) for CERHR Evaluation Process:** This study indicates that bisphenol A is able to  
49 induce vitellogenin in male fish and intersex gonads. This study exhibited classic dose responses in the  
50 affected endpoints. Because this study was conducted in fish, it is not useful in the evaluation.

51

## 4.0 Reproductive Toxicity Data

1 **Lahnsteiner et al. (531)**, supported by the Austrian Federal Ministry of Agriculture, Forestry,  
2 Environment, and Water Management, examined the effects of bisphenol A exposure on reproduction of  
3 male and female brown trout (*Salmo trutta f. fario*). Fish were caught and acclimated for 2 weeks prior to  
4 starting the study. Ten males/group and 6 females/group were exposed in a flow-through system to  
5 bisphenol A at 0 (DMSO vehicle), 1.75, 2.4, or 5.00 µg/L beginning in the late prespawning period and  
6 continuing through the remainder of the spawning season [**No information on purity or culture ware**  
7 **was provided**]. The bisphenol A concentrations selected were said to occur in the Austrian water system.  
8 Endpoints examined included time point of spawning, sperm count and motility, ability of sperm to  
9 fertilize eggs from non-treated females, and numbers and viability of eggs produced by treated females.  
10 Statistical analyses included ANOVA and Tukey *b* post hoc test.

11  
12 Throughout the entire spawning period, only 1 male in the high bisphenol A dose group produced semen  
13 and it was of low quality as indicated by significantly reduced sperm density, motility rate, swimming  
14 velocity, and fertility. In the low- and mid-dose groups, sperm density was significantly reduced in the  
15 early spawning period but was not affected in the mid or end part of the spawning period. Additional  
16 significant effects observed in the low-dose group included decreased sperm motility in the early  
17 spawning period, reduced swimming velocity in the early and middle spawning period, and increased  
18 circular motion and decreased linear motion in the middle of the spawning period. In the mid-dose group,  
19 sperm motility and swimming velocity were significantly decreased in the early and mid-spawning  
20 period, and a significant increase in circular motion and a decrease in linear motion occurred in the mid  
21 and late part of the spawning period. The study authors interpreted the sperm effects as representing a 4-  
22 week delay in spawning. Fertility of males in the low- and mid-dose group was not affected by bisphenol  
23 A treatment. In females, no eggs were produced by fish in the high-dose group. In all other dose groups,  
24 there were no significant effects on egg volume, viability, mass, mass increase during hardening, or on  
25 numbers of eggs produced by females. However, ovulation was delayed by 2 weeks in the low-dose group  
26 and by 3 weeks in the mid-dose group. The study authors concluded that exposure of trout to bisphenol A  
27 resulted in negative effects on semen and egg quality.

28  
29 **Strengths/Weaknesses:** In this study of fish, alterations in sperm motility were observed consistent with  
30 those observed in mice. Fertility effects in the female were also similar to those observed in other species.  
31 Weaknesses include a failure to determine the actual bisphenol A concentrations in the test system, the  
32 narrow dose range examined (1.75 to 5 µg/L), and the small number of fish/dose level assessed.

33  
34 **Utility (Adequacy) of CERHR Evaluation Process:** This study suggests that fish are sensitive to  
35 bisphenol A-induced abnormalities in reproductive endpoints. Because this study was conducted in fish, it  
36 is not useful in the evaluation.

37  
38 **Ortiz-Zarragoitia and Cajaraville (532)**, supported by the European Commission, examined the effects  
39 of bisphenol A exposure on the reproductive and digestive systems of adult blue mussels. For a period of  
40 3 weeks, mussels were exposed to bisphenol A in acetone vehicle at 0 or 50 ppb [µg/L] [**no information**  
41 **on purity or culture ware was provided**]. Additional compounds were also tested but will not be  
42 discussed. Ten mussels/sex/group were examined at the end of the exposure period. The digestive gland  
43 was examined for volume of peroxisomes and peroxisomal proliferation. Gonads were histologically  
44 evaluated and assessed for alkali-labile phosphate level, a vitellogenin-like protein that is a possible  
45 biomarker of endocrine disruption. Statistical analyses included ANOVA followed by Duncan post hoc  
46 test, Kruskal-Wallis, and Mann-Whitney *U* test. Bisphenol A had no effect on gonadal development,  
47 gonadal alkali-labile phosphate levels, or digestive gland peroxisomal proliferation or peroxisomal  
48 volume. However, observations of follicular brown cell aggregates and gonadal hemocyte infiltration in  
49 35% of male and female mussels indicated severe gamete resorption.

## 4.0 Reproductive Toxicity Data

1 **Strengths/Weaknesses:** This study evaluated bisphenol A-induced alterations in several reproductive  
2 endpoints in adult mussels. Severe gamete resorption was observed. Weaknesses include the failure to  
3 confirm bisphenol A concentrations in the test water and the use of only 1 concentration.  
4

5 **Utility (Adequacy) for CERHR Evaluation Process:** Because this study was conducted in the mussel, it  
6 is not useful in the evaluation.  
7

### 8 **4.3 Utility of Reproductive Toxicity Data**

9

#### 10 *4.3.1 Human*

11 One high utility study of 42 men occupationally exposed to bisphenol A diglycidyl ether and 42  
12 unexposed men evaluated the relationship between urinary levels of bisphenol A and plasma LH, FSH,  
13 and free testosterone, found reduced FSH levels among the exposed men. No fertility endpoints were  
14 evaluated. Three studies were considered to have low utility in the evaluation process due to limitations in  
15 design and analysis but suggest directions for future research. Two of these studies measured serum  
16 bisphenol A in healthy women, women with polycystic ovary syndrome and healthy men and evaluated  
17 correlations with serum gonadotropins, prolactin, testosterone, and other androgens. No fertility endpoints  
18 were included in these studies. The third study of 37 women found significantly lower bisphenol A  
19 concentrations among women with endometrial cancer and complex endometrial hyperplasia compared to  
20 healthy women and women with simple hyperplasia.  
21

#### 22 *4.3.2 Experimental animal*

23 Female reproductive toxicity testing using multiple dose levels has been evaluated in 2 rat, 1 mouse, and  
24 1 gerbil study. Endpoints affected in these studies included brain progesterone receptor, estrous cyclicity,  
25 resorptions, and social sniffing. Male reproductive toxicity testing using multiple dose levels has been  
26 evaluated in 7 rat and 2 mouse studies. Affected endpoints in males included reproductive organ weight  
27 and histology, serum testosterone, daily sperm production, sperm motility, sperm concentration, percent  
28 pregnant females after mating, and females with resorptions after mating. There are 4 multigeneration  
29 tests, 2 in rats and 2 in mice, involving gavage or dietary treatments with bisphenol A with dose levels as  
30 low as 0.0009 mg/kg bw/day. There are also 2 reproductive assessments by continuous breeding, 1 of  
31 which involved subcutaneous implants for bisphenol A delivery and 1 of which used dietary  
32 administration in which the lowest dose level was ~437.5 mg/kg bw/day.  
33

### 34 **4.4 Summary of Reproductive Toxicity Data**

35

36 The hypothesis has been advanced that the Charles River SD rat is insensitive to estrogens and other  
37 EDCs and therefore it should not be used for developmental EDC studies and the studies of the effects of  
38 BPA that used this strain should be discounted. In order to address this important issue Expert Panel  
39 members reviewed the literature on estrogen-sensitivity among rat strains and the following is a summary  
40 of our findings.  
41

42 Different strains of rats show clear, robust reproducible differences in responses to potent estrogens and  
43 antiandrogens. Several traits have been shown to be estrogen sensitive in rats including prolactin  
44 regulation in the pituitary, thymic involution, uterine pyometra, and liver carcinogenesis to name a few. It  
45 is evident that the SD rat and other rat strains are less sensitive to the effects of estrogens than the F344  
46 rat. However, for some traits, the reverse is true. In addition, while the SD was less sensitive than the  
47 F344 to estrogen, the reverse was true for sensitivity to tamoxifen.  
48

49 The sensitivity to estrogens has been mapped to specific chromosomes for several traits. In no case has it  
50 been demonstrated that the SD strain is completely insensitive to any known estrogen. It is evident that

## 4.0 Reproductive Toxicity Data

1 different traits map to different chromosomes and the degree of estrogen sensitivity varies from tissue to  
2 tissue, likely depending upon the tissue-specific gene regulated by ER on the chromosome.

3  
4 Therefore, one cannot conclude that the SD is insensitive to estrogens and the results of BPA studies with  
5 BPA should be ignored. In fact, there are several papers reporting low dose effects that used the SD rat. A  
6 comparison of the uterotrophic data from the OECD study with EE, BPA and other estrogens does not  
7 indicate that the SD rat is less sensitive to any estrogen versus the Wistar. In this study, oral EE at 1  
8 microgram/kg/d for 3 days stimulated uterine weight whereas 0.3 micrograms/kg/d was uterotrophic  
9 when administered sc. In addition, in the pubertal female rat assay, EE, the antiestrogen tamoxifen and the  
10 estrogenic pesticide methoxychlor produced equivalent responses in the Long Evans and SD female rats.

11  
12 While some have hypothesized that the CrI: CD (SD) rat is more insensitive to estrogens than SD rats  
13 from other suppliers, there are no data supporting this assertion.

### 14 15 4.4.1 Human

16 Human reproductive studies are summarized in [Table 103](#). A study of 42 men occupationally exposed to  
17 an epoxy hardening agent containing bisphenol A diglycidyl ether found higher urinary bisphenol A  
18 concentrations, corrected for creatinine, than were found in 42 men who worked in the same factory but  
19 did not have known exposure to the hardening agent (116). Differences were not detected between the  
20 worker groups in plasma testosterone or LH, but plasma FSH was significantly lower in exposed workers  
21 [BPA: 0.043 µg/kg bw] than in workers not exposed to the hardening agent [BPA: 0.021 µg/kg bw]. A  
22 significant correlation was noted between total urinary bisphenol A concentration and decreased FSH  
23 when adjusted for age and alcohol intake ( $r=0.23$ ,  $p=0.045$ ).

24  
25 Two papers from Takeuchi et al. (90, 94) suggested a relationship between serum bisphenol A  
26 concentration and serum testosterone (total and free). The first study (90) included women with and  
27 without polycystic ovary syndrome (POS), and healthy men. Statistically significant positive correlations  
28 were observed for women with and without POS (0.559 for total testosterone and 0.598 for free  
29 testosterone,  $p<0.01$ ), and with all participants (0.595 and 0.609, respectively,  $p<0.001$ ). The second  
30 study (94) reported only cycling women with and without obesity and women with POC, with and  
31 without obesity, hyperprolactinemia and hypothalamic amenorrhea. Statistically significant positive  
32 correlations were found for bisphenol A and total testosterone ( $r=0.391$ ,  $p<0.001$ ), free testosterone  
33 ( $r=0.504$ ,  $p<0.001$ ), androstenedione ( $r=.684$ ,  $p<0.001$ ), and dehydroepiandrosterone sulfate (DHEAS,  
34  $r=0.514$ ,  $p<0.001$ ). Although these studies used ELISA, which may over-estimate bisphenol A compared  
35 to HPLC, significant correlations between bisphenol A levels and higher serum testosterone levels were  
36 found. The authors speculated that androgens either may affect bisphenol A metabolism or the reverse.

37  
38 A study of 37 women found differences in bisphenol A concentrations by health status. Significantly  
39 lower mean bisphenol A concentrations were found among women with endometrial cancer (1.4 ng/ml,  
40  $n=7$ ) and complex endometrial hyperplasia (1.4 ng/ml,  $n=9$ ) compared to healthy women (2.5 ng/ml,  
41  $n=11$ ) and women with simple hyperplasia (2.9 ng/ml,  $n=10$ ) (95).

### 42 43 4.4.2 Experimental animal

44 Reproductive toxicity studies of high and limited utility are summarized in [Table 100](#) and [Table 101](#)  
45 respectively. (single and multiple dose level studies in the same utility category are combined within a  
46 table). Based on reproductive studies using a single dose level, the lowest dose level at which an effect  
47 was seen in these studies was 0.04 mg/kg/day fed to female rats during pregnancy and lactation and  
48 resulting in a decreased duration of licking/grooming pups (477). This study of neural and behavioral  
49 effects is shown here for convenience but has been included with other papers focused on these endpoints  
50 for further discussion in Section 3.

## 4.0 Reproductive Toxicity Data

1 For high utility female reproductive studies using multiple doses, the lowest effect level, for altered  
2 estrous cycle, was  $\geq 600$  mg/kg bw/day by gavage in rat for 28 days (158). For high utility male  
3 reproductive studies, the lowest effect level, for histologic alterations in the testis, was 235 mg/kg bw/day  
4 by gavage in rat for 28 days (497). The value of the histologic observations may be limited due to the  
5 fixation and embedding techniques employed, raising some concern over the validity of this endpoint.  
6

7 The reproductive assessments by continuous breeding included a study using very high dose levels (524),  
8 and this study is not the most informative for reproductive risk assessment. In a multigeneration study,  
9 CD rats did not show statistically significant or dose-related reproductive effects over 2 generations with  
10 bisphenol A gavage doses of 0.0002, 0.002, 0.020, or 0.200 mg/kg bw/day (337). In Sprague Dawley rats  
11 treated for 3 generations, adverse reproductive effects consisted of decreased F<sub>1</sub> epididymal sperm  
12 concentration, decreased F<sub>3</sub> daily sperm production, decreased live pups/litter, decreased pup body  
13 weight, and delayed vaginal opening at an average dose level of 475 mg/kg bw/day. Delayed preputial  
14 separation was seen in F<sub>1</sub> and F<sub>2</sub> males at an average dose level of 47.5 mg/kg bw/day (338, 475). In CD-  
15 1 mice given bisphenol A for 2 generations in the diet at dose levels as low as  $\sim 0.003$  mg/kg bw/day, the  
16 most sensitive effect was a reduction in F<sub>2</sub> seminal vesicle weight relative to brain weight at 50 mg/kg  
17 bw/day. Effects on F<sub>0</sub> epididymal sperm concentration, gestation length, and relative testis weight  
18 occurred at 600 mg/kg/day, the next highest dose level(436).  
19

20 A summary of LH and testosterone effects observed in bisphenol A-exposed experimental animals and in  
21 humans are included in [Table 102](#).  
22

### 23 **Data sufficiency statement for human data**

24 In summary, there are insufficient data to evaluate whether bisphenol A causes male or female  
25 reproductive toxicity in humans. However, several studies collectively suggest hormonal effects,  
26 including one study of exposed male workers likely to have multiple routes of exposure including  
27 inhalation (116).  
28

### 29 **Data sufficiency statement for animal data**

30 In summary, the experimental animal literature was assessed for its utility (high utility, limited utility, or  
31 no utility) based on the criteria established by this expert panel, including an evaluation of experimental  
32 design and statistical procedures. Studies with high and limited utility were further grouped according to  
33 female and male reproductive toxicity, their use of single or multiple dose levels, a multigenerational  
34 exposure paradigm, and the measurement of various hormonal endpoints. Greater weight was given to  
35 studies using the oral route of exposure, because of evidence that oral exposure predominates in humans  
36 and that target tissue exposure to parent compound (bisphenol A) is very low after oral exposure and first-  
37 pass metabolism as compared to subcutaneous or other routes of exposure.  
38

39 There is sufficient evidence in rats and mice that bisphenol A causes female reproductive toxicity,  
40 characterized as delayed vaginal opening with subchronic or chronic oral exposure NOAELs of 47.5  
41 mg/kg bw/day and a LOAEL of 475 mg/kg bw/day (338).  
42

43 There is sufficient evidence in rats and mice that bisphenol A causes male reproductive toxicity,  
44 characterized as delayed preputial separation, with subchronic or chronic oral NOAEL of 4.75 mg/kg  
45 bw/day and a LOAEL of 47.5 mg/kg bw/day (338).  
46

47 There is inconsistent evidence in rats and mice that bisphenol A alters testosterone and gonadotropin  
48 levels in males after oral postnatal exposure.  
49

50 There is inconsistent evidence in male and female mice that bisphenol A produces aneugenic effects in  
51 germ cells after exposure.

4.0 Reproductive Toxicity Data

**Table 100. Summary of High Utility Reproductive Toxicity Studies (Single and Multiple Dose Levels)**

Model & Treatment (doses in mg/kg bw/day)	Endpoint	Bisphenol A Dose Level (mg/kg bw/day)						Reference
		NOAEL	LOAEL	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>	
<b>High Utility Reproductive Toxicity Studies</b>								
<b>Female</b>								
Sprague Dawley rat (fed during pregnancy and lactation)	↓Duration of licking/grooming pups		0.04 (single dose)					Della Seta et al. (477)
CD rat (gavage, 40, 200, or 600/1000 × 28 days)	Altered estrous cycle	Unclear	≤1000/ 600				Data presentation does not permit modeling	Yamasaki et al. (158)
<b>Male</b>								
Sprague Dawley rat (gavaged, 0.020, 0.200, 2, 20, or 200 × 6 days)	No effect on daily sperm production, sperm count or reproductive organ weight	200	>200					Ashby et al. (499)
F344 rats (drinking water with 0.011, 0.116, 1.094 or 11.846 × 13 weeks)	No adverse effects reported	11.846	-					Kim et al. (154)
C57BL/6N mouse (gavaged with 0.002, 0.020, or 0.200 × 6 days)	No effect on reproductive organ weight or epididymal sperm count	≥0.200	-					Nagao et al. (428)
F344 rat (diet 235, 466, 950)	Histologic alterations in testis	<235	235				No dose response	Takahashi and Oishi (497)
CD rat gavaged with 40, 200, or 600/1000 × 28 days	↓Relative ventral prostate weight ↑Relative testis weight	200 200	600/1000 600/1000				Data presentation does not permit modeling Data presentation does not permit modeling	Yamasaki et al. (158)
<b>Multigeneration</b>								
CD rat (gavaged with 0.0002, 0.002, 0.020, or 0.200 prior to mating and × 2 generations)	No significant or dose-related reproductive effects	≥0.200	-					Ema et al. (337)
Sprague Dawley rat (dietary with ~0.0009, 0.018, 0.27, 4.5, 45, or 450 (male) and ~0.001, 0.02, 0.3, 5, 50, or 500 (female) × 3 filial generations)	↓F <sub>1</sub> epididymal sperm concentration	47.5 <sup>a</sup>	475	317	216	700	469	Tyl et al. (338, 476)
	↓F <sub>3</sub> daily sperm production	47.5	475	469	255	524	481	
	↓Live pups/litter <sup>b</sup>	47.5	475	236	174	376	286	
	↓Pup body weight <sup>b</sup>	47.5	475	183	163	177	153	
	Advanced vaginal opening <sup>b</sup>	47.5	475	394	343	206	176	

#### 4.0 Reproductive Toxicity Data

Model & Treatment (doses in mg/kg bw/day)	Endpoint	Bisphenol A Dose Level (mg/kg bw/day)						Reference
		NOAEL	LOAEL	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>	
<b>High Utility Reproductive Toxicity Studies</b>								
	Advanced F <sub>1</sub> preputial separation	4.75	47.5	466	411	188	163	
CD-1 mouse [F0 diet with ~840 or 1669 (male) and ~1055 or 1988 (female)]	↓Number of live pups	840/1055	1669/1988	1116	727	1925	1189	Tyl et al. (527)
	↓Female pup body weight (trend test)			2281	1728	2332	1733	
CD-1 mouse (diet with ~0.003, 0.03, 0.3, 5, 50, or 600 from 6 weeks of age × 2 filial generations)	↓F <sub>0</sub> epididymal sperm concentration	50	600	567	319	607	549	Tyl et al. (436)
	↑Gestation length <sup>b</sup>	50	600	3619	639	1144	599	
	↓Relative testis weight <sup>b</sup>	50	600	562	339	905	605	
	↓Seminal vesicle weight relative to brain weight, F <sub>2</sub>	30	50	405	223	1914	615	
<b>Reproductive assessment by continuous breeding</b>								
CD-1 mouse (diet with ~437.5, 875, or 1750 over 14-week continuous breeding period)	↓Litters/breeding pair	437.5	875	1750	1295	1680	1155	NTP (524)

<sup>a</sup>Dose levels expressed as a mean of the estimated male-female target dose levels

<sup>b</sup>Benchmark doses are shown for the generation with the lowest values.

↑, ↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

## 4.0 Reproductive Toxicity Data

**Table 101. Summary of Limited Utility Reproductive Toxicity Studies (Single and Multiple Dose Levels)**

Model & Treatment (doses in mg/kg bw/day)	Endpoint	Bisphenol A Dose Level (mg/kg bw/day)				Reference
		NOAEL	LOAEL	BMD <sub>10</sub>	BMDL <sub>10</sub>	
<b>Limited Utility Reproductive Toxicity Studies</b>						
<b>Female</b>						
Wistar rat (ovariectomized; sc dosed with ~40 × 1 day)	Altered progesterone receptor mRNA in different brain regions		~ 40 (single dose)		Single dose study	Funabashi et al. (483, 486)
Wistar rat (ovariectomized; sc dosed with ~0.004, 0.04, 0.4, or 4 sc, single dose)	↑ Progesterone receptor in brain regions	0.04	0.4		Data presentation does not permit modeling.	Funabashi et al. (485)
Wistar rat (ovariectomized; sc dosed with 11, 78, 128, or 250 × 7 days)	↓ body weight and body weight gain ↑ Blotted uterine weight (compared to ovariectomized controls) ↑ Pituitary weight (compared to ovariectomized controls) ↑ Serum prolactin (compared to ovariectomized controls) ↑ Prolactin immunopositive cells in anterior pituitary		250 11 (low dose) 128 128 250			Goloubkova et al. (240)
ICR mice, ip every 3 days over 2 wks	No effect on body weight, uterine or ovarian histology, or hematological endpoints ↓ Blood urea nitrogen ↓ ovarian weight (right) ↓ ovarian weight (left)	5	> 5 0.05 (low dose) 0.5 0.5 only			Park et al. (487)
Mongolian gerbil, fed 0.002 or 0.02 from 1 <sup>st</sup> through 21 <sup>st</sup> day of cohabitation	↑ Social sniffing	<0.002	0.002		No dose response	Razzoli et al. (478)
Sprague Dawley (pseudopregnant; sc)	↑ uterine wet weight and protein content on days 1–4 with ~60% ↓ on days 5–8		20 (single dose)		Single dose study	Spencer et al. (484)
<b>Male</b>						

#### 4.0 Reproductive Toxicity Data

Model & Treatment (doses in mg/kg bw/day)	Endpoint	Bisphenol A Dose Level (mg/kg bw/day)					Reference	
		NOAEL	LOAEL	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>		BMDL <sub>1SD</sub>
<b>Limited Utility Reproductive Toxicity Studies</b>								
Long Evans rat Leydig cells (cell culture)	↓ testosterone production; various effects on mechanistic endpoints (e.g., LH-stimulated and basal testosterone production, mRNA expression)		0.0023 µg/L		No dose response		Akingbemi et al. (350)	
Swiss mouse, gavaged with 0.005, 0.025, and 0.1 × 30 days	↓Body weight ↑relative testis weight <sup>b</sup> ↓seminal vesicle weight <sup>b</sup>	0.005 0.005	0.005 0.025 0.025				Al-Hiyasat et al. (508)	
Wistar rat, gavage for 45 days with 0.0002, 0.002 or 0.02	↓Relative testis weight ↓Relative epididymis weight ↓Relative ventral prostate weight		0.0002 0.0002 0.0002	0.056 0.011 0.014	0.021 0.0082 0.0083	0.014 0.0069 0.015	0.0087 0.0050 0.0089	Chitra et al. (501)
ICR mice, ip every 3 days over 2 wks	↓sperm concentration  ↑sperm abnormalities	0.5  0.5	5.0  5.0				Park et al. (487)	
Sprague Dawley rat, gavaged with 0.020, 0.200, 2, 20, or 200 × 6 days	↓Daily sperm production (absolute and per g testis)	<0.020	0.020		No dose response		Sakaue et al. (498)	
Sprague Dawley rat, gavaged with 0.000002, 0.00002, 0.0002, 0.002, 0.020, 0.200, or 2 × 6 days	↓Daily sperm production (absolute and per g testis)	0.002	0.020	Data presentation does not permit modeling.				Sakaue et al. (498)
Wistar or Holtzman SD rat (diet)	No effect on reproductive organ histopathology, daily sperm production, epididymal sperm reserves, or serum testosterone				Single dose study		Takahashi and Oishi (504)	
Wistar rat (sc)	↓ Terminal body weight, absolute and relative reproductive organ weight; altered testicular histopathology		~ 200 (single dose)		Single dose study		Takahashi and Oishi (504)	
CD-1 (ICR) mouse (diet)	↑ Absolute testis weight, ↓absolute epididymis weight. No effect on testis histopathology, epididymal sperm reserves, daily sperm production, or serum testosterone		~ 400 (single dose)		Single dose study		Takahashi and Oishi (504)	

#### 4.0 Reproductive Toxicity Data

Model & Treatment (doses in mg/kg bw/day)	Endpoint	Bisphenol A Dose Level (mg/kg bw/day)						Reference
		NOAEL	LOAEL	BMD <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>1SD</sub>	BMDL <sub>1SD</sub>	
<b>Limited Utility Reproductive Toxicity Studies</b>								
C57BL/6CrSlc mouse (diet)	No effect on reproductive organ weights. No effect on testis histopathology, epididymal sperm reserves, daily sperm production, or serum testosterone.	~ 400 (single dose)						Takahashi and Oishi (504)
Wistar rat, 2 or 20 ip 4 days/week × 1 month	↓ventral prostate weight	2	20	7	5	9	6	Takahashi and Oishi (504)
	↓Serum testosterone	2	20	3	2	16	9	
	↓Preputial gland relative weight	<235	235	124	86	171	114	
<b>Reproductive assessment by continuous breeding</b>								
CD-1 mouse, ~2.4, 4.2, or 8.1 over 18 week continuous breeding period, sc implant	No adverse effects on fertility	≤8.1						NTP (522, 523)

<sup>a</sup>Dose levels expressed as a mean of the estimated male-female target dose levels

<sup>b</sup>Benchmark doses are shown for the generation with the lowest values.

↑, ↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

**Table 102. Summary of Blood LH and Testosterone Changes in Experimental Animal Studies**

Endpoints/protocol	LH effects <sup>a</sup>	Testosterone effects <sup>a</sup>	Reference
<b>High Utility</b>			
Experimental animal studies with oral exposure			
Adult male and female rats gavaged for 28 days	↔ at 40–1000 mg/kg bw/day	↔ at 40–1000 mg/kg bw/day	Yamasaki et al. (158)
Four-week-old male rats fed bisphenol A in diet for 44 or 60 days	Not examined	↔ at 235–950 mg/kg bw/day or 200 mg/kg bw/day	Takahashi and Oishi (497, 504)
Multiple generation gavage dosing study in rats	↓ in F0 adult females at 0.0002, 0.002, and 0.020 mg/kg bw/day but not at high dose (0.2 mg/kg bw/day); not considered treatment-related.	↔	Ema et al. (337)
<b>Experimental animal studies with parenteral exposure</b>			
Female lambs im injected at 4–11 weeks of age; ovariectomy at 9 weeks of age	↔ on blood levels during treatment; ↓ pulsatile secretion following treatment with 3.5 mg/kg bw biweekly	Not examined	Evans et al. (442)
<b>Limited Utility</b>			
<b>Experimental animal studies with oral exposure</b>			
Male rats were gavaged from PND 21 through 35	↓ at 0.0024 mg/kg bw/day but ↔ at higher doses (0.010–200 mg/kg bw/day)	↓ at 0.0024 mg/kg bw/day but ↔ at higher doses (0.010–200 mg/kg bw/day)	Akingbemi et al. (350)
Male rats gavaged from PND 21 through 90	↑ at 0.0024 mg/kg bw/day	↔ at 0.0024 mg/kg bw/day	Akingbemi et al. (350)
Four-week-old mice fed bisphenol A through diet for 2 months	Not examined	↔ at 400 mg/kg bw/day	Takahashi and Oishi (504)
<b>Experimental animal studies with parenteral exposure</b>			
Four-week-old male rats sc dosed on 4 days/week for 1 month.	Not examined	↔ at 200 mg/kg bw	Takahashi and Oishi (504)
Four-week-old male rats ip injected for 1 month.	Not examined	↓ at 20 mg/kg bw	Takahashi and Oishi (504)

↑, ↓ Statistically significant increase/decrease compared to controls; ↔ no statistically significant effects compared to controls

<sup>a</sup>Unless otherwise stated, animals were examined immediately after the treatment period

**Table 103. Summary of Serum Hormone Changes in Human Studies**

Study Members	Hormone Effects	Other	Reference
<b>High Utility</b>			
Urine in male workers 42 exposed 42 non-exposed	↓ FSH (exp median 5.3 mIU/ml vs 7.6 in controls) No difference LH, free testosterone	BPA exposure Exposed men: 1.06 umol/mol creatinine [0.043 μg/kg bw) Non-exposed men:	Hanaoka et al, (116)

#### 4.0 Reproductive Toxicity Data

		0.52 umol/mol creatinine [0.02 µg/kg bw)	
<b>Limited utility</b>			
Serum samples from 14 healthy women 11 healthy men 16 women with PCOS	↑ total testosterone (r=0.595, all subjects) ↑ free testosterone (r=0.609, all subjects) No difference LH		Takeuchi and Tsutsumi (90)
Serum Samples from 26 healthy women 19 women with PCOS 28 women with other conditions	↑ total testosterone (r=0.391) ↑ free testosterone (r=0.504) ↑ androstenedione (r=0.684) ↑ dehydroepiandrosterone sulfate (DHEAS) (r=0.514) no difference LH		Takeuchi et al, (94)
Serum samples from women 11 controls 10 simple hyperplasia (HP) 9 complex hyperplasia (HP) 7 endometrial cancer (EC)		dec BPA in complex HP and EC patients compared to controls	Hiroi et al (95)

## 5.0 SUMMARIES, CONCLUSIONS, AND CRITICAL DATA NEEDS

### 5.1 Developmental Toxicity

No data on the effects of human developmental exposure to Bisphenol A are available. There is a large literature describing studies in rodents and some work in other species. A large experimental animal literature was reviewed, assessed for its utility, and weighed based on the criteria established by this panel.

From the rodent studies we can conclude that Bisphenol A:

- Does not cause malformations or birth defects in rats or mice at levels up to the highest doses evaluated: 640 mg/kg/d (rats) and 1250 mg/kg/d (mice).
- Does not alter male or female fertility after gestational exposure up to doses of 450 mg/kg bw/d in the rat and 600 mg/kg bw/d in the mouse (highest dose levels evaluated).
- Does not permanently affect prostate weight at doses up to 475 mg/kg/d in adult rats or 600 mg/kg/d in mice.
- Does not cause prostate cancer in rats or mice after adult exposure at up to 148 or 600 mg/kg/d, respectively.
- Does change the age of puberty in male or female rats at high doses (ca. 475 mg/kg/d).

Rodent studies suggest that Bisphenol A:

- Causes neural and behavioral alterations related to disruptions in normal sex differences in rats and mice. (0.01-0.2 mg/kg/d).

The data on bisphenol A are insufficient to reach a firm conclusion about:

- A change in the onset of puberty in male rats or mice at doses up to 475 – 600 mg/kg/d.
- An acceleration in the age of onset of puberty at a low dose in female mice at 0.0024 mg/kg/d, the only dose tested.
- Whether Bisphenol A predisposes rats toward prostate cancer or mice towards urinary tract deformations.

### 5.2 Reproductive Toxicity

There are insufficient data to evaluate whether bisphenol A causes male or female reproductive toxicity in humans. A large experimental animal literature was reviewed, assessed for its utility, and weighted based upon the criteria established by this expert panel, including an evaluation of experimental design and statistical procedures. These animal data are assumed relevant for the assessment of human hazard.

Female effects: There is sufficient evidence in rats and mice that bisphenol A causes female reproductive toxicity with subchronic or chronic oral exposures with a NOAEL of 47.5 mg/kg bw/day and a LOAEL of  $\geq 475$  mg/kg bw/day.

Male effects: There is sufficient evidence in rats and mice that bisphenol A causes male reproductive toxicity with subchronic or chronic oral exposures with a NOAEL of 4.75 mg/kg bw/day and a LOAEL of  $\geq 47.5$  mg/kg bw/day.

### 5.3 Human Exposures

Bisphenol A is FDA-approved for use in polycarbonate and epoxy resins that are used in consumer products such as food containers (e.g., milk, water, and infant bottles) food can linings [reviewed in (3, 18)] and in dental materials(22). Resins, polycarbonate plastics, and other products manufactured from

## 5.0 Summaries, Conclusions, and Critical Data Needs

1 bisphenol A can contain trace amounts of residual monomer and additional monomer may be generated  
2 during breakdown of the polymer (2).

### 3 4 Environmental Exposures

5 Bisphenol A emitted from manufacturing operations is unlikely to be present in the atmosphere in high  
6 concentrations. However, it was found in 31-44% of outdoor air samples with concentrations of < LOD  
7 (0.9) to 51.5 ng/m<sup>3</sup> (32). Indoor air samples found concentrations ≤29 ng/m<sup>3</sup>.(31, 32); (33, 34). Limited  
8 U.S surface water sampling found bisphenol A in 0-41% of samples ranging from <0.1 to 12 ug/L (25,  
9 26). Twenty-five to 100% of indoor dust samples contained bisphenol A with concentrations of <  
10 detectable to 17.6 µg/g (31-34).

### 11 12 Exposures through Food

13 The highest potential for human exposure to bisphenol A is through products that directly contact food  
14 such as food and beverage containers with internal epoxy resin coatings and through the use of  
15 polycarbonate tableware and bottles, such as those used to feed infants (2). Studies examining the  
16 extraction of bisphenol A from polycarbonate infant bottles in the U.S. found concentrations < 5 ug/L.  
17 Canned infant formulas in the U.S. had a maximum levels of 13 ug/L in the concentrate that produced a  
18 maximum of 6.6 ug/L when mixed with water (48, 60). Breast milk studies in the U.S. have found up to  
19 6.3 ug/L free bisphenol A in samples(37). Measured bisphenol A concentrations in canned foods in the  
20 U.S are less than 39 ug/kg (32, 48). Limited drinking water sampling in the U.S. indicates that bisphenol  
21 A concentrations were all below the limit of detection (<0.1 ng/L) (25).

### 22 23 Biological Measures of Bisphenol A in Humans

24 The panel finds the greatest utility in studies of biological samples that use sensitive and specific  
25 analytical methods (LC-MS or GC-MS) and report quality control measures for sample handling and  
26 analysis. The panel further focused on biological monitoring done in U.S. populations. In the U.S, adult  
27 urine concentrations of free bisphenol A are less than 0.6 ug/L and total bisphenol A concentrations are <  
28 19.8 ug/L (15, 96, 97). The 95<sup>th</sup> percentile total bisphenol A concentration for 394 adult volunteers (males  
29 and females; 20–59 years old) from the NHANES III survey was 5.18 ug/L (15). Girls age 6-9 in the U.S.  
30 have concentrations of total bisphenol A < 54.3 ug/L, with median concentrations ranging from 1.8-2.4  
31 ug/L (86, 97). No U.S. studies have examined blood or semen concentrations of bisphenol A. Amniotic  
32 fluid total bisphenol A concentrations in the U.S are less than 1.96 ug/L. Dental sealant exposure to  
33 bisphenol A occurs primarily with use of the dental sealant bisphenol A dimethylacrylate. This exposure is  
34 considered an acute and infrequent event with little relevance to estimating general population exposures.

### 35 36 Bisphenol A Intake Estimates.

37 The panel found that previous oral intake estimates for infants fed formula and breast milk did not use  
38 levels reported for the U.S. population, so the panel estimated intake based on typically-used parameters.  
39 The panel found the food intake estimates made by the European Commission(106) used concentrations  
40 of bisphenol A comparable to U.S. food concentrations in their intake estimates, so have included these  
41 estimates as well (Table 104). Estimates from duplicate diets in U.S. children (31, 32) found lower  
42 bisphenol A concentrations in foods than those estimated by the European Commission ,therefore the  
43 aggregate estimates of intake by Wilson were somewhat lower than those estimated by the European  
44 Commission. However, the aggregate intake estimates by Wilson et al. (31, 32) are in line with the  
45 estimates based on urinary metabolite measurements for children described above.

46  
47 Estimates of intake based on occupational air concentrations of bisphenol A from U.S powder paint  
48 workers suggest exposures up to 100 ug/kg bw/day (115). Estimates of intake based on urinary metabolite  
49 levels among Japanese workers spraying epoxy coatings resulted in a mean estimate of exposure of 0.043  
50 µg/kg bw/day (<0.002 pg to 0.45 µg/kg bw/day) (116).

1 **Table 104. Estimates of U.S. General Population Intake of Bisphenol A**

Exposure Source	Population	BPA mg/kg bw/day	Notes	Source
<b>Estimates based on Intake</b>				
Formula	Infant	0.001	Assumes 4.5 kg bw, 700 ml formula at 6.6 ug/L BPA (U.S. canned formula max)	Expert Panel
Breast milk	Infant	0.001	Assumes 4.5 kg bw, 700 ml at 6.3 ug/L (U.S. breast milk max)	Expert Panel
Food	Infant 0-4 mo	0.0016	European Commission	<a href="#">Table 11</a>
	Infant 6-12mo	0.0008- 0.00165		<a href="#">Table 14</a>
	Child (4-6 years old)	0.0012	European Commission	<a href="#">Table 11</a>
Aggregate	Adult	0.00037 (canned food)-0.00048 (canned food + wine)	European Commission	<a href="#">Table 14</a> <a href="#">Table 11</a>
	Child (1.5-5 years old)	0.00004-0.00007	Max 0.00007-0.00157 Assumes 50% absorption	Wilson et al. (31, 32)
<b>Estimates based on Urinary Metabolites</b>				
Aggregate	Child	0.00007	U.S. 6-8 yr old girls (max 0.00217)	<a href="#">Table 15</a>
	Adult	0.000026	U.S. population 95 <sup>th</sup> %ile 0.0.00159	<a href="#">Table 15</a>

2  
3

## 5.4 Overall Conclusions

The panel spent a considerable amount of time attempting to interpret and understand the inconsistent findings reported in the “low dose” literature for bisphenol A. Conducting low dose studies can be challenging because the effects may be subtle and small in magnitude and therefore more difficult to statistically distinguish from background variability. The inherent challenge of conducting these types of studies may be exacerbated with bisphenol A because the endpoints of concern are endocrine-mediated and potentially impacted by factors that include phytoestrogen content of the animal feed, extent of bisphenol A exposure from caging or water bottles, and the alleged sensitivity of the animal model to estrogens. The panel believed that high dose studies are less susceptible to these types of influences because the toxicologic response should be more robust and less variable. While the panel did not necessarily expect a specific effect to display a monotonic dose response (e.g., consistently increasing organ size), many members of the panel expected the high dose studies with bisphenol A to detect *some* manifestation of toxicity (e.g., altered weight, histopathology) in tissues reported to be affected at low doses even if the study could not replicate the reported low dose effect. There are several large, robust, well designed studies with multiple dose groups using several strains of rats and mice and none of these detected any adverse reproductive effects at low to moderate dosage levels of BPA administered via the relevant route of human exposures. Further, none of these studies detected changes in prostate weight, age at puberty (rat), pathology or tumors in any tissue, or reproductive tract malformations. For this reason, panel members gave more weight to studies that evaluated both low and high doses of bisphenol A compared to low-dose-only studies in cases where the target tissues were comparably assessed.

Every chemical that produces low dose cellular and molecular alterations of endocrine function also produces a cascade of effects increasing in severity resulting in clearly adverse alterations at higher doses, albeit the effects can be different from those seen at low doses. With these endocrine disruptors, but not BPA, the low dose effects are often causally linked to the high dose adverse effects of the chemical. This is true for androgens like testosterone and trenbolone, estrogens like DES, 17 $\beta$ -estradiol and ethinyl estradiol, xenoestrogens like methoxychlor and genistein, and antiandrogens like vinclozolin, for example. Hence, the failure of BPA to produce reproducible adverse effects via a relevant route of exposure, coupled with the lack of robustness of the many of the low dose studies (sample size, dose range, statistical analyses and experimental design, GLP) and the inability to reproduce many of these effects of any adverse effect strains the credibility of some of these study results. They need to be replicated using appropriate routes of exposures, adequate experimental designs and statistical analyses and linked to higher dose adverse effects if they are to elevate our concerns about the effects of BPA on human health. The lack of reproducibility of the low dose effects, the absence of toxicity in those low-dose-affected tissues at high doses, and the uncertain adversity of the reported effects led the panel to express “minimal” concern for reproductive effects.

In contrast, the literature on bisphenol A effects on neural and behavioral response is more consistent with respect to the number of “positive” studies although it should be noted that the high dose studies that proved to be the most useful for evaluating reproductive effects did not adequately assess neural and behavioral responses. In addition, even though different investigators assessed different neural and behavioral endpoints, the panel concluded that the overall findings suggest that bisphenol A may be associated with neural changes in the brain and behavioral alterations related to sexual dimorphism in rodents. For this reason, the panel expressed “some” concern for these effects even though it is not clear the reported effects constitute an adverse toxicological response.

Concerns are expressed relative to current estimates of general population exposure levels in the U.S. 1. For pregnant women and fetuses, the Expert Panel has different levels of concern for the different developmental endpoints that may be susceptible to bisphenol A disruption, as follows:

- For neural and behavioral effects, the Expert Panel has some concern

## 5.0 Summaries, Conclusions, and Critical Data Needs

- 1       • For prostate effects, the Expert Panel has minimal concern
- 2       • For the potential effect of accelerated puberty, the Expert Panel has minimal concern
- 3       • For birth defects and malformations, the Expert Panel has negligible concern
- 4 2. For infants and children, the Expert Panel has the following levels of concern for biological processes
- 5 that might be altered by Bisphenol A, as follows:
- 6       • some concern for neural and behavioral effects
- 7       • minimal concern for the effect of accelerated puberty
- 8 3. For adults, the Expert Panel has negligible concern for adverse reproductive effects following
- 9 exposures in the general population to Bisphenol A. For highly exposed subgroups, such as
- 10 occupationally exposed populations, the level of concern is elevated to minimal.

### 5.5 Critical Data Needs

- 14 1. Neural and behavioral endpoints. A concerted effort is needed to better understand the effects of
- 15 gestational and lactational exposure to bisphenol A on maternal behavior and offspring brain structure
- 16 and behavior. This effort should include molecular and cellular studies to ascertain the sensitivity of
- 17 the developing brain to bisphenol A-induced structural and biochemical alterations. The association
- 18 between bisphenol A and neural and behavioral endpoints should also be examined in longitudinal
- 19 studies of pregnancy and child development in humans.
- 20
- 21 2. Human exposure assessment. Additional data are needed to clarify bisphenol A exposures and
- 22 internal dosimetry in the general population, newborns, and occupationally-exposed individuals.
- 23 Available data demonstrate that a large fraction of children and adults have detectable levels of
- 24 bisphenol A metabolites in their urine. What are needed are duplicate diet studies to identify in detail
- 25 the sources and routes of exposure of bisphenol A. For example, while research suggests diet is the
- 26 major source of BPA for U.S. infants and young children, the detailed analysis of BPA levels has
- 27 primarily focused on polycarbonate baby bottle leachates and canned food.. The contributions of non-
- 28 canned food and drinking water routes of exposure for U.S. youth and adults not occupationally-
- 29 exposed to BPA remain unknown and in need of further study. Levels of BPA in residential drinking
- 30 water wells and community water sources have not been systematically studied. Also unknown is the
- 31 impact of landfill leachates on levels of bisphenol A in U.S. drinking well waters and whether
- 32 chlorinated congeners of bisphenol A are found in U.S. municipal water supplies. Finally, more
- 33 measurement are needed of free and total bisphenol A, its glucuronide conjugate, and other
- 34 metabolite concentrations from maternal, fetal, and neonatal tissues or fluids (i.e., placenta, amniotic
- 35 fluid, breast milk, urine, serum). These data would provide insight into the roles of metabolism and
- 36 exposure route on internal dose.
- 37
- 38 3. Human studies relating adult exposure to reproduction and development, including effects on
- 39 hormone levels.
- 40
- 41 4. Physiologically-based pharmacokinetic (PBPK) models. PBPK models are needed to facilitate the
- 42 interpretation and applicability of animal studies, including rodents and nonhuman primates, for
- 43 human risk assessment.
- 44
- 45 5. Effects on prostate and mammary gland development. Additional data are needed to understand the
- 46 susceptibility to disruption of prostate and mammary gland development in humans and animals by
- 47 bisphenol A exposure. Laboratory animal studies should initially focus on the oral route of exposure
- 48 and should be informed by any new knowledge about human exposure and human internal dosimetry.
- 49 A particular data need is an improved understanding of the biology of PIN (prostatic intraepithelial
- 50 neoplasia) in animal models and its relationship to prostate cancer. Similarly, bisphenol A-induced

## 5.0 Summaries, Conclusions, and Critical Data Needs

1 alterations in mammary gland development and their potential relationship to mammary cancer  
2 should be investigated across a broad range of internal concentrations and external doses.  
3

- 4 6. Altered puberty. The robustness and biologic basis for altered puberty following bisphenol A  
5 exposure should be evaluated in mouse, rat, and gerbil. In laboratory animals, this evaluation should  
6 be performed following combined gestational and lactational exposure, and following pubertal  
7 exposure alone, and should include an assessment of any changes in hormonal responsivity at later  
8 ages, and all related to internal and tissue concentrations of bisphenol A. In addition, longitudinal  
9 cohort studies examining the potential modulation by bisphenol A of the onset, progression, and  
10 control of puberty in humans should be performed.  
11
- 12 7. Biological Mechanism for Low-Dose-Only Effects. Most useful would be data which provided a  
13 biologically-plausible explanation for effects which appear at low doses but not higher doses. This  
14 might involve the membrane-bound estrogen receptor and it's possible activation by Bisphenol A.  
15
- 16 8. More work directed toward urinary tract morphological and histologic changes after developmental  
17 exposure would be helpful to determine the robustness and relevance of the limited report of these  
18 effects in one study.  
19
- 20 9. Inter-laboratory replication of studies. Inter-laboratory replication of critical findings is a *sine qua*  
21 *non* for enhancing confidence in experimental results. Such studies should be supported by funding  
22 agencies, and should be facilitated by the open sharing of experimental details and approaches. The  
23 future reproducibility should also be considered by investigators as they design their studies.  
24
- 25 10. Critical design components for all future research on BPA
  - 26 a. Appropriate experimental design and statistical analysis, especially accounting for litter effects.
  - 27 b. Appropriate route (oral) of exposure. Studies with non-oral route of administration should include  
28 internal dose measurements of free BPA
  - 29 c. Multiple dose groups ranging from low to high.
  - 30 d. Linkage of effects to adverse effects.
  - 31 e. Relevant endpoints, with biologically plausible outcomes especially for estrogen-mediated effects  
32 on reproduction and behavior.  
33  
34

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