DRAFT ASSESSMENT OF BISPHENOL A FOR USE IN FOOD CONTACT APPLICATIONS

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Executive Summary

This document describes the Food and Drug Administration's (FDA) safety assessment of Bisphenol A (BPA) as it relates to exposure through use in food contact materials. This assessment is particularly focused on the concerns for developmental toxicity identified in recent assessments of BPA, including those of the National Toxicology Program and their expert panel. BPA is an impurity in FDA-regulated food additives, including epoxy-based food can liners and polycarbonate baby bottles. FDA estimates that BPA exposure from use in food contact materials in infants and adults is 2.42 µg/kg bw/day and 0.185 µg/kg bw/day, respectively. FDA has determined the appropriate no observed adverse effect level (NOAEL) for its assessment of BPA to be the NOAEL for systemic toxicity of 5 mg/kg bw/day (5000 µg/kg bw/day) derived from two multigenerational rodent studies. This NOAEL results in adequate margins of safety of approximately 2,000 and 27,000 for infants and adults, respectively. The data reviewed on highlighted endpoints, such as the prostate gland and developmental neural and behavioral toxicity, were insufficient to provide a basis to alter the NOAEL used to calculate the margins of safety. FDA has concluded that an adequate margin of safety exists for BPA at current levels of exposure from food contact uses. At a later date, FDA will publish a separate document that provides a safety assessment of BPA exposure from other FDA-regulated products.

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Introduction

Bisphenol A (2, 2'-bis(4-hydroxyphenyl)propane; CAS Reg. No. 80-05-7; BPA) is regulated by the Food and Drug Administration (FDA) for use in food contact applications. BPA is not itself a food additive, but a monomer used in the manufacture of food additives. Once BPA is reacted with other chemicals in the manufacturing process very little residual BPA remains. In evaluating the safety of food contact materials, FDA considers exposure to the food additive and any impurity/constituent which migrates to food. Safety for food additives is defined in 21 CFR §170.3(i): *Safe or safety means that there is reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use.* This definition goes on to state that complete certainty of absolute harmlessness is scientifically impossible to establish.

Exposure of adults or infants to residual BPA through uses in food additives is relatively low (i.e., no more than 11 µg/person/day for any segment of the population). Traditionally, FDA's evaluation of chemical migrants to food from the use of food contact materials at exposures of ≤ 150 µg/person/day focuses primarily on carcinogenicity and on genetic toxicity as an indicator of carcinogenicity[1], unless data are available (biological or predictive) that indicate a concern for another endpoint of toxicity at this level.

It is well documented that BPA binds to estrogen receptors (ERα and ERβ), although its affinity is orders of magnitude lower than that of endogenous estrogen[2,3]. In addition, several *in vitro* studies have indicated that BPA may also interact with other receptors, including membrane bound ER and estrogen-related receptor γ (ERR γ)[4]. Since the late 1990s, a large volume of research has been generated suggesting a possible 'low' dose effect for weakly estrogenic environmental contaminants, such as BPA. The National Toxicology Program (NTP) defines 'low' dose for BPA as ≤ 5 mg/kg bw/day[5].

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[1] FDA's Preparation of Food Contact Notifications for Food Contact Substances: Toxicology Recommendations Final Guidance April 2002 (accessible at http://www.cfsan.fda.gov/~dms/opa2pmnt.html).

[2] Krishnan, AV, Stathis P, Permuth SF, Tokes L, and Feldman D. (1993) Bisphenol A: An estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology* 132(6): 2279-2286.

[3] Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, Van Der Saag PT, Van Der Burg B, and Gustafsson J-A. (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* 139(10) 4252-4263.

[4] Summarized data cited in CERHR final report NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A, dated November 2007 (accessible at http://cerhr.niehs.nih.gov/chemicals/bisphenol/BPAFinalEPVF112607.pdf) and published as Chapin *et al.* (2008) NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A *Birth Defects Research* (Part B) 83:157–395.

[5] Melnick R, Lucier G, Wolfe M, Hall R, Stancel G, Prins G, Gallo M, Reuhl K, Ho SM, Brown T, Moore J, Leakey J, Haseman J, Kohn M (2002) Summary of the National Toxicology Program's report of the endocrine disruptors

A complicating aspect of evaluating the potential adverse effects of endocrine active compounds, especially at low doses, are dietary confounders, i.e. the potential presence of high levels of estrogenically active phytoestrogens and lignans in laboratory[6], adult[7] and formula-fed infant[8] diets. As BPA has been discussed as binding and acting through ERs (α and β), it is important to consider that *in vivo* BPA is therefore competing for binding to ERs with endogenous estrogen (17β-estradiol, E2) and with much higher levels of these dietary compounds. In fact, BPA has an approximately 1000 - 10,000 fold lower affinity for ERα and ERβ as compared to E2[9], whereas genistein, a phytoestrogen, has a much higher affinity than BPA for ERα and ERβ[10]. Accordingly, if equal concentrations were available, the assumed order of binding to the ERs would be E2, genistein, and then BPA.

In the last few years, several organizations have published risk assessments on BPA[11], commenting on the low dose effect data. These include the National Toxicology Program's (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) expert panel report[12], the NTP's draft Brief on BPA[13] and Environment Canada (EC)[14] draft screening assessment for BPA. None of these documents indicate a concern for adult exposures; however, some of them (NTP and EC) indicate potential concerns for developmental exposures on select

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low-dose peer review. *Environ Health Perspect.* 110(4): 427-431.

[6] Reviewed in Jensen, NM and Ritskes-Hoitinga, M. (2007) How isoflavone levels in common rodent diets can interfere with the value of animal models and with experimental results *Lab Anim* 41(1):1-18; Brown, NM and Setchell, KD. (2001) Animal models impacted by phytoestrogens in commercial chow: implications for pathways influenced by hormones. *Lab Invest* 81(5):735-47. Thigpen JE, Setchell KD, Ahlmark KB, Locklear J, Spahr T, Caviness GF, Goelz MF, Haseman JK, Newbold RR, Forsythe DB (1999) Phytoestrogen content of purified, open- and closed-formula laboratory animal diets. *Lab Anim Sci* 49(5):530-6.

[7] Reviewed in Setchell, KD (1998) Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones *Am J Clin Nutr* 68:1333S-1346S; Mazur, W and Adlercreutz, H. (2000) Overview of Naturally Occurring Endocrine-Active Substances in the Human Diet in Relation to Human Health *Nutrition* 16:654–687; and Cassidy, A and Setchell, KDR (1999) Dietary Isoflavones: Biological Effects and Relevance to Human Health *J Nutr* 129(3):758S-767S.

[8] Reviewed in Bhatia J, Greer, F and the Committee on Nutrition (2008) Use of Soy Protein-Based Formulas in Infant Feeding *Pediatrics* 121:1062-1068.

[9] Summarized data cited in CERHR final report NTP-CERHR Expert Panel Report (see footnote 4).

[10] Kuiper, GG ,Lemmen, JG, Carlsson, B, Corton, JC, Safe, SH, Van Der Saag, PT, Van Der Burt, B and Gustafsson, J-A. (1998) Interaction of Estrogenic Chemicals and Phytoestrogens with Estrogen Receptor β *Endocrinology* 139 (10): 4252.

[11] Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-Bis(4-Hydroxyphenyl)Propane (Bisphenol A) Question number EFSA-Q-2005-100 Adopted on 29 November 2006, *The EFSA Journal* (2006) 428:1 - 75; Bisphenol A Risk Assessment Document, November 2005, (AIST Risk Assessment Document Series No. 6) New Energy and Industrial Technology Development Organization (NEDO) and Research Center for Chemical Risk Management (CRM) Japanese National Institute of Advanced Industrial Science and Technology (AIST) original 2005 and 2007 documents.

[12] CERHR final report NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A (see footnote 4).

[13] Draft NTP Brief on Bisphenol A, April 14th, 2008. Accessible at http://cerhr.niehs.nih.gov/chemicals/bisphenol/BPADraftBriefVF_04_14_08.pdf

[14] Government of Canada, Environment Canada draft screening assessment and risk management documents dated April 2008 accessible at http://www.chemicalsubstanceschimiques.gc.ca/challenge-defi/batch-lot_2_e.html#ReleaseofDraft.

endpoints. More recently the European Union completed an updated Risk Assessment Report[15] on BPA concluding that data do not indicate a concern for BPA at current exposure levels. As noted in a footnote in this report[16], the Nordic environmental agencies (Norway, Sweden and Denmark) who participated in the discussions disagreed with the no observed adverse effect level (NOAEL) stated with regard to its applicability to the endpoint of developmental neurotoxicity. However, since the publication of that report, the Norwegian Scientific Committee on Food Safety[17] published their own assessment of the studies highlighted in the footnote in the EU RAR, concluding that the results of these data do not provide sufficient evidence to set a robust lower NOAEL. Lastly, in July 2008, the European Food Safety Authority (EFSA), published their updated assessment including an evaluation of the toxicokinetics and the concerns raised by NTP and EC, stating that the previous assessment (2006), which did not indicate a concern at current exposure levels, remains unchanged and that the differences in age-dependent toxicokinetics of BPA in animals and humans would have no implication for the EFSA 2006 risk assessment of BPA[18].

In addition to evaluations by government organizations, two other evaluations have been made public in the past year indicating a concern for BPA exposure. The Environmental Working Group[19] (EWG) posted web site documents examining the toxicity of BPA and a group of BPA researchers, the '*Chapel Hill group*', met in late 2006 and published their findings in *Reproductive Toxicology*[20]. These groups have articulated several additional endpoints of concern beyond those identified by the NTP or other international regulatory bodies. Noteworthy, these groups and the previously noted governmental agencies have all examined the same toxicology data set with regard to BPA.

FDA has never established an acceptable daily intake (ADI) for BPA exposure through food additive use; however, the U.S. Environmental Protection Agency (EPA) has published a reference dose (RfD, 0.05mg/kg/day) for BPA[21] and conducted a mode of action cross-species

[15] Updated European Risk Assessment Report 4,4'-Isopropylidenediphenol (Bisphenol-A) CAS Number: 80-05-7 EINECS Number: 201-245-8, final approved version awaiting for publication, accessible at http://ecb.jrc.it/documents/Existing-Chemicals/RISK_ASSESSMENT/ADDENDUM/bisphenola_add_325.pdf.

[16] Updated European Risk Assessment Report 4,4'-Isopropylidenediphenol (see footnote 15) – with regard to the opinion on the four studies (Negishi 2004, Carr 2003, Ryan and Vandenberg 2006, and Adriani 2003) - see footnote located on page 120 and repeated elsewhere in the document.

[17] Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food Safety (18 June 2008): Assessment of four studies on developmental neurotoxicity of bisphenol A, accessible at http://www.vkm.no/eway/default.aspx?pid=266&trg=MainLeft_5419&MainLeft_5419=5468:17924::0:5420:1:::0:0.

[18] European Food Safety Authority, Toxicokinetics of Bisphenol A Scientific Opinion of the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food (AFC) (Question No EFSA-Q-2008-382): Toxicokinetics of Bisphenol A Scientific Opinion of the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food (AFC). Adopted on 9 July 2008. accessible at http://www.efsa.eu.int/EFSA/efsa_locale-1178620753812_1211902017492.htm Full text of the 2006 opinion of the former AFC Panel accessible at http://www.efsa.eu.int/EFSA/efsa_locale-1178620753812_1178620772817.htm .

[19] Accessible at http://www.ewg.org/node/20936 and http://www.ewg.org/reports/bpaformula.

[20] Several manuscripts reviewing different endpoints included in *Reproductive Toxicology* 24(2):August-September 2007.

[21] Integrated Risk Information System (IRIS), last update 1993 accessible http://www.epa.gov/ncea/iris/subst/0356.htm.

informational assessment[22]. Recently, two multigenerational studies have become available that followed regulatory guidelines and included doses that would be considered low. In early 2007, FDA's Center for Food Safety and Applied Nutrition (CFSAN), which is responsible for evaluating the safety of food contact substances, formed a task force to review the available pharmacokinetic (PK) data on BPA, the two recently completed multigeneration studies performed using international regulatory protocols/guidelines, and the peer reviewed literature with regard to specific endpoints which had been highlighted in recent regulatory reviews or which were highlighted by the CERHR expert panel, and to determine uncertainties and data gaps in the completed safety assessment based on available data.

Subsequently, in light of the findings of the NTP and EC in April of 2008, Commissioner von Eschenbach formed an FDA Task Force to evaluate the safety of all BPA-containing FDA-regulated products. As a component of the work of this task force, FDA/CFSAN has conducted this safety assessment to determine if current exposure to BPA through the use of food additives is safe, meaning that there is reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use in food contact applications. This assessment is focused on the endpoints of carcinogenesis and reproductive and developmental toxicity of BPA. At a later date, FDA will publish a separate document that provides a safety assessment of BPA exposure from other FDA-regulated products.

Exposure Assessment[23]

BPA is regulated for use as a monomer in the manufacture of polycarbonate and epoxy-based enamels and coatings used in food contact applications. Specific regulations which mention BPA as a monomer used in the production of food additives include 21 CFR §172.105 (anoxomer), §175.300 (resinous and polymeric coatings), §177.1580 (polycarbonate resins), §177.1585 (polyester carbonate resins), §177.2600 (rubber articles intended for repeated use), §177.2280 (4,4′-isopropylidenediphenol-epichlorohydrin thermosetting epoxy resins), §177.2420 (polyester resins, cross-linked), §177.1655 (polysulfone resins), and §177.1440 (4,4′-isopropylidenediphenol-epichlorohydrin resins with a minimum molecular weight 10,000). In addition, since 2000 several Food Contact Notifications (FCNs) have become effective for which BPA is used in the manufacture of the notified food contact substances[24]. FDA does not maintain a list of all the specific products manufactured from BPA nor does it maintain a list of the various processors for the BPA-containing products, this is due to the fact that FDA evaluates information based on manufacturing and use conditions. The listing of BPA in 21 CFR §170-199 permits any manufacturer or processor to manufacture and market a food-contact article made from BPA as long as the conditions of use and specifications, such as identity and extractable limitations, in the applicable regulation(s) are met. In fact, FDA's exposure estimate for BPA considers 100% market capture of the applicable products, since it is generic in nature. Conversely, the listings of BPA containing products on the Inventory of Effective Food Contact

[22] http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=22445.
[23] References cited specific to a study reviewed are not referenced using footnotes but can be found under the correlating review heading in Appendix 3.
[24] Effective FCNs include 362, 363, 404, 463, 517, 624, 702, and 737; details are available at http://www.cfsan.fda.gov/~dms/opa-fcn.html.

Notifications are specific to notifiers[25]. Although FDA obtains more specific information on manufacturers of food contact substances and their individual manufacturing processes through this program, as with the petitioned uses, FDA does not have a specific list of BPA-containing end products as provided to consumers.

Previous exposure assessment

FDA previously calculated the cumulative estimated daily intake (CEDI)[26] of BPA for adults and infants from food additive uses to be 0.185 µg/kg bw/day and 0.7 µg/kg bw/day, respectively (assuming 60 kg and 10 kg for adults and infants, respectively)[27]. These estimates were based on studies conducted by FDA laboratories in the early 1990s that focused on BPA migration from polycarbonate (PC) infant bottles and BPA levels in vegetables[28] and infant formula[29] packed in epoxy-coated cans. Migration studies were conducted on reusable PC infant bottles under conditions simulating actual household use in the preparation of infant formula. In addition, FDA laboratories surveyed selected canned vegetables and infant formulas for levels of BPA.

Pertinent to infant exposure, PC bottles were tested according to two migration protocols designed to model "common" and "worst case" use scenarios in the preparation of infant formula. Residual levels of BPA in commercially available PC infant bottles were reported to range from 7 to 30 ppm.

In the first study[30], Biles *et al.* determined BPA levels in 14 samples of infant formula (liquid concentrates) representing 5 brands purchased in metro Washington, DC supermarkets. At least one interior surface of each container (sidewall and/or ends) was found to contain a BPA-based epoxy coating. BPA levels in the formula concentrates ranged from 0.1 to 13.2 ppb, with an

[25] Ibid.

[26] Although exposure estimates are sometimes qualified with 'upper-bound' or 'average', FDA does not routinely use these terms for food packaging. Food packaging estimates use a variety of factors, such as the migration of a substance into fatty or aqueous foods, the types of packaging for respective types of foods and may include the distribution of the types of foods in the diet. For food packaging, FDA does not routinely consider that a person's diet will result from a sole-source, such as an individual subsisting only on canned tuna, but considers that a diet will be of a variety of foods packaged in a variety of food contact materials. As numerous conservatisms are used in the exposure calculation, they assume beyond an 'average' exposure; however, they should not necessarily be characterized as 'upper-bound' exposure estimates.

[27] FDA memoranda - FAP 9Z4681 (MATS #1070 M2.0 and 2.1): National Environmental Trust; submission of 5/13/99. Migration of di(2-ethylhexyl) adipate from polyvinyl chloride cling film and bisphenol-A from can coatings and polycarbonate baby bottles and tableware. Paquette/Smith, 05/2/2000; Cumulative exposure estimates for bisphenol A (BPA). Bailey/Cheeseman, 08/13/2001; Cumulative exposure estimates for bisphenol A (BPA), individually for adults and infants, from its use in epoxy-based can coatings and polycarbonate (PC) articles. Verbal request of 10-23-95. Bailey/Diachenko, 03/13/2006.

[28] A summary of FDA's Chemistry Methods Branch's (CMB) studies on BPA migration from PC infant bottles and BPA level in vegetables was provided to the Chemistry Review Branch (CRB) on 9-26-95 by CMB (Henry Hollifield) in a draft report entitled "Bisphenol-A: Status Summary Report."

[29] FDA CMB's studies on BPA levels in infant formula are described in a FDA CMB memorandum dated 3-5-96 (J. Biles to G. Diachenko).

[30] Biles J, McNeal T, Begley T, Hollifield H. (1997) Determination of Bisphenol-A in Reusable Polycarbonate Food-Contact Plastics and Migration to Food-Simulating Liquids. *Journal of Agricultural and Food Chemistry* 45(9): 3541-3544.

average of 5 ppb. Label directions specify a 1:1 dilution with water. Thus, BPA levels in prepared formula ranged from 0.05 to 6.6 ppb, with an average of 2.5 ppb.

In the second study[31], Biles *et al.* conducted migration tests with intact bottles (1-sided migration) or cut-up bottle strips (2-sided migration) in contact with various food simulants (water; 8%, 10%, 50%, or 95% ethanol; Miglyol 812) or real foods (infant formula or apple juice) under various time and temperature conditions designed to represent exaggerated, repeat, typical and "exaggerated typical" uses. The test solutions were then analyzed for BPA by high performance liquid chromatography (HPLC) with fluorescence detection. Only the "typical" tests were deemed to simulate *normal use* of baby bottles:

1) *Typical use condition with a whole bottle:* Intact bottles were washed with soap and water, rinsed, boiled in HPLC grade water for 5 minutes, filled with formula or apple juice, and then refrigerated at 4°C for up to 72 hours. BPA was not detected at a limit of detection (LOD) of 100 ng/mL (100 ppb)[32].

2) *Exaggerated typical migration experiment[33]:* Pieces of bottles (6x4 cm) were cut from a bottle that had been analyzed for residual BPA, washed, and then boiled in HPLC grade water for 5 minutes. Each bottle piece (folded to fit through the mouth of the vial) was tared into a 40-mL vial, the weight of the polymer and vial was determined and the weight of polymer was calculated. To each vial, 20.0 mL of water or 10% (v/v) ethanol/water (care was taken to ensure that the entire polymer was immersed) was added and the vial sealed. The vials were placed into a preheated 100°C forced air oven for 30 minutes and cooled to room temperature. An aliquot was then removed with a microliter syringe, diluted with methanol, and analyzed by HPLC. The vials were placed in a refrigerator (4°C) and aliquots were removed after 48 and 72 hours. The BPA level in the 10% ethanol and water food simulants was about 2 µg/kg (2 ppb), after correction for the food mass-to-surface area typical of baby bottles[34]. The LOD for BPA was determined to be 2 ng/mL (2 ppb) in ethanolic simulants and water.

FDA estimated the cumulative exposure for infants to BPA from the use of epoxy-based can enamels containing liquid infant formula and PC infant bottles used by the consumer to prepare infant formula and milk to be the maximum values of 6.6 ppb and 1.7 ppb BPA, respectively. Although the analytical method used for analysis of formula and juice for BPA dictated a high LOD due to the effects of the matrix used, BPA was nonetheless not detected in these foods. FDA would not expect BPA at levels as high as the LOD given that the studies in water and 10% ethanol were conducted at a higher temperature and BPA was not detected at a lower LOD. An

[31] Biles J, McNeal T, Begley T. (1997) Determination of Bisphenol-A Migrating from Epoxy Can Coatings to Liquid Infant Formula Concentrates. *Journal of Agricultural and Food Chemistry* 45(9): 4697-4700.

[32] As elaborated upon in Biles *et al.* 1997, limits of detection for BPA in fruit juices, infant formula, and Miglyol were higher, ca. 100 ng/mL as a result of matrix effects. FDA acknowledges that this is a less sensitive experiment due to dilution with water required by matrix effects (detection limit of 100 ng/mL, which is equivalent to ca. 2% of residual BPA migrating from the bottle). Measurable BPA was not present in either the juice or formula.

[33] FDA considers this exaggerated due to greater contact between the polymer and given volume of simulant resulted in increased sensitivity (sensitivity for BPA increased by measuring double-sided as opposed to a single-sided migration experiment with whole bottles).

[34] Using the simulant volume-to-sample surface area (3.4 mL/sq in) and our standard assumption (10 g food/in^2).

average cumulative exposure for BPA was calculated by multiplying the mean, eaters-only daily consumption of infant formula (820 g, based on a 3 day survey of actual users) for infants up to 12 months of age by the maximum BPA level in infant formula which was assumed to be prepared in a PC bottle (6.6 ppb+1.7 ppb), resulting in a cumulative exposure of 8.3 ppb.

In estimating adult exposure, FDA analyzed BPA levels in select canned vegetables purchased in Washington, D.C. metro supermarkets and packed in imported and domestic manufactured cans containing epoxy-based coating enamels. The test samples consisted of canned mushrooms (3 samples), tomatoes, artichokes, and mixed vegetables (1 sample each) and included both the pureed vegetable and liquid. The test samples were analyzed for BPA by HPLC with fluorescence detection, with an LOD of 5 ppb. BPA levels ranged from 5-39 ppb in vegetables, with an average value of 16 ppb for all 6 samples. FDA also considered a study by Brotons *et al.*[35] in which BPA levels were analyzed in select canned vegetables purchased in U.S. or Spanish supermarkets. Test samples consisted of the liquid phases of canned peas, artichokes, green beans, mixed vegetables, corn, mushrooms, asparagus, palm hearts, peppers, and tomatoes. The test samples were analyzed for BPA by HPLC and ranged from 12-76 ppb (four samples were non-detect), with an average value of 22 ppb for all 10 samples. FDA considers that an individual's diet typically consists of a variety of canned vegetables; therefore, an average level of 22 ppb BPA in vegetables is sufficiently conservative for estimating exposure to BPA from epoxy-based can enamels. FDA assumed that the levels are representative of all food (i.e., aqueous, acidic, alcoholic, and fatty) packed in coated cans. This is known as the "weight-averaged" concentration of BPA in food (<M>), i.e., <M>average is 22 ppb. FDA has determined that 17% of all food available to consumers for purchase is packaged in polymer coated metal packaging[36] and; therefore, the appropriate consumption factor (CF) for calculating exposure is 0.17. Using FDA's traditional approach of combining migration values and CF, the corresponding average dietary concentration of BPA from can enamels has been calculated by multiplying 22 ppb by 0.17 (22 ppb x 0.17) resulting in a dietary concentration of 3.7 ppb (equivalent to an intake of 0.185 μg/kg bw/day for a 60 kg person consuming 3 kg of food per day).

Although the average value was used in estimating exposure, FDA considers this conservative in that it assumes canned foods are consumed daily and that *all* food types (including beverages) are packed in cans coated with BPA-based enamels. Although epoxy-based can enamels dominate the market, other major types of can enamels, such as oleoresinous and vinyl, are used depending on the particular packaging application. Moreover, beverage cans contain thinner coatings which are not known to result in detectable migration of BPA and are not thermally processed in the same manner as "food cans," Accordingly potential exposures to BPA are lower for beverage cans and the fact that beverage cans are not included in the estimation of average migration values from food cans increase the conservativism of FDA's exposure estimates. PC-

[35] Brotons JA, Olea-Serrano MF, Villalobos M, Pedraza V, and Olea N. (1995) Xenoestrogens Released from Lacquer Coatings in Food Cans, *Environmental Health Perspectives* 103:609-612. The limit of detection was not detailed in the published report; FDA has assumed a limit of detection of 5 ppb, as achievable by FDA and others at the time of publication. Furthermore, no information on the origin of individual cans (i.e., U. S. or Spanish) or exact breakdown on can construction (i.e., 2- or 3-piece; identity of end and body coating) was reported.

[36] Guidance for Industry Preparation of Premarket Submissions for Food Contact Substances: Chemistry Recommendations (accessible at http://www.cfsan.fda.gov/~dms/opa3pmnc.html).

based polymers are intended for repeated-use by the adult consumer. Given the large quantity of food processed over the service lifetime of a typical PC-based food-contact article, dietary exposure to BPA from this use for adults would be insignificant in comparison to BPA exposure for can enamels. Therefore, PC-based polymer exposure, if added, would not significantly change the cumulative exposure of BPA of 11 µg/person/day resulting from the use of epoxy-coated cans.

Updated exposure assessment

As identified in recent assessments, the focus of concern for BPA ingestion is developmental exposure. Accordingly, FDA has updated the CEDI for infants (less than 12 months of age) to consider if the assumptions used in the FDA's previous assessments are still valid and to consider recently published evaluations[37]. In updating the exposure estimate, FDA considered studies conducted by FDA, data reported in the literature, data reported by consumer groups (the Environmental Working Group and Canada's Environmental Defence), and data conducted by or reviewed in the EFSA, EU and Environment Canada's recently released assessments. Additionally, in conducting the updated assessment, FDA re-evaluated the current practices of infant formula preparation and consumption. Several points are worth highlighting in this re-analysis:

- Formula intake: FDA evaluated mean daily intakes for various infant age groups using food consumption databases from the U.S. Department of Agriculture (USDA) from 1994-96 & 1998 Continuing Survey of Food Intakes by Individuals (CSFII) and National Health and Nutrition Examination Survey (NHANES 2003-2004)[38] using the Exponent Food Analysis and Residue Evaluation (FARE) software (version 8.12; NFCS food code #117 for infant formula). The mean, eaters-only intakes are shown in Table 1 (row 5). Based on the analysis of the available data, by 12 months of age a high percentage of infants have stopped consuming liquid formula. FDA's previous analysis (circa 1996) focused on the period when the most infant formula is actually consumed, i.e., the first year of an infant's life. Based on the updated information, this assumption is considered to still be valid.

- PC baby bottles: In re-evaluating the exposure assessment for infants, FDA surveyed the current practices in infant formula preparation. FDA's review of available information indicates that infant formula preparation may or may not include thermal sterilization of bottles and water. A survey of the available information on infant feeding practices indicates that a conservative estimate is to assume that PC bottles are thermally sterilized in the preparation of infant formula during the first 2 months of life, though thermal sterilization may not be used at all. FDA also analyzed the PC bottle studies summarized by EFSA, the Environmental Working Group and the Canadian Government and concluded that only those

[37] FDA memorandum - Memorandum to the File. Update on cumulative exposure to BPA for infants from epoxy-based container coatings and polycarbonate (PC) bottles in contact with infant formula. Verbal request dated 4/29/08. Bailey/Twaroski, 06/02/2008.

[38] USDA Continuing Survey of Food Intakes by Individuals data accessible at http://www.ars.usda.gov/main/site_main.htm?modecode=12-35-50-00 and National Health and Nutrition Examination Survey, 2003-2004 data accessible at http://www.cdc.gov/nchs/about/major/nhanes/nhanes2003-2004/exam03_04.htm

studies on PC infant bottles that used time/temperature conditions that are representative of realistic PC baby bottle use are of use in estimating exposure. FDA concludes that BPA migration levels are represented as follows:

1) 1 µg/kg under room temperature use conditions and used to represent BPA level from PC bottles from the use of typical heating in the preparation of infant formula in months 3-12 and;
2) 10 µg/kg under use conditions as high as 100ºC (thermal sterilization) and used to represent BPA levels from PC bottles from the use of thermal sterilization in the preparation of infant formula in months 1-2.

FDA updated its assessment to include a migration value of 10 µg/kg BPA (10 ng/g BPA) for the contribution from PC bottles subject to thermal sterilization in the preparation of infant formula for infants 0-2 months of age. For the remaining months (3-12), FDA considers a migration value of 1 µg/kg to represent BPA levels from the use of PC bottles from typical uses to be sufficiently conservative based on general recommendations for infant formula preparation for this age group. These values are shown in Table 1 (row 2).

- Infant formula:

Powdered: Inspection of select powdered infant formula cans available in the US[39] indicates that they are composite cans made of paper and aluminum foil and, as such, would not be expected to contain any BPA-based coatings. In fact, composite cans are not intended for use in high temperature food processing or holding applications, rather they are only intended for use in applications that are at or below room temperature. Moreover, powdered formulas are not heat sterilized in the same manner as liquid ready-to-feed and concentrates and; therefore, would not require the use of more expensive BPA-based epoxy coatings as are used in the manufacturing of ready-to-feed and concentrate formulas. Because there is no BPA to migrate into formula, FDA did not consider powdered infant formula a source of BPA exposure.

Liquid: FDA's previous assessment relied on studies conducted by FDA laboratories (summarized above) in which the BPA levels measured in 14 samples of prepared formula ranged from 0.05 to 6.6 ppb, with an average of 2.5 ppb. An updated review of the literature indicated that the results of other studies were consistent with these figures, and as such, the average value of 2.5 ppb is used in the updated analysis. These values are shown in Table 1 (row 3).

Exposure estimates for infants based on this updated analysis are presented in Table 1. The calculated exposure estimates consider a mass of BPA migration (ng/g) per mass of infant formula (g/person/day), and therefore, are independent of the number or size of PC bottles used per day. For instance, FDA has assumed that for every gram of infant formula prepared for a 0 –

[39] Results from W. Limm (HFS-706) on two brands of infant formula packaged in composite containers indicates that the linings not based on epoxy chemistry. FDA Memorandum dated 5/28/08, W. Limm to A. Bailey.

2 month old infant, 10 ng of BPA will be present in the formula from thermal sterilization of the PC bottle used to feed the infant.

Table 1: BPA exposure for infants up to 12 months

Source	Age Range (months)											
	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12
BPA level in formula from PC bottles (ng/g)	10	10	1	1	1	1	1	1	1	1	1	1
BPA level in formula from can coatings (ng/g)[a]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Maximum total BPA level in formula (ng/g) [b]	12.5	12.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Mean Eaters-only consumption[c] (grams/person/day)	705	882	923	916	853	832	736	772	798	717	564	435
BPA exposure[d] (µg/person/day)	8.8	11	3.2	3.2	3	2.9	2.6	2.7	2.8	2.5	2	1.5
♂Mass (kg)[e]	4.00	4.88	5.67	6.39	7.04	7.63	8.16	8.64	9.08	9.48	9.84	10.16
♂BPA exposure[f] (µg/kg bw/day)	2.20	2.25	0.56	0.50	0.43	0.38	0.32	0.31	0.31	0.26	0.20	0.15
♀ Mass (kg)[e]	3.80	4.54	5.23	5.86	6.44	6.97	7.45	7.90	8.31	8.69	9.04	9.36
♀ BPA exposure (µg/kg bw/day)	2.32	2.42	0.61	0.55	0.47	0.42	0.35	0.34	0.34	0.29	0.22	0.16

a- BPA in prepared formula from ready-to-feed and liquid concentrates, not powder. FDA study results where BPA levels in prepared formula ranged from 0.05 to 6.6 ppb, with an average of 2.5 ppb.
b- Total migration derived from adding PC bottle and can coating levels (i.e., <10 ng/g + 2.5 ng/g = 12.5 ng/g).
c- Numbers used are NHANES 2003-2004 data for infants who consume only infant formula
d- Example calculation for 0-1 month age group: (10 ng/g + 2.5 ng/g) x 705 grams/person/day) = 8812.5 ng/person/d ÷ 1 µg/1000 ng = 8.8 µg/person/day
e- CDC infant body mass data (accessible at http://www.cdc.gov/nchs/about/major/nhanes/growthcharts/datafiles.htm)
f- Example calculation for 0-1 month age group: 8.8 µg/person/day ÷ 4.00 kg/person = 2.2 µg/kg bw/day

Biomonitoring data

As summarized by NTP in their draft Brief[40], there are several publications detailing measurements in biological fluid for BPA. Although FDA is aware of these data and considers them extremely useful, FDA also understands the experimental limitations that have been identified with regard to these data (issues with regard to sample stability and deconjugation, environmental contamination, and methods of analysis, such as enzyme-linked immunosorbent assay). FDA's updated safety assessment is focused on a subpopulation, infants. Accordingly, the currently available data, which consider exposure to adults or young children (6 years of age or older), were not used or relied upon in FDA's safety assessment. However, as an example of data available, FDA notes that of the CDC NHANES, published by Calafat *et al.*[41] and

[40] NTP Brief on Bisphenol A, April 14th, 2008 (see footnote 13).
[41] Calafat AM, Ye X, Wong Y-L, Reidy JA and Needham LL. (2008) Exposure of the U.S. Population to Bisphenol A and 4-tertiary-Octylphenol:2003–2004. *Environ Health Perspect* 116:39–44.

commented on in the NTP draft Brief. The CDC NHANES urinary measurements indicate a ubiquitous exposure to BPA at a concentration of approximately 0.289 – 0.233 µg/kg bw/day based on a 50 kg default assumption for adults aged 20 – 60+ years at the 95th percentile. Although this value is approximately slightly higher than FDA's estimate for adult exposure (60 kg default) of 0.185 µg/kg bw/day, these numbers are within a relative range and do not suggest a concern that FDA's estimate is less than conservative. Applying FDA's default value for body weight to the CDC estimate, the difference is even smaller (0.241 – 0.194 µg/kg bw/day versus 0.185 µg/kg bw/day). Additionally, it is unclear if these differences may be attributable to non-food contact sources, such as environmental contamination through landfill leachates as these areas of exposure are not as well characterized or researched as those of food contact materials.

Conclusion

FDA's conservative approach is to consider the highest value to represent all exposure. Accordingly, the highest µg/kg bw/day CEDI estimated by FDA is 2.42 µg/kg bw/day (females, 1-2 months of age). FDA has reviewed the documents cited in the NTP draft Brief, as well as other sources of information, and considers this estimate to be conservative. FDA's conclusion is based on the fact that this estimate assumes 1) that all infant formula is packed in cans coated with BPA-based enamels; 2) that all formula is in a liquid form as purchased and used by the consumer; 3) that the consumer prepares and delivers all formula in thermally sterilized PC bottles; and 4) that the repeat use scenario of the PC baby bottle results in continuous exposure at the 10 µg/kg level. FDA is aware that both powdered and infant formula are available to consumers and consumers may use a mixture of formula types or only powdered formula, that alternatives to PC baby bottles are available, including polypropylene bottles and those with polymeric liners that do not contain BPA and are convenient to use, and that it is conservative[42] to assume that consumers will thermally sterilize their PC bottles and migration over the life of the bottle would continue to occur at 10 µg/kg/use, even if some depolymerization were to take place. These assumptions all increase the likelihood that actual infant exposure to BPA is lower than FDA's estimated exposure[43].

[42] FDA's typical repeat use scenario assumes that typical residual migration levels are extrapolated to the entire service lifetime of the article. Some studies have reported potential continued migration of BPA during repeat use but findings and protocols are inconsistent; therefore, FDA has used a conservative approach in modeling this exposure.

[43] FDA recognizes that different methods and assumptions are used by regulatory agencies to determine dietary exposure to food contact materials based on particular regulatory frameworks. For example, the NTP's draft Brief cites a maximum BPA estimate for infants from 0-6 months of 11 µg/kg bw/day which was calculated in the 2006 EFSA assessment. The EFSA estimate differed from FDA's in that it used different sources of data for formula consumption, data from a survey of epoxy-coated cans from the Taiwanese market [Kuo and Ding (2004)], and an estimate of 50 µg/L that the assessment described as "conservative" (as opposed to the "typical" estimate of 10 µg/L) for PC bottle contribution in deriving an exposure estimate for BPA. As stated in the EFSA assessment "Available data were not adequate to assess average migration from PC bottles but a migration value of 10 µg/L was considered to complement this conservative scenario with a more typical situation" (EFSA report page 18, see footnotes 11 and 18) noting that in two 2003 studies, the measured levels of BPA migration were lower than the upper value of 50 µg/L BPA (studies cited on page 17 of the EFSA report). Based on FDA's updated review of the literature (2008), including the EFSA assessment (2006), FDA considers that data currently available are sufficient for FDA to conclude an estimate of PC bottles leaching BPA at 10 µg/L for every use is sufficiently protective and conservative. (See footnote 37 for FDA's review of updated literature.) FDA also notes that in general EFSA's approach to estimating exposures to food contact materials and pre/post market assessments of food contact

In conclusion, the highest CEDI estimated by FDA is 2.42 µg/kg bw/day, for female infants 1-2 months of age (highest estimate for males is 2.25 µg/kg bw/day). In addition to the conservative assumptions discussed above, the use of this maximum exposure estimate of 2.42 µg/kg bw/day to represent infant exposure, as opposed to using an average based on the entire 0 - 12 month infant formula consumption period, introduces an additional level of conservatism into this assessment.

Toxicological Profile

Toxicity data on BPA have been summarized in numerous reviews[44] and assessments prepared by regulatory bodies. Discussion as to whether the low µg/kg bw/day exposure from BPA leaching from food contact articles is hazardous to human health has continued for more than a decade. As summarized in the CERHR expert panel report, the concern for adult toxicity at low doses is "negligible"[45]. Conversely, the CERHR expert panel and others have concluded "some concern" exists for developmental toxicity at the low doses humans encounter from food contact articles with regard to neural and behavioral effects. The NTP draft Brief extends their "some concern" finding to the prostate gland[46], mammary gland, and the age at which females attain puberty. However, in a meeting of NTP's Board of Scientific Counselors on June 11th, 2008, the Counselors voted to reduce the concern level for the findings regarding puberty and mammary gland to minimal[47]. Additionally, FDA is aware that a group of BPA researchers, the '*Chapel Hill group*', met in late 2006 and published their findings in *Reproductive Toxicology*[48]. Although they have articulated several additional endpoints of concern beyond those identified by the NTP or other international regulatory bodies, the CERHR expert panel, the NTP, and the international regulatory bodies which have recently updated their assessments, all considered the same data on which the conclusions of this group of researchers were based.

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materials differs from FDA's and these differences stem in part from differences in the regulatory frameworks and, as such, the terms "typical" and "conservative" though similar may not equate between the respective agencies.

[44] Goodman,JE, McConnell EE, Sipes IG, Witorsch RJ, Slayton TM, Yu CJ, Lewis AS, Rhombert LR. (2006) An Updated Weight of the Evidence Evaluation of Reproductive and Developmental Effects of Low Doses of Bisphenol A. *Critical Reviews in Toxicology*, 36:387-457; Haighton LA, Hlywka JJ, Doull J, Kroes R, Lynch BS, and Munro, IC. (2002) An Evaluation of the Possible Carcinogenicity of Bisphenol A to Humans, *Regulatory Toxicology and Pharmacology* 35:238-254; Several manuscripts reviewing different endpoints included in *Reproductive Toxicology* 24(2):August-September 2007; CERHR final report NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A (see footnote 4); Willhite CC, Ball GL and McLellan CJ. (2008). Derivation of a bisphenol A oral reference dose (RfD) and drinking-water equivalent concentration. *Journal of Toxicology and Environmental Health*, Part B, 11(2): 69 – 146; Gray GM, Cohen JT, Cunha G, Hughes C, McConnell EE, Rhomberg L, Sipes IG, Mattison, D (2004) Weight of the evidence evaluation of low-dose reproductive and developmental effects of bisphenol A. *Human and Ecological Risk Assessment* 10(5): 875-921; and Huff, J. (2001) Carcinogenicity of Bisphenol A in Fischer 344 and B6CC3F1 mice. *Odontology* 89:12-20.

[45] The five levels of concern used by NTP are from highest to lowest: serious concern, concern, some concern, minimal concern, and negligible concern. Definitions of these levels are not defined by NTP. http://www.niehs.nih.gov/news/media/questions/sya-bpa.cfm.

[46] The conclusions mention only prostate, but the text on page 9 and elsewhere covers both altered prostate and urinary tract development.

[47] Actions on the Draft NTP Brief on Bisphenol A by the NTP Board of Scientific Counselors (BSC), June 11, 2008, accessible at http://ntp.niehs.nih.gov/files/BSCactionsBPA_508.pdf.

[48] Several manuscripts reviewing different endpoints included in *Reproductive Toxicology* 24(2):August-September 2007.

FDA notes that the activities of the CERHR expert panel and NTP draft Brief are hazard identifications and not quantitative safety or risk assessments. As the NTP and FDA are both part of the Department of Health and Human Services (DHHS), the activities of these agencies are complementary but independent. Accordingly, FDA uses data and information produced by the NTP in safety evaluations of FDA products, but only after Agency scientists have reviewed and considered the information under the applicable policies, procedures and laws. BPA represents a unique food contact substance based on the volume of scientific studies available. FDA has monitored data on BPA for years and considered its own preliminary evaluations of the peer reviewed literature as well as other assessments performed by international counterparts in determining a process of review. FDA's activities were focused on studies with available raw data and concerns identified following in-depth analysis of peer reviewed literature for which the CERHR expert panel, NTP or other agencies have identified. FDA's safety evaluation of BPA considered an identification of 'some concern' or higher in the expert panel, NTP draft Brief, or other similar designation by an international counterpart in their review as an endpoint requiring our own independent analysis.

FDA has conducted an assessment of BPA including a review of studies performed for regulatory bodies to support safety assessments and of published literature, focused on data FDA previously reviewed by the CFSAN task group (initiated March 2007) and on studies identified by the NTP draft Brief specifically for endpoints for which "some concern" was identified. FDA's review of the literature was focused on the endpoints identified by the recent reviews (NTP, CERHR, and Canada) and not the entire body of information on BPA. This review is focused on issues/concerns that are of current discussion regarding BPA safety and is not an exhaustive review of BPA. For the particular endpoints in question, FDA performed an independent assessment of the supporting information on which the NTP draft Brief was based[49]. As part of the FDA/CFSAN updated safety assessment for BPA, data which had been previously reviewed for other endpoints are included for completeness of record; however, if the endpoints were not considered relevant to the exposure level or deemed of some concern by NTP, an exhaustive update of the available literature has not been performed.

A comment on the nature of studies used to support safety assessments for the regulation of food additives ("guideline studies") is required prior to a discussion of the large body of data on BPA. FDA has published guidance on the conduct of studies for submission to the agency to support the safe use of food additives (Redbook 2000)[50]. The reason for such guidance is to ensure that studies that follow the guidance use sufficient and relevant dosing protocols, adequate replicates of animals for meaningful statistical analysis, interim analysis when applicable, and analysis of endpoints (organ weights, clinical chemistry, histopathology, etc.) which are considered validated by the FDA or other international regulatory organizations for use in safety assessment. These studies also follow good laboratory practices (GLP, 21 CFR Part 58)[51] and contain quality assurance (QA) statements. A typical GLP study submitted to FDA contains all raw data

[49] NTP reviewed data available up to April/May 2008.
[50] Guidance for Industry and Other Stakeholders Toxicological Principles for the Safety Assessment of Food Ingredients Redbook 2000 accessible at http://www.cfsan.fda.gov/~redbook/red-toca.html.
[51] As described in 21 CFR §58.1, following GLP's is intended to ensure the quality and integrity of the safety data.

collected during the course of the study, thereby allowing FDA to review and audit the study and reach an independent conclusion on the findings reported in the study author's report.

When adequately reported and performed, FDA does use published studies in the safety assessment of food contact materials. FDA's review of such studies is similar to that of GLP studies, comparing the protocol to published guidelines/recommendations, analyzing the data for results, and critiquing the author's conclusions. However, because journal publications typically are limited in the thoroughness in which they are reported, FDA is often unable to validate the performance quality or data integrity of these studies, as is FDA's standard procedure for reported GLP/QA studies. This reporting limitation limits FDA's ability to independently reach the authors' conclusions or arrive at alternative interpretations of the data/findings presented. Even in cases where this limitation severely affects FDA's confidence in the findings or the endpoints analyzed are unclear with regard to human adverse effects, such studies can be very useful in assisting FDA in determining if additional GLP data should be generated on a food additive to ensure safe use.

As detailed in Appendices 1 and 2, many of the studies in the published BPA literature have limitations with regard to their protocol designs as compared to recommended guidelines for the types of endpoints examined. Limitations cited in the assessments of many, but not all, of these studies included single dose administration, experimental designs lacking in reported details or otherwise flawed[52], a lack of use of a positive control or the lack of/abnormal response of a positive control, inappropriate vehicle used, lack of control/measurement of confounding environmental estrogens, and inappropriate route of exposure (subcutaneous) without measurement of an internal dose, or calculation of an oral dose to the animal (drinking water studies).

Two of the commonly cited limitations in mode of action studies are lack of a positive control or internal dose measurement. Positive control and internal dose measurement are usually not recommended for routine assessments in Redbook 2000[53] protocols for developmental, reproductive, systemic or cancer studies because these studies are designed to measure relevant endpoints in a robust study design (they are not mode of action analysis) and the recommended route of exposure is the relevant route of exposure (dietary). The limitation regarding the use of a positive control is important because authors are usually hypothesizing a mode of action similarity to a chemical with a known mode of action (estrogenic compounds) and usually only measuring a discrete number of endpoints. In studies only measuring a few select endpoints, a positive control evaluated on these same endpoints can be essential to conclusions of a common mode of action or a compound related effect. In guidance studies, all validated endpoints for evaluating toxicity are recommended, providing information on multiple pathways (target organs and systems) and concurrent measurements of observed toxicity. Still, positive controls can also be useful for determination of the sensitivity of the test species and strain when preliminary information regarding the mode of action or structure activity relationship data are available.

[52] Assessment observations included insufficient replicates, a lack of control for litter effects, insufficient dose information (the dose was not calculated by author), lack of control for bias (relevant assessments not performed blind), the use of protocols which lack measurements of common concomitant endpoints of analysis for the endpoint under investigation, underreporting of statistical analysis, evaluation of only one sex, or lack of histochemistry data.
[53] FDA Redbook 2000 protocols include positive controls in short term genetic toxicity assays.

However, a lack of sensitivity would not necessarily result in invalidation of a GLP study which reported data based on the validated protocols. Instead, such data would be put in the context of human safety assessment based on the relevant findings and the animal model used. The lack of measurement of an internal dose is relevant to the ability to compare multiple routes of exposures to a chemical for which the relevant route is oral. Therefore, though the lack of measurement of an internal dose is noted, this limitation is highlighted because the pharmacokinetics will be affected by the route of administration and findings reported would be more clearly comparable to well performed guideline studies in which BPA was administered by the relevant route (orally) if internal dose measurements were available. Accordingly, many of the studies cited in the literature failed to control for numerous issues that validated regulatory protocols eliminate by design and these shortcomings limit the utility of these studies in an overall safety analysis of the use of BPA in food contact applications.

Pharmacokinetics (PK)

Numerous studies have examined the absorption, disposition, metabolism and elimination of BPA. Importantly, the main metabolite of BPA, BPA-glucuronide (BPAG), has no significant estrogenic activity in either *in vitro* or *in vivo* test systems[54]. As a consequence, determination of unconjugated BPA concentrations in target tissues of test species at sensitive life stages is of critical importance in the analysis of BPA safety. An overview of the cross-species PK properties was produced by FDA[55] which considered *in vivo* data generated in 12 studies in rats, 3 studies in mice, 4 studies in monkeys and 3 studies in humans. Additional *in vitro* data and physiologically-based models were also considered in the assessment.

PK studies of BPA conducted in mice, rats, monkeys and humans all indicate rapid intestinal absorption, and very rapid conjugation of BPA with UDP-glucuronic acid, forming BPAG. BPAG formation is followed by a slower process of its elimination. Human (and monkey) BPAG elimination is relatively rapid ($t_{1/2}$ = 3-4 hr) and primarily via urine. Elimination of BPAG in rodents is complicated by the fact that it is routed primarily into bile rather than urine (as in primates), and consequently enters the intestines, where bacterial glucuronidases hydrolyze BPAG to re-form free BPA, allowing BPA to be reabsorbed and re-circulated. The extent of re-absorption of BPA from rat intestines may be less than 50 %, but is sufficiently large to make kinetic measurements in the rat confusing and emphasizes the fact that additional free, estrogenically-active BPA may be available in this animal model as compared to humans. Additionally, Zalko *et al.* (2003) demonstrated the potential for alternative metabolites in the mouse, suggesting species variability. The half-life for BPAG, the estrogenically inactive main BPA metabolite, may be a few hours, but could be as long as 17 hours in humans. A summary of the kinetic characteristics by species is presented in Table 2.

[54] Matthews JB, Twomey K, Zacharewski TR. (2001) *In vitro* and *in vivo* interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta. *Chem Res Toxicol* 2:149-57.
[55] FDA Memorandum - Compact Summary of Bisphenol A (BPA) Pharmacokinetics. Roth and Komolprasert/Twaroski, 06/01/2007; revised 05/23/2008 see Appendix 2 for studies cited within document.

Table 2: BPA - Basic Kinetic Characteristics by Species

Species	F (fraction absorbed) Reported	*Range of $t_{1/2}$	Elimination Routes
Mouse	100 % (estimate from Taylor *et al.*, 2008)	< 5 hr (total BPA) 9-18 hr (free BPA)	Primarily fecal
Rat	80 - 100 %	1 hr (free BPA) 10 - 21 hr (BPAG)	50 - 80 % fecal 20 - 40 % urinary
Monkey	80 + %	1 hr (free BPA) 10 - 14 hr (BPAG)	80 - 90 % urinary 5 – 10 % fecal
Human	84 + %	3.4 - 17 hr (BPAG)	80 - 90 % urinary 10 + % fecal

* These parameters represent the range reported in studies reviewed here, or estimated by FDA from the data.

Data indicate that some free BPA enters the fat and other tissues, where it may have an extended residence time in the free form. BPA has been shown to cross the placenta and enter the fetus in rats, mice, monkeys and humans. Depending on the study and sampling methodology, concentrations of fetal BPA residues have been reported as much lower or as much higher than in the mother. Fetal-stage animals do not have the ability to conjugate BPA rapidly, so the residence time of free BPA in the conceptus can be longer than that in maternal circulation.

BPA appears in the milk of lactating animals in substantial quantities. Previous reports indicate that BPA in milk is found to be primarily in the form of BPAG; however, more recent data (which is limited in interpretation by the small sample size employed) published by the CDC[56] indicates that this ratio may be less well defined than previously thought. Nonetheless, if BPAG is excreted in milk, it may be hydrolyzed by intestinal (bacterial) glucuronidases to form free BPA which would be rapidly absorbed by the neonate. Milk excreted free BPA would also be readily absorbed. The ability of free BPA to be conjugated by neonates is an unresolved question because glucuronidating capability is low at birth and develops with age[57]. For instance, Domoradzki *et al.* (2004) studied the pharmacokinetics of BPA in 4 - 21 day post-natal (PND) rats, using 10 mg/kg dose and oral administration. This study demonstrated that PND 4 neonates had much less ability to glucuronidate BPA than PND 7 or PND 21 rats, but also demonstrated that significant glucuronidation capability was present in PND 4 rats.

[56] Ye X, Bishop L, Needham L, and Calafat AM. (2008) Automated on-line column-switching HPLC-MS/MS method with peak focusing for measuring parabens, triclosan, and other environmental phenols in human milk *Anal Chem Acta* 622(1-2):150-6 and Ye, X, Kuklenyik, Z, Needham, LL and Calafat, AM. (2006) Measuring environmental phenols and chlorinated organic chemicals in breast milk using automated on-line column-switching–high performance liquid chromatography–isotope dilution tandem mass spectrometry. *J. Chromatography* B 831:110-115.

[57] European Food Safety Authority, Toxicokinetics of Bisphenol A Scientific Opinion of the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food (AFC) (Question No EFSA-Q-2008-382) Adopted on 9 July 2008 (see footnote 18).

Considerable discussion regarding BPA PK has centered on the routes of administration in rodent laboratory studies and their relevance to human oral exposure. The relative concentration of unconjugated BPA is lower following oral administration compared to subcutaneous (s.c.) or intraperitoneal administration. This fact and differences in routes of BPAG elimination between species indicate that caution should be used in the interpretation of studies using non-oral routes of exposure. FDA has considered a recent study by Taylor *et al.* (2008) which compared the s.c. route to the oral route in mice. Parameters estimated from plasma ^{3}H-BPA concentration data included C_{max}, first-order disappearance rates, initial and terminal half-lives ($t_{1/2}$) and areas under the concentration versus time curves (AUCs) at two doses, 35 µg/kg and 395 µg/kg. The authors found no differences in these computed parameters between dosing routes or doses. Although the data contain useful information that may be combined with data from other studies for use in modeling, several errors were noted in the experimental analysis. For example, the initial disappearance slope is a composite of several processes, including continuing absorption, distribution from blood into tissues, and glucuronide and sulfate conjugation, making interpretation difficult; the shapes of the plasma disappearance curves are characteristic of enterohepatic recirculation following s.c. injection and when combined with a lack of samples 24 hours after dosing may invalidate the terminal half-life estimate; and the mass balance cannot be reconciled due to the fact that tissue and excreta analyses are lacking. Accordingly, based on the available data and considering that human route of exposure comparison data are not available, FDA concludes that safety assessments comparing possible levels of human exposure to no-effect or lowest effect levels should be based on laboratory animal studies using oral routes of exposure since this is the most relevant route of human exposure for food contact materials. Studies based on other routes of exposure, such as intraperitoneal or s.c. injections, are likely not comparable to typical human exposures to food contact materials and will not produce results relevant to safety assessments of food contact materials.

A considerable amount of uncertainty exists in the existing PK data on BPA. This includes:

- The relevance of rodent doses in relation to human doses due to the enterohepatic recirculation of free BPA in rodents that does not occur in humans following oral administration (excretion is primarily urinary).
- The neonatal activity of UDPGT for which BPA is a substrate in glucuronidation;
- The relevance of non-oral route of administration studies involving neonatal exposure (cross species and cross route of administration studies which include internal dosimetry measurements for multiple endpoints/target tissues are lacking) in the safety assessment of BPA exposure from the use of food contact materials (human oral exposure); and
- The systemic steady-state levels of unconjugated BPA in humans and test animals.

These uncertainties must be considered in evaluating the overall safety of BPA.

Carcinogenesis

The NTP conducted a BPA carcinogenicity study in mice and rats which was completed in 1982[58]. This study was reviewed by FDA[59]. A brief description of the study design and conclusion follows:

[58] Carcinogenesis Bioassay of Bisphenol A in F344 rats and B6C3F1 mice - Feed Study, NTP Technical Report 215,

Rats: 50/sex/group were administered 0, 1000, or 2000 ppm BPA in the diet for 103 weeks beginning at 5 weeks of age. Concentrations were calculated to be 74 and 148 mg/kg bw/day for males and 74 and 135 mg/kg bw/day for females, respectively. Treatment with BPA resulted in decreased body weight gain in both sexes. NTP concluded that there was no convincing evidence of carcinogenicity associated with BPA treatment; however, the technical report authors pointed out that the incidence of leukemia was elevated in high dose males and slightly elevated in low and high dose females, while the incidence of interstitial cell (Leydig) tumors in males exhibited a statistically significant trend with the incidences in treated males increased compared to that of the controls. The NTP considered that these findings may have resulted from an unusually low incidence in the concurrent controls.

Mice: 50/group of male mice were administered 0, 1000 or 5000 ppm BPA in the diet while groups of 50/group of female mice were administered 0, 5000 or 10,000 ppm BPA in the diet, beginning at approximately 5 weeks of age for 103 weeks. Corresponding concentrations were not calculated by FDA based on the limited data available. The European Union estimated the mouse doses of BPA, using default factors, to be 120 and 600 mg/kg bw/day in males, and 650 and 1,300 mg/kg bw/day in females[60]. BPA reduced body weight in high dose animals and low dose females. In males, the incidence of multinucleated giant hepatocytes was increased, but an increase in liver tumors was not observed. Regarding neoplastic findings, the combined incidences of lymphomas or leukemia were slightly higher in treated males than controls; however, this finding did not reach statistical significance. The NTP concluded that the study did not provide conclusive evidence of the carcinogenicity of BPA.

As mentioned in its draft Brief, NTP concluded that some concern exists for perinatal BPA exposure and for susceptibility to tumors of the mammary gland and for hormonally-induced pre-neoplastic lesions of the prostate later in life. It is noteworthy that the 1982 NTP study did not include *in utero* exposure and NTP has concluded that the conventional rodent bioassay is insensitive with regard to prostate tumors (see NTP draft Brief).

As part of this safety assessment, CFSAN's Cancer Assessment Committee (CAC) evaluated BPA based on the available bioassay data and recent peer-reviewed publications on BPA, specifically those that reported evidence of pre-neoplastic and neoplastic changes in animal models that were administered BPA orally at various dose levels[61]. The CAC concluded that the findings reported in the 1982 NTP study on BPA do not provide any evidence that BPA is carcinogenic to F344 rats or $B6C3F_1$ mice of either sex as tested under the conditions of this bioassay. In re-evaluating the study, the CAC commented that due to the high and variable background incidence of mononuclear cell leukemia in Fischer 344 rats and variable incidence of

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NIH Publication No. 82-1761.

[59] FDA Review Memorandum -Acceptance of Final TDERs for review of NTP's Carcinogenesis Bioassay of Bisphenol A in F344 rats and B6C3Fl mice (Feed Study) (NTP TR 215). Shackelford/Food Additive Master file 580. 07/24/2007

[60] EU Risk Assessment Report on BPA – Final Report, 2003 accessible at http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/bisphenolareport325.pdf

[61] FDA Memorandum - CAC Meeting Dates: 04/24/2008, 05/09/2008 CFSAN Cancer Assessment Committee (CAC), Full CAC Review – Bisphenol A (BPA)

lymphoma/leukemia in $B6C3F_1$ mice, tumors of the hematopoietic system reported in these studies were not considered treatment-related or suggestive of an effect of BPA. CAC noted the age of this NTP study (more than 20 years old) and the significant limitations in its experimental design (e.g. limited number of dose groups, limited clinical observations made throughout the study, lack of organ weight data, and lack of an *in utero* phase).

All of the studies highlighted in recent assessments, though interesting with regard to potential modes of action or target organs for BPA, are difficult to interpret with regard to long-term effects (chronic exposure) and oral safety assessment for regulatory purposes as they were mainly non-oral exposure and short term in duration. As none of the studies were carried out long-term, there is an absence of data indicating progression of the observed lesions or data indicating that the observed lesions are adverse in nature. Based on an evaluation of the literature, the CAC in 2008 concluded that the studies available, which focus on rodent models with regard to BPA's effects on male prostate gland and the female mammary gland, are more mechanism-driven studies rather than safety evaluation studies and, as such, several limitations were noted with regard to the confidence in the reported results. In addition, the available PK data indicate that routes of exposure for BPA are critical to any carcinogenic outcome. Reported studies on BPA had several inconsistencies and inadequacies, such as non-oral routes of administration, limited endpoints, lack of proper histopathological evaluations and inappropriateness of models used, including a lack of continuous exposure as would occur in the human population. Furthermore, of the studies available for which BPA was administered orally, findings are limited in their interpretation and assessment applicability. In addition, the CAC could not validate the performance quality or data integrity of these studies in their available published format. Because of these limitations, the CAC concluded that the totality of the information contained in these reports is of questionable usefulness for a determination of potential enhancement of neoplastic effects of BPA on the rodent prostate and mammary gland.

Systemic Toxicity

The systemic toxicity of BPA has been examined in numerous studies, some of which were reviewed by FDA; others were not fully reviewed due to either their dose selection or lack of relevant findings based on the margin of exposure. Studies fully reviewed included a 2-week aerosol toxicity study with Fischer 344 rats, a 90-day oral toxicity study in dogs, and a 13-week aerosol toxicity study with Fischer 344 rats[62]. Several other subchronic studies in FDA's records are summarized by the study authors, but were not fully reviewed by FDA. The *Systemic Toxicity Summary Tables* in Appendix 1 cites the relevant findings. Some summaries were omitted as they are mentioned elsewhere in this document as full reviews. It is noted, however, that the multi-generation studies discussed under *Reproductive Toxicity* contained a subchronic period preceded by *in utero* exposure. These studies reported NOAELs of 5 mg/kg bw/day for systemic effects.

Although FDA had previously reviewed BPA studies in which the method of exposure was aerosol administration, these were not considered useful in evaluating oral exposure, but were

[62] FDA Review Memorandum - Acceptance of Final TDERs for studies reviewed under contract with ICF Consulting (Contract Number 223-96-2302) for Food Additive Master File No 580 under Work Assignment 2000-20 (ICF 020) Tasks Number 1, 2 and 3. Shackelford/Food Additive Master File 580, 07/24/2007.

evaluated due to their robustness for the identification of potential target organs. As the reviews of these data are present in FDA's files and have not been commented on previously, they have been summarized herein.

None of the reviewed or cited studies indicate a concern at the current CEDI. Furthermore, all recent reviews of BPA have focused on the pivotal endpoints of reproductive and developmental toxicity. As such, this review was not expanded to include a search of the currently available literature for general toxicity.

Reproductive Toxicity

FDA reviewed two studies concerning the reproductive toxicity of BPA in rodents: a two generation reproductive toxicity study in CD-1® Swiss Mice and a three generation reproductive study in CD Sprague-Dawley rats. These studies were chosen for full review based on their comprehensive dosing, adherence to accepted guidelines and inclusion of several additional endpoints. Pivotal aspects of the study review are included below.

Two-Generation Reproductive Toxicity Evaluation of Bisphenol A Administered in the Feed to CD-1® Swiss Mice[63]

The study was conducted by RTI International, Research Triangle Park, NC and was sponsored by the American Plastics Council.[64] The in-life portion of the study occurred in 2005 – 2007; the study report was finalized 03/01/2007. BPA was administered via feed to 9 groups of 6 week old mice at doses of 0 (2 groups), 0.018, 0.18, 1.8, 30, 300, or 3500 ppm BPA (equivalent to intakes of 0, 0.003, 0.03, 0.3, 5, 50, or 600 mg/kg bw/day, respectively). 17β-estradiol was used as a positive control and was administered at 0.5 ppm (intake of 0.08 mg/kg bw/day) to a separate group. F0 animals were exposed for eight weeks prior to mating, during the mating period, through gestation, and during the three week lactation period. F1 offspring (28/sex/group) were exposed through premating, mating, gestation and lactation. F0 dams were necropsied after weaning occurred, F1 dams and F2 offspring were necropsied at the time of weaning F2 offspring. F0 and F1 males were necropsied at the end of the gestation of their respective F1 and F2 litters. In addition, 1 F1 male/litter was randomly selected at weaning for retention and treatment for 3 months. These animals were evaluated for andrology, necropsy, and histopathology concurrent with F1 parental males. (This resulted in an additional 21-27 *n* in BPA treatment groups and 50 in control.) Treatment related effects at 3500 ppm included the following: decreased epididymal sperm concentration; decreased paired epididymal weights (did not achieve statistical significance) (F0 males); significantly reduced absolute paired epididymal weights (F1 males); significantly increased gestational length (F0 and F1 females); reduced pup body weight (PND 7 – 21, F1); reduced absolute and relative spleen weights (F1 and F2 weanlings); increased incidence of undescended testes, seminiferous tubule hypoplasia, and decreased testes weight (F1 and F2 male weanlings); delayed preputial separation (F1 male

[63] FDA memoranda Shackelford/Twaroski, 06/24/2007: Review of Two-Generation Reproductive Toxicity Evaluation of Bisphenol A Administered in the Feed to CD-1® Swiss Mice; RTI study number (Study number 65C-09301.000.003/0209301.000.003)

[64] The study has since published: Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Hentges SG, and Waechter JM Jr (2008) *Toxicol Sci.* Two-generation reproductive toxicity study of dietary bisphenol A (BPA) in CD-1 (Swiss) mice. *Tox Sci* 104(2):362-384.

offspring); increased liver weights (absolute and relative), increased incidence in minimal to mild centrilobular hepatocyte hypertrophy, increased kidney weights (absolute and relative), increased minimal to mild nephropathy (F0 and F1 adults and retained F1 adult males); day of acquisition (vaginal patency) was statistically significantly accelerated when adjusted by body weight on PND 21 (F1 females only animals measured). Results at 300 ppm included increased incidence in minimal to mild centrilobular hepatocyte hypertrophy (adult F0 males, retained F1 males and F1 females). FDA calculated the following NOAELs for the study:

- Systemic: 30 ppm (5 mg/kg bw/day)
- Reproductive: 300 ppm (50 mg/kg bw/day)
- Offspring[65]: 300 ppm (50 mg/kg bw/day).

Three-Generation Reproductive Toxicity Evaluation of Bisphenol A in the Feed of CD® (Sprague-Dawley) Rats.[66]

The study was conducted by RTI International, Research Triangle Park, NC and was sponsored by the Society of Plastics Industry. The in-life portion of the study occurred in 1998-2000; the study report was finalized 10/05/2000. BPA was administered via feed to CD-SD virgin rats (30/sex/dose) at doses of 0, 0.015, 0.3, 4.5, 75, 750, or 7500 ppm BPA (equivalent to intakes of 0, 0.001, 0.02, 0.3, 5, 50, or 500 mg/kg bw/day, respectively). F0 animals were exposed for 10 weeks prior to mating, during the mating period, through gestation, and during the lactation period until weaning (PND 21). F1 litters were culled to 10 pups (equal sex ratio) at PND4. F1 and F2 offspring (30/sex/group) were exposed through premating (13-15 weeks), mating, gestation and lactation. F0 males were sacrificed and necropsied after F1 delivery. F3 weanlings were sacrificed after approximately 10 weeks of continued dietary exposure. Treatment-related reproductive effects at 7500 ppm included reduced absolute paired ovarian weights (all females); reduced relative paired ovarian weights (F0, F1 and F2); increased paired ovarian primordial follicle counts (F0); reduction in number of implants, total and live pups per litter at birth (F1, F2, F3); reduction in epididymal sperm concentration (F1 males); decreased testicular homogenization-resistant spermatid head counts (DSP, F3 males). A reduction in number of implants total and live pups per litter at birth was also seen at 0.3 ppm for F3. Offspring effects included decreased pup body weights per litter during lactation (7500 ppm, F1, F2, and F3; 75 ppm and 4.5 ppm, F2), delayed absolute age of vaginal patency and delayed absolute age at preputial separation (7500 ppm, F1, F2 and F3). Systemic effects included reduced body weight and body weight gain (7500 ppm, F0, F1, F2, and F3); reduced body weight during gestation and lactation (7500 ppm, F0, F1 and F2 females); decreased terminal body weights (7500 ppm, all); increased slight to mild renal tubular degeneration and chronic hepatic inflammation (7500 ppm, F1 and F2 females); chronic hepatic inflammation (7500 ppm,

[65] FDA considers the comprehensiveness of the study, including the use of multiple generations, relevant to the analysis of developmental endpoints though not in complete agreement with the Redbook 2000 developmental protocols. The major difference is the time of administration and the sacrifice period. FDA notes that the protocol of continuous exposure is more consistent with human exposure scenarios for BPA.

[66] FDA memorandum Gu/Twaroski, 07/18/2007: Review of study entitled "Three-Generation Reproductive Toxicity Evaluation of Bisphenol A in the Feed of CD® (Sprague-Dawley) Rats" and email "About AGD" dated 06/06/2008 (Gu/Twaroski). RTI study number 65C-07036-000. This study was also submitted in manuscript form with the same title: Tyl RW, Myers CB, Marr MC, Thomas BF, Keimowitz AR, Brine DR, Veselica MM, Fail PA, Chang TY, Seely JC, Joiner RL, Butala JH, Dimond SS, Cagen SZ, Shiotsuka RN, Stropp GD, Waechter JM (2002) Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. *Toxicol Sci.* 68(1): 121-146.

F0 males); at 750 ppm, effects observed included reduced body weights during lactation (F1 females), reduced body weights during gestation and lactation (F0 and F2 females), and decreased terminal body weights [F1 (all) and F2 (males)]. An observation of increased anal genital distance was made only in F2 females, all doses except for 75 and 7500 ppm. This observation was considered sporadic based on the lack of dose response and lack of finding in F3 females and; therefore, was not considered treatment related. FDA calculated the following NOAELs for the study:

- Systemic: 75 ppm (5 mg/kg bw/day)
- Reproductive: 750 ppm (50 mg/kg bw/day)
- Offspring: 750 ppm (50 mg/kg bw/day).

An additional GLP study by Ema *et al.*[67] was also identified, but the original study report and raw data are not available to FDA, only the published report. Briefly, developmental and reproductive toxicity of BPA was examined in a 2-generation study in Crj:CD(SD) rats. Animals (25/sex/dose) were gavaged daily with 0, 0.2, 2, 20, 200 μg/kg bw/day BPA throughout premating, mating, gestation, and lactation. Stainless steel cages were used for housing. Bedding/diet (< 0.003 μg/g, LOD) and drinking water (0.03 μg/L) were analyzed for BPA. Endpoints included clinical observations, body weight, food consumption in F0, F1 and F2 generations; estrous cyclicity (adult females only in F0, F1 and F2); reproductive effects (parents/offspring-F0/F1 and F1/F2); developmental parameters (F1 and F2), behavioral effects (F1); necropsy and histopathology (F0, F1 and F2); organ weight; serum hormone levels (F0 and F1 adults; and sperm parameters (F0 and F1). Some statistically significant changes were observed; however, those changes were sporadic, inconsistent or non-dose-dependent and, accordingly findings were considered non-treatment-related. BPA exposure did not cause compound-related reproductive or developmental changes in this 2-generation rat study.

Based on the reviewed studies in rodents, the NOAEL for reproductive and offspring toxicity is 50 mg/kg bw/day in both rats and mice. A NOAEL for systemic toxicity was determined to be 5 mg/kg bw/day in both species.

Developmental Toxicity

FDA has reviewed teratology studies conducted by the NTP[68,69]. Based on the data presented in these NTP studies, a developmental no observed adverse effect level (NOAEL) of 1280 mg/kg/day (highest dose tested) was identified for CD® rats administered BPA on gestation days (GD) 6-15; a developmental NOAEL of 1000 mg/kg/day and a developmental lowest observed adverse effect level (LOAEL) of 1250 mg/kg/day was identified for CD-1 mice administered BPA on GD days 6-15. Maternal LOAELs were lower than the developmental NOAELs (160

[67] Ema M, Fujii S, Furukawa M, Kiguchi M, Ikka T, and Harazono A. (2001): Rat two-generation reproductive toxicity study of bisphenol A. *Reprod Toxicol* 15:505-523

[68] FDA Memorandum - Table of NOAELS and LOAELS from Bisphenol A Toxicity Studies in FMF 580. Shackelford/Twaroski, 06/12/2007.

[69] FDA Memorandum - Acceptance of Final TDERs for studies reviewed under contract with ICF Consulting (contract No. 223-96-2302) for Food Additive Master File No. 580 under Work Assignment 2000-19 (ICF 419), Task Numbers 3, 4, 5, 6. Shackelford/Twaroski, 06/11/2007.

mg/kg/day in rats and 500 mg/kg/day in mice) in these studies. Two other studies were reviewed; however, their protocols are limited with regard to endpoints beyond fertility.

In the aforementioned studies listed under *Reproductive Toxicity*, a NOAEL for offspring was determined to be 50 mg/kg bw/day in both species and sexes. Although these studies were not considered full teratology studies as described in Redbook 2000 developmental protocols, FDA considers the comprehensiveness of these studies, including the use of multiple generations, relevant to the analysis of developmental endpoints. FDA notes that the protocol of continuous exposure is more consistent with human exposure scenarios for BPA. The NTP draft Brief indicated some concern for the current level of exposure to BPA and developmental toxicity to the prostate, urinary tract and early onset of puberty in females. Some of these developmental endpoints were addressed in the multigenerational studies performed by RTI (Tyl 2002 and 2008), though by different methodologies (discussed below). Accordingly, FDA evaluated the literature on these endpoints.

Specific Developmental Endpoint Analysis (Summarized in Appendices 1 and 2)

In most of the studies reviewed, limitations were cited that decreased FDA's confidence in their usefulness in a safety assessment. Some studies had only small numbers of replicates, some used only 1 or 2 doses of BPA so a dose-response relationship could not be determined, some used a non-oral route of administration, which would have affected blood levels and embryonic exposures, and several lacked experimental details that would allow complete analysis of the reported results or independent conclusions based on an evaluation of the raw data. One of the most common weaknesses among these studies is a lack of a measure of internal dose, which is important for comparing the reported findings in published studies which used different routes of exposure (see *PK* regarding importance) and various protocol designs. Because even the highly relevant, regulatory guideline studies, which administered BPA in the diet (the most relevant exposure route), did not measure internal dose, FDA cannot compare the published studies using various routes of exposures and study protocols to the relevant guideline studies with regard to dose of BPA administered and reported finding. In addition, effects have been reported after direct s.c. injections of neonates with low doses of BPA. Because of the relatively low glucuronidation capacity of neonates, it is unclear if, in this subpopulation, results of experiments in which exposure to BPA using the s.c. route are relevant to oral exposure assessments. However, data currently available suggests that studies based on other routes of exposure, such as intraperitoneal or s.c. injections, may not be comparable to possible human exposures through food contact materials and will not produce realistic safety assessments for this route of exposure (oral). Additionally, it has been suggested that sulfotransferase activity is high in neonatal animals and may play an active role in BPA detoxification[70]. Additional experimental shortcomings identified in reviewing the current literature included selective use of only male or female offspring for testing, inadequate control procedures, lack of positive controls, absence of correlative morphochemical and functional endpoints, and failure to consider litter as the appropriate statistical unit.

[70] European Food Safety Authority, Toxicokinetics of Bisphenol A Scientific Opinion of the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food (AFC) (Question No EFSA-Q-2008-382) Adopted on 9 July 2008 (see footnote 18)

Another issue with the interpretation of these studies is the procedure of limiting exposure to only select portions (days or weeks) of development, for example during critical periods of nervous system development. Use of a limited exposure protocol may be useful in mechanistic studies of developmental neurotoxicity or, possibly, in the safety assessment of certain types of substances with expected human exposures occurring only during discrete periods of development. However, FDA considers that BPA exposure to a mother will be continuous, occurring throughout her entire life. Exposure to any offspring will therefore occur throughout gestation, during infancy (whether through breast milk, PC bottles, or infant formula) and on through later development and adulthood. In the presence of continuous exposure, changes or adaptations may occur that impact the potential toxicity of the substance. Accordingly, as is the case for BPA, FDA considers a more accurate assessment of a food additive's potential developmental neurotoxicity to be more relatable to human exposure when examined with exposure occurring throughout the period of development. This is not to imply that shorter term studies are not informative for hazard characterization; however, given conflicting results in short term versus long term studies, the relative exposure pattern to humans (chronic versus acute) and the quality of the study must be considered in the assessment .In addition, since select critical periods may occur at various times during development, the variety of exposure regimens used may have contributed to some of the inconsistent or conflicting findings reported in studies on developmental toxicity potential of BPA.

Acceleration of puberty in female rodents[71]

Three studies were judged to be useful in performing a safety assessment for BPA exposure through the use of food contact materials; these are the multigeneration studies by Tyl *et al.* (2002, 2008, both reviewed above) and Ema *et al.* (2001). All three studies were conducted under GLP conditions and examined only the day of vaginal opening as the endpoint for determination of the onset of puberty in the female. Tyl *et al.* (2008) used mice; the other studies used rats. The study by Ema *et al.* (2001) reported no effects on the day of vaginal opening at oral doses up to 200 µg/kg bw/day. Although the authors did not identify a NOAEL, it appears that 200 µg/kg bw/day would be a NOAEL for the timing of female puberty; this was the maximum dose used in this study. The study by Tyl *et al.* (2002) reported a delay in vaginal opening at 7500 ppm; this appeared to be due to a decrease in body weight. The study by Tyl *et al.* (2008) used CD-1 mice and reported no effect on the day of vaginal opening at any dose, including the maximum dose of 3500 ppm; however, as indicated in Table 9 of the published study absolute day of acquisition was not statistically significant at 3500 ppm. Day of acquisition was statistically significantly accelerated when adjusted by body weight on PND 21 for F1 (only animals measured). Again, no findings were reported at the lower doses. Due to the very thorough nature of these studies, FDA has a high level of confidence in their results. However, FDA acknowledges that it has been argued that the more appropriate endpoint for the determination of puberty in the female mouse is first estrus as indicated by the presence of cornified epithelial cells in the vaginal lavage rather than the day of vaginal opening[72]. In the

[71] FDA Memorandum – Acceptance of "Bisphenol A – Effects on onset of puberty in female and prostate and urinary tract in male rodents" reviewed by Drs. K. Barry Delclos (HFT-110) and Deborah K. Hansen (HFT-130) at FDA's National Center for Toxicological Research (NCTR). Twaroski/Gu/Food Master File 580. 05/27/2008.

[72] Safranski ,TJ, Lamberson, WR and Keisler, DH. (1993) Correlations among three measures of puberty in mice and relationships with estradiol concentration and ovulation *Biology of Reproduction,* 48: 669-673.

female rat, vaginal opening occurs at the same time as puberty (first estrus); however, these events are not as well coordinated in the mouse[73]. Although vaginal opening is not a direct measure of puberty as first estrus, it is an indicator of sexual maturation that is estrogen responsive[74]. The studies FDA considers of high confidence did not use vaginal lavage.

Of the studies that were reviewed, only 1 rat study and 3 mouse studies used the day of first estrus as their endpoint for the onset of puberty. The rat study (Tinwell *et al.*, 2002) reported no effect at doses up to 50 mg/kg bw/day which is the same dose at which Tyl *et al.* (2002) reported no adverse effects using the day of vaginal opening as the endpoint. Tinwell *et al.* (2002) did observe a delay in vaginal opening at 50 mg/kg bw/day in the Alderley Park strain of rats, but there was no effect on the day of first estrus at this dose. This suggests that the slight delay observed in the day of vaginal opening had no consequence on the subsequent attainment of estrus. Two mouse studies (Ryan and Vandenbergh, 2006 and Honma *et al.*, 2002) reportedly observed acceleration of the day of first estrus; however, it is noteworthy that the Honma *et al.* study used s.c. exposure and the reported effects were of questionable significance (~1 day). An additional study, Howdeshell *et al.*, 1999, reported a reduction in the number of days between vaginal opening and first estrus; however, neither the age of vaginal opening nor the age at first estrus were accelerated. Accordingly, this study, though interesting, did not report a potential acceleration in puberty.

As detailed in comments provided to the NTP peer review, several issues have been raised regarding the measurements used in these studies as indicators of puberty as well as their magnitude of response[75]. Based on FDA's review of the data, only Honma *et al.* (2002) evaluated the fertility of the animals demonstrating a slight acceleration in first estrus and found no effect on fertility. Although the multigeneration study by Tyl *et al.* (2008) did not evaluate the time of first estrus, they observed no adverse effects on fertility. Ashby *et al.* (1999) also did not observe a change in vaginal opening following treatment of CF-1 mice on GD 11–17 with 0, 2 or 20 μg BPA/kg bw/day[76]. Taken together, these results suggest that within the context of laboratory animal studies, limited evidence exists regarding an acceleration of puberty and none of the studies indicate an adverse affect on the ability of the mice to reproduce. The relationship of the increment of the responses observed in these studies to human effects as well as other possible adverse effects which may be associated with accelerated puberty in humans have not been correlated using rodent study data or examined in rodent studies, respectively, for BPA. In fact, a recent expert meeting formed to discuss environmental factors and puberty concluded changes in the onset or progression of puberty were an adverse outcome; however "*the increment*

[73] Nelson, JF, Karelus, K, Felicio, LS, and Johnson TE (1990) Genetic influences on the timing of puberty in mice. *Biol of Reproduction* 42: 649-655

[74] Cooper RL *et al*. In: Heindel JJ, Chapin RE (Eds.), Female Reproductive Toxicology. Methods Toxicol., Vol. 3B. Academic Press, pp. 45–56

[75] Written comments provided by Gray LE (former member of the CERHR BPA Expert Panel) on the NTP draft brief (05/23/2008, accessible at http://cerhr.niehs.nih.gov/chemicals/bisphenol/pubcomm/BPA(37)Gray23May2008best.pdf.

[76] FDA's review notes that the naive group and the positive control group (diethylstilbestrol, DES) both demonstrated a delay in vaginal opening. The responses of the naive and DES groups limit the utility of this study; it is only presented here for completeness of discussion of the varied data on this endpoint. FDA notes that the dose of DES used in this study (0.2 μg/kg bw/day) may have been too low for use as a positive control for reproductive effects.

of change in puberty timing considered biologically meaningful was not agreed on for either humans or an animal model." [77]

Only a very small number of studies evaluated blood levels of BPA and/or its metabolites. The lack of this information complicates the interpretation of conflicting study findings and is demonstrated in the inability to compare the findings with regard to the age of vaginal opening in the studies of Honma *et al*. (2002) and Ashby *et al*. (1999) in which the same doses of BPA were administered during the same gestation period. Honma *et al*. observed an acceleration of vaginal opening at 20 µg/kg bw/day whereas Ashby *et al*. observed no effect at the same dose; Honma *et al*. used the s.c. route while Ashby *et al*. used the oral route. There were other differences in experimental design that may have contributed to the different observations (differences in mouse strains used, in environmental exposure, and in numbers of animals examined), but the different routes of administration cannot be eliminated as a major contributor to the differing results.

Altered prostate and urinary tract development in males[78]

Guideline GLP studies using oral exposure (Tyl *et al*., 2002; Tyl *et al*., 2008, reviewed above) throughout the life span, including gestation and weaning, show no evidence of selective reproductive toxicity or effects on male development or prostate at doses at or below 750 ppm (approximate intake of 50 mg/kg bw/day) in the rat or 300 ppm (approximate intake of 50 mg/kg bw/day) in the mouse. Although there were no effects on the prostate at this dose, there was evidence of adverse effects on other male reproductive tissue endpoints, including decreased testis weight and delays in preputial separation and testicular descent. As discussed in *Reproductive Toxicity*, the NOAEL for reproductive and offspring toxicity was 50 mg/kg bw/day. A third such study (rat two generation reproductive study with Sprague-Dawley rats, Ema, *et al*., 2001), likewise found no effect on prostate weight or histology at doses up to 200 µg/kg bw/day. These studies clearly contain datasets that are most useful in a safety assessment because of their size, comprehensive endpoint evaluation, rigorous attention to the certification of doses, and control of experimental conditions. The study of Tyl *et al.* (2008) is particularly important because it utilizes a strain of mouse that has been reported by others to be sensitive to BPA under different treatment conditions. These studies indicate that perinatal BPA exposure does not adversely affect prostate weight or histology at doses of 0.2 – 50 mg/kg bw/day. Timms *et al.* report a decrease in the diameter of the urethra near the bladder neck following oral exposure at 10 µg/kg bw/day; this finding has not been assessed in other studies and associated findings in the kidney which may arise from severe constriction have not been reported at low doses in other studies. It is noted that prostate weight and histology are somewhat crude but validated endpoints. Functional endpoints, such as those examined in some of the smaller studies might uncover more subtle effects of BPA exposure and would need to be assessed for their long term consequences and relevance to human toxicity prior to utilization in a safety assessment.

[77] As discussed in Euling SY, Selevan SG, Pescovitz OH, and Skakkebaek NE. (2008) Role of Environmental Factors in the Timing of Puberty. *Pediatrics* 121;S167-S171.

[78] FDA Memorandum – Acceptance of "Bisphenol A – Effects on onset of puberty in female and prostate and urinary tract in male rodents"… (see footnote 71).

There are conflicting results on the effects of BPA on the mouse prostate after oral dosing of dams during gestation only. Some studies report effects at doses between 2 and 50 µg/kg bw/day, while others show no effects at these doses using reportedly similar conditions or even at much higher doses. Similar conflicting results have been observed in direct dosing experiments. For example, the effects observed by Nagel *et al.* (1997) and vom Saal *et al.* (1998) at 2 µg BPA/kg bw/day were not repeatable in a GLP study with doses of 0.2 – 200 µg/kg bw/day, although effects of 0.2 µg/kg bw/day diethylstilbestrol (DES) were also not observed in this study[79]. As discussed in these publications and in Appendix 2, the use of this low a dose of DES as a positive control for this endpoint is questionable. Additionally, attempts to replicate the Nagel *et al.* (1997) study with regard to both BPA and DES also resulted in negative results as reported by Ashby *et al.* (1999). As has been concluded by other review groups, there is no clear reason why the results of these studies differ, although the effects of environmental factors, including background diet estrogenic activity, animal strain and/or genetic background remain as possible contributory factors.

Smaller studies with reported findings often used non-traditional dose routes and endpoints. The debate surrounding the relevance of these findings persists since several of the endpoints reported to be affected in these studies would not be readily detectable in standard toxicology studies utilizing organ weights and histopathology on hematoxylin and eosin sections, such as the GLP studies conducted by Tyl *et al.* (2002, 2008). This is particularly true for effects on male endpoints, since a variety of non-traditional endpoints have been reported to be adversely impacted by BPA; however, the relevance of these findings to safety assessment is unresolved. Ho *et al.*, 2006 and Ogura *et al.*, 2007 touch on the issue of effects in the prostate that would not be detected in standard assays. As discussed under *Carcinogenicity*, several of the available prostate studies focus on the sensitization to later hormonal stimulation rather than overt toxicity to the prostate, with only subtle treatment-related changes in control of gene expression evident prior to hormonal challenge.

Developmental Neurotoxicity[80]

Three recently released assessments of BPA note concerns for developmental exposure and neural and behavioral effects of BPA. In 2004, the Society of the Plastic Industry (SPI) submitted to FDA a review of the neurobehavioral effects of BPA, "*Exponent: Literature Review of Neurobehavioral Effects of Bisphenol A*". FDA has reviewed and audited the review submitted by SPI and performed an updated review for neurotoxicity as a whole.

Most of the studies considered by FDA used the oral route of exposure (gavage, micropipette, diet, water), which is the most relevant route of exposure to humans, in considering food contact

[79] FDA memorandum – Sprando/Biddle, Review of MPI report on bisphenol A, 02/04/1999, cover memo (Twaroski/Food Master File 580, 01/22/2008). Published as Cagen SZ, Waechter JM, Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE, and Harris LR. (1999) Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A, *Tox Sci* 50: 36-44.

[80] FDA Review Memorandum - FDA Memorandum - Acceptance of updated reviews of the developmental neurotoxicity potential performed by Oak Ridge National Laboratory (ORNL, FDA Interagency Agreement #224-00-2615, Task #2007-20) and by Drs. Sherry A. Ferguson and Merle G. Paule at FDA's National Center for Toxicological Research Food Master File 580. 05/28/2008.

applications. Several non-oral studies injected BPA into the experimental animals by a variety of routes (s.c., intracisternal, and intracerebral). A variety of exposure regimens were used in which animals were given BPA for various intervals during gestation, lactation and/or after weaning. Others exposed dams to BPA throughout gestation and/or lactation; the non-oral studies used exposure durations of 1 to several days during select periods of gestation or lactation. Studies in which intracisternal or intracerebral dosing was used are considered unrelated to intact organism exposure and were not included in the overall evaluation.

The varied treatment-related findings in a majority of the reviewed studies collectively appear to suggest that developmental BPA exposure in rodents may have the potential to alter brain development and behavior. However, in view of the limitations in study design and study conditions that confound the interpretability of the study findings, without appropriate confirmation of these findings using well-designed experimental protocols and/or clarification of their biological significance, the utility and relevance of the study findings to an assessment of the safety of BPA from food contact uses is unknown. Until these disparate experimental findings are examined in well-designed safety assessment studies using appropriate biomarkers of effect, FDA has concluded that the reviewed studies are inadequate for use in supporting a safety assessment determination or regulatory decision for BPA.

Among the findings reported in these studies, a number of behaviors were identified for which there existed little or no clear or consistent credible evidence of significant effects of BPA treatment in juvenile or adult experimental offspring. These included ontogeny of sensory/motor behaviors and reflexes, self-grooming, open-field defecation scores, social play/non-social behaviors, aggression, stress/anxiety, and maternal behavior. Behavioral measures of learning and memory were found to show no consistent reliable evidence of adverse effects in experimental offspring, although schedule-controlled operant behavior was reported as being improved in rat offspring. There was no consistent evidence that BPA adversely affects sexual behavior in rodents. There were equivocal findings of BPA-related changes in sexually dimorphic behaviors. However, these particular findings are difficult to interpret with regard to potential human effects since rodent hormonal sexual differentiation is primarily controlled by estrogens whereas these pathways appear to be regulated by androgens in primates[81].

A number of studies reviewed (see *Exponent* and Appendix 1) reported findings that collectively appear to suggest several general types of effects that might be attributable to developmental exposure to BPA: (1) the possible effects on morphochemical development of brain and sexual differentiation are suggested by reported findings of altered patterns of neuronal differentiation and migration in neocortical and thalamocortical connections, sex-dependent changes in the number of neurons in the locus coeruleus, and altered distribution of neurons with ERs or tyrosine hydroxylase immunoreactivity in sexually-dimorphic regions of the brain in offspring of BPA treated dams; (2) altered endocrine function in offspring of BPA exposed dams is suggested by reports of decreased testosterone levels in male offspring, altered thyroxin levels in postnatal pups, and conflicting reports of changes in expression of RC3/neurogranin mRNA (a thyroxine responsive gene), retinoid receptor levels and steroid hormone receptor coactivator-1 mRNA;

[81] Reviewed in Wilson, C.A. and Davies, D.C. (2007) The control of sexual differentiation of the reproductive system and brain. *Reproduction* 133:331-359.

and (3) the possibility that developmental exposure to BPA may modulate the development of monoaminergic neural pathways is suggested by reported findings of significant changes in the behavioral responses of adult offspring to challenge with dopaminergic/noradrenergic pharmacologic agents (amphetamine, tranylcypromine and methamphetamine) and a series of immunohistochemical and biochemical studies of the effects of BPA on developmental distribution of tyrosine hydroxylase neurons, dopamine activation of G-related proteins, neurotransmitter levels, and the expression of brain dopamine receptor and dopamine transporter mRNA. Additionally, non-estrogen related pathways, such as the mRNA for the arylhydrocarbon receptor (AhR) and its associated proteins have also been shown to be altered in the mouse brain. However, in view of the caveats regarding limitations in experimental design and the questionable confidence in the data and their interpretability, it is premature to make firm conclusions about the utility and significance of these findings without appropriate confirmation of the findings using well-designed experimental protocols and/or clarification of their biological relevance.

Conclusions

BPA exposure in humans through food contact applications may occur through adult use of food contact articles or infant exposure through maternal transfer, the use of ready made (liquid) infant formula or the use of PC plastic bottles. Discussion and investigation about whether BPA causes adverse reproductive and developmental effects has been ongoing since the discovery that BPA was weakly estrogenic. As detailed in the NTP CERHR expert panel report, a large volume of information has been generated with mixed results regarding potential low dose effects of BPA. A goal of this Task Force was to examine BPA data to determine if the safety standard for food additives was still met with regard to the continued use of BPA. The safety standard is defined in 21 CFR§170.3(i): *Safe or safety means that there is reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use.* This definition goes on to state that complete certainty of absolute harmlessness is scientifically impossible to establish.

FDA's approach was to review the PK of BPA to assist in determining the most appropriate animal model for human safety assessment; to examine robust studies conducted using regulatory protocols which incorporated low doses; and to examine the literature regarding the concerns brought forth in the recent NTP CERHR and NTP draft Brief reviews of BPA. As adult toxicity to BPA from food contact applications is not a concern at low doses, the Task Force has focused on developmental exposures.

Assumptions

Several assumptions are considered in making conclusions regarding the data on BPA as it relates to human toxicity in evaluating the safety of exposure to BPA as a result of the use of food contact materials. These assumptions and the accompanying uncertainties and limitations surrounding them are:

- *The rodent animal model is appropriate for assessing the safety of BPA in humans (primates).*

In comparison to primates, which eliminate the main metabolite of BPA (estrogenically inactive BPAG) through urine, rodent BPAG elimination is confounded by the fact that it is routed primarily into bile and consequently, enters the intestines, where bacterial glucuronidases hydrolyze BPAG to re-form free, active BPA, allowing BPA to be reabsorbed and re-circulated. Accordingly, given a defined dose, rodents will have a prolonged exposure to free, estrogenically active BPA as compared to primates. In addition, PK analysis indicates that the metabolic profile may be unique in the mouse and that different species/strains of rodents have varying sensitivities to estrogens that are likely endpoint specific. Additionally, hormonal control of sexually dimorphic brain development in higher organisms is more dependent on androgens as opposed to estrogens. Data concerning the toxicity of BPA have been primarily limited to the rodent animal model. Thus the impact of using the rodent model likely leads to an overestimate of effects in humans.

- *Studies using oral exposure are the most relevant studies to the weight of evidence safety assessment of BPA from food contact uses. Furthermore, studies using dietary exposure are the most relevant for safety assessment of a food contact substance because human exposure will occur through migration of the substance to food.*
  The first-pass metabolism of BPA is considerable and results in inactive BPAG. Very little data have been generated combining biological measurements and internal dosimetry, thereby limiting the ability to compare non-orally derived BPA data to human safety assessments for oral exposure.
- *Regulatory guidance studies such as Redbook 2000 reproductive and developmental protocols adequately model human infant BPA exposure.*
  PK data indicates that embryonic/neonatal animals lack the adult capacity to conjugate BPA. Maternal exposure to BPA results in embryonic/neonatal animals receiving BPA via placental transfer, milk or excreta either through the direct transfer of BPA or hydrolysis of transferred BPAG (the major form in milk) to BPA. Exposure of infants to BPA directly, in the absence of maternal transfer or excretion, such as occurs through bottle feeding and/or infant formula feeding, does not occur in animal models until the animals begin to feed. Guideline studies used a large range of doses; however, internal dose measurements were not made in the neonatal animals in these studies (this is not a Redbook 2000 recommendation for this type of study).
- *The development of the nervous system and its responses to exogenous hormonally active compounds is comparable (model-able) across species such as rodents and primates.*
  Many of the endpoints examined regarding observed neural changes following BPA treatment are of unclear relevance to human adverse effects. FDA considers appropriately designed neurodevelopment studies in laboratory species informative in assessing the safety of products. Toxicity studies (systemic, reproductive toxicity, developmental toxicity, neurotoxicity) on BPA using non-human primates are currently unavailable.
- *BPA acts mainly as an estrogenic compound.*
  It is known that steroid receptors are susceptible to cross-talk that may have indirect effects on ligand bound receptors; alternatively the data reviewed also indicate the potential for other pathways to be involved in BPA's mode of action. It is known that BPA has a much lower affinity for ERα and ERβ than estrogen[82] and that BPA is rapidly metabolized to BPAG, an estrogenically inactive compound. Accordingly, some of the low dose effects reported are at

[82] Kuiper *et al.*; Summarized in the CERHR report (see footnote 4)

levels for which endogenous estrogen or dietary phytoestrogens may likely inhibit binding of BPA to the ERs (see *Introduction*). FDA has not modeled BPA's potential binding to ERs in the presence of endogenous estrogen to analyze this endpoint in combination with PK data for determination of the internal dose and corresponding external dietary concentration of BPA necessary to compete with and bind to ERs in the presence of endogenous estrogen.

- *Exposure to BPA as a food additive impurity will be continuous throughout life.*
  Many of the mechanistic studies considered in this assessment administered BPA at various critical periods. Although critical developmental periods exist, such "windows" of exposure to BPA are not expected to occur in the human population as exposure is continuous. For this reason, studies which use multiple generations, which include continuous exposure throughout mating, gestation and development are most relatable to a safety assessment for BPA. Conversely, it can be difficult to rely on findings from laboratory animal studies that use discrete periods of exposure to predict potential human health effects when exposure is continuous/lifetime. However, FDA considers these short term studies to be informative for potential hazard assessment and informative for endpoints which should be accessed in multigenerational studies.
- *Exposure to BPA as a food additive impurity to an individual infant will occur from all potential sources of food contact materials.*
  FDA's estimate of exposure to infants 0-2 months of age assumes that the feeding of infants always occurs with the use of thermally sterilized PC bottles filled with ready-made liquid formula from cans coated with BPA-based enamels. If PC bottles are not thermally sterilized, if powdered formula is used, or if liquid formula is obtained from non-BPA coated cans, exposures to BPA will be considerably lower. However, FDA uses this maximum CEDI in the overall assessment; therefore, maintaining the assessment's conservativeness. It is clear from the information detailed in Table 1 that infant formula consumption and BPA exposure decrease with age for this subpopulation; however, FDA has considered this maximum exposure for the full exposure period.

Sufficient data are lacking to eliminate the uncertainties surrounding the assumptions that have been used in reaching conclusions on BPA's safety for use in food contact materials. However, FDA notes that there are always uncertainties associated with safety decisions; such uncertainties are accounted for in safety assessments through the application of uncertainty factors in a margin of safety analysis.

Margins of Safety

Based on FDA's review of the data, the NOAELs from studies suitable for safety assessment are 5 mg/kg bw/day for systemic toxicity and 50 mg/kg bw/day for reproductive and offspring toxicity. FDA uses the term acceptable daily intake (ADI) to define the estimated maximum amount of a food additive to which individuals in a population may be exposed daily over their lifetimes without an appreciable health risk with respect to the endpoint from which the NOAEL is calculated. Since BPA is an impurity and not a food additive, FDA does not consider the use of the term ADI appropriate in this case; instead FDA considers the use of a margin of safety approach appropriate for evaluating the safety of BPA. The margin of safety (MOS) is the dose at which a NOAEL in animals was defined divided by the dose to which humans will be exposed (the CEDI). The MOS is compared to the uncertainty factors (UF) typically used for the

associated endpoint in deeming if the substance is safe for the expected exposure. A MOS higher than the relevant UF indicates that the margin of safety is "adequate". CFSAN's typical safety/uncertainty factors are 10 for intraspecies variability and 10 for interspecies variability for reproductive or developmental effects that are reversible (10x10 for a total of 100); for reproductive or developmental effects that are considered severe or irreversible, an additional factor of 10 is used (10x10x10 for a total of 1,000)[83]; and for systemic toxicity in which exposure is less than chronic, an additional factor of 10 is used to extrapolate from subchronic to chronic exposure (10x10x10 for a total of 1,000)[84]. The MOS for BPA with regard to systemic, reproductive and offspring toxicity in comparison to infant and adult CEDIs for BPA are shown in Table 3.

Table 3: Margins of Safety for BPA[85]

Endpoint	NOAEL	Maximum infant CEDI (♀, 1-2 months)	Infant MOS	Adult CEDI	Adult MOS	Typical Total UF
Systemic*	5 mg/kg bw/d	2.42 µg/kg bw/day	2,066	0.185 µg/kg bw/day	27,027	1,000
Reproductive	50 mg/kg bw/d	2.42 µg/kg bw/day	20,661	0.185 µg/kg bw/day	270,270	1,000
Offspring**	50 mg/kg bw/d	2.42 µg/kg bw/day	20,661	0.185 µg/kg bw/day	270,270	100

*Based on the lowest NOAELs for systemic toxicity which were observed in the RTI studies (Tyl *et al.* 2002 and 2008) for which liver and body weight effects were observed in animals administered BPA in utero and ~ 3 months (subchronic duration).
**Effects observed at the LOAELs in these studies (RTI, Tyl *et al.* 2002 and 2008; Tabulated in Appendix 1) for offspring were not considered severe or irreversible. "Offspring" toxicity in these studies is referring to the toxic effects observed in offspring during perinatal (around birth) and postnatal (after birth) period of time.

The MOS calculated for BPA are based on the validated guidance studies, which are not designed to analyze molecular level changes, but which are designed based on internationally recognized endpoints in toxicology. FDA considers that the use of a typical UF as opposed to a modified UF is sufficiently protective and in fact conservative. Sufficient information is available on BPA or the identified findings of the studies to indicate that lower UFs may be appropriate for use in a safety assessment of BPA[86]. FDA has used unmodified, typical study type UFs and considers them conservative based on the large body of knowledge for BPA and the findings observed in the pivotal studies.

Conclusions Regarding Specialized Endpoints

[83] Collins TFX, Sprando RL, Shackelford ME, Gruber MF, and Morse DE. (2006) Principles of Risk Assessment – FDA Perspective. In Developmental and Reproductive Toxicology- A Practical Approach, Taylor & Francis (Ed. Ronald D. Hood). p. 877-909.
[84] Twaroski ML, Batarseh LI, and Bailey AB. The Regulation of Food Contact Substances in the United States. Chemical Migration and Food Contact Materials. Woodhead Publishing (2007) Barnes, Sinclair, and Watson (Eds.).
[85] MOS is used for BPA; however, for clarity, if an 'ADI' were calculated for BPA for systemic effects it would be 5 µg/kg bw/day. Since the 'ADI' is greater than the CEDI for infants, this level of exposure is considered safe.
[86] Discussed in Updated European Risk Assessment Report 4,4'-Isopropylidenediphenol (Bisphenol-A) (see footnote 15)

Several recently released BPA assessments suggest a concern for BPA at levels of current exposure for a select group of endpoints. Based on the NTP's Board of Scientific Counselor's (BSC) review of NTP's draft Brief[87] and EC's draft screening assessment[88], concerns have been raised for developmental exposure to BPA at the current consumer levels with regard to the prostate gland and neural and behavioral effects. Some of these endpoints had not been previously addressed in guideline studies, such as those that are behavioral and brain related. FDA has considered each of these endpoints, as well as the concerns for developmental exposure with regard to the mammary gland and with regard to early onset of puberty in females, which were included as "some concern" in the original NTP draft Brief but recommended to be reduced to "minimal concern" by NTP's BSC. FDA has considered the endpoints of concern in the context of the available data and what is known about the PK of BPA as detailed above. It is noted that many of the studies for which recent assessments are based are mechanism-driven studies which do not readily lend themselves to safety assessments, but are informative with regard to mode of action analysis. Furthermore, as cited in Appendix 2, numerous experimental design flaws exist in these studies such that confidence in them for decision making purposes is limited.

- Carcinogenesis: A concern for predisposition to carcinogenicity of the mammary and prostate glands has been suggested. The CFSAN CAC does not concur that the data reported in the literature are adequate to draw any conclusions based on the nature of the endpoints examined, limitations in study designs, and the quality of the data. The available bioassay data, conducted in mice and rats by NTP, does not indicate a concern for this endpoint; however, the lack of an *in utero* exposure period in the NTP study is a limitation.

- Male reproductive tract and early onset of puberty in females: Effects on the prostate/male reproductive tract have been reported for doses as low as 2 µg/kg bw/day; however, these findings have not been confirmed in GLP studies using low doses. The effects on puberty have centered on visual examination versus cellular examination for day of first estrus. Of the studies reviewed, only the mouse studies reported a decrease in the days to first estrus; however, at least one of those was of limited magnitude and used s.c. as the route of exposure. It is noteworthy that none of the studies reviewed reported effects on fertility at low doses and many of the studies suffered from serious protocol limitations that decrease confidence in their results. These data suggest that fertility is not affected at these doses. Until effects on puberty are repeated in an appropriately controlled study, FDA considers the current data of limited use for a safety assessment.

- Neurotoxicity: Many of the studies reviewed appear to suggest that developmental BPA treatment can cause alterations in brain development and behavior; however, the limitations noted for individual studies ranged from mild to severe. The majority of the studies appeared focused on mechanism testing, rather than safety assessment, and many of the study authors did not clearly define the criteria used in the analysis and had a tendency to inappropriately

[87] Actions on the Draft NTP Brief on Bisphenol A by the NTP Board of Scientific Counselors (BSC) (see footnote 47). The five levels of concern used by NTP are from highest to lowest: serious concern, concern, some concern, minimal concern, and negligible concern. These definitions of these levels are not defined by NTP.
[88] Government of Canada, Environment Canada draft screening assessment and risk management documents (see footnote 14)

anthropomorphize behaviors or make exaggerated conclusions regarding the relevance of the results shown. Additionally, many of the studies employed various exposure periods, conditions that would not be expected to occur in human exposure scenarios. The endpoints examined in these studies (behavioral changes related to stress, pharmacological challenges and sexual dimorphism) represent an emerging area in developmental neurotoxicity for which validated protocols are currently unavailable. Major limitations of many of the studies reviewed in this area included a lack of concurrent examination of endpoints used for validating findings (histomorphologic evaluations, hormonal analyses, or neurochemical assessments with which to correlate the treatment-related behavioral effects of perinatal BPA exposure and vice versa) or examining only one sex. In rats dosed orally during development, effects were reported at doses as low as 2.4 µg/kg bw/day (Akingbemi et al 2004; dosed from PND 21 – 35, decreased serum testosterone and luteinizing hormone at PND 35 (no effect at higher doses) and decreased estradiol at 2.4 and $1x10^5$ µg/kg bw/day (no effect at $2x10^5$ µg/kg bw/day)). These data suggest findings at relevant doses; however, species/strain differences appear to exist, the dosing regimen utilized is not indicative of the human exposure scenario, and the reporting limitations (lack of experimental details or raw data allowing for critical or independent analysis) of the studies inhibit their use in regulatory decision making. Studies demonstrating BPA-related changes at the molecular level with regard to receptor distribution are interesting from an investigational point of view, but do not readily lend themselves to regulatory decision making. These data collectively suggest that more research, using validated studies with feeding protocols modeling human exposure are necessary prior to establishing a NOAEL for this endpoint for use in regulatory safety assessments.

Overall Conclusion

In conclusion, the results of FDA's assessment indicate that the data reviewed on endpoints highlighted as of potential concern in recent reports, such as developmental effects on the prostate gland and developmental neural and behavioral toxicity, are insufficient to provide a basis to alter the NOAEL used to calculate the margin of safety. FDA's lowest calculated margins of safety are approximately 2,000 and 27,000 for infants and adults, respectively. FDA concludes that an adequate margin of safety exists for BPA at current levels of exposure from food contact uses, for infants and adults.

It should be noted, however, that this conclusion is based on the articulated assumptions and limitations of the studies examined. This assessment does not represent a comprehensive review of BPA, but represents a full examination of data considered pivotal to the relevant exposure levels associated with food contact substances.

FDA is proposing a tiered testing strategy in order to decrease the uncertainties surrounding this assessment of BPA exposure from the use of food contact materials. It is important that future studies be conducted based on accepted/validated protocols with appropriate replicates and endpoints for examination.

Recommendations

As described in this document, the utility or relevance of a portion of the current body of data on BPA to human safety assessment for food contact substances has not been established. The findings associated with these data have not been replicated in well-designed safety assessments and/or these data are characterized by inconsistencies and inadequacies which limit the interpretations of the findings. In some studies, these deficiencies are compounded by the lack of inclusion of multiple doses or the examination of unvalidated endpoints/multiple endpoints. Some uncertainties that arise from these data may be reduced by future testing. In addition, future testing may provide FDA with additional information that could enhance the overall understanding of the effects of BPA and provide information useful in future safety assessments of BPA exposure resulting from all FDA regulated products.

Our understanding of exposure to BPA in both laboratory animals and humans must be improved prior to the initiation of additional toxicology studies. PK studies of BPA conducted in mice, rats, monkeys and humans all indicate rapid intestinal absorption, and very rapid conjugation of BPA with UDP-glucuronic acid, forming BPAG. BPAG formation is followed by a slower process of elimination of BPAG. In primates BPAG elimination is relatively rapid ($t_{1/2}$ = 3-4 hr) and primarily to the urine. Elimination of BPAG in rodents is complicated by the fact that it is routed primarily into the bile, rather than the urine and consequently enters the intestines, where bacterial glucuronidases hydrolyze BPAG to re-form free BPA; thereby free, active BPA can be re-absorbed. The extent of re-absorption of BPA from rat intestines may be less than 50 %, but is sufficiently large to confound the kinetic measurements in rodents.

Accordingly, the following tiered testing approach is recommended based on short term analysis (*Tier 1*) which would determine if additional toxicology studies are necessary (*Tier 2*), and the results of which would influence any toxicology studies' design and analysis.

Tier 1:

1) The utility of the current body of knowledge in the rodent model with regard to BPA human safety assessment would be improved by the development of PK and toxicity data measuring:

    a. internal dose with respect to multiple routes of administration (oral, s.c. and intravenous) and multiple animal models (adult, pregnant and neonatal rodents) which includes organ concentrations of free and conjugated BPA as well as full kinetic analysis;
    b. an evaluation of the concentration of unconjugated BPA accumulating in neonatal animals prior to their attainment of adult UDP-GT activity; and
    c. concurrent evaluations of neural and behavioral developmental endpoints in laboratory animals.

2) FDA's safety assessment would be improved if biomonitoring data were used to determine the internal concentrations of free BPA in human fetuses, infants, and adults by:

a. working with the CDC's NHANES program or other partners to develop biomonitoring analytical techniques for measuring and obtaining data on free BPA levels in adults and the most critical population (< 6 years of age); and
b. using PK animal data developed in the studies outlined in 1), above, and human physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling that account for differences in excretion patterns to estimate free BPA concentrations based on FDA's exposure estimates.

3) FDA's estimates of current exposure to BPA in infants may be improved by updating information or developing data with regard to:

a. the consumption of different types of infant formula and current packaging practices; and
b. an updated analysis of packaged infant formula and PC bottles using methods with improved limits of detection in food matrices.

Tier 2:

4) FDA's safety assessment may be improved if data were available in the non-human primate model. Based on PK analysis, the non-human primate model may be more relevant in evaluating the potential human toxicity of BPA. Such a model should consider:

- multiple routes of administration which take into consideration all potential exposures from FDA regulated products (oral and intravenous);
- adult, pregnant, and neonatal animals;
- internal dose measurements;
- measurements of developmental onset of adult UDP-GT activity;
- measurements of the systemic equilibrium ratios of unconjugated BPA to BPAG as they relate to human exposure;
- continuous exposure through gestation (*in utero* exposure);
- the potential for oral exposure to neonatal animals from non-maternal transfer, such as occurs through bottle feeding (dietary feeding of BPA in neonatal animals would more closely model FDA's human exposure assumptions for infants 0-2 months of age); and
- the inclusion of an examination of neural and behavioral developmental endpoints.

Appendix 1: Summary Data Tables

Systemic Toxicity Summary Tables

GLP Study Data Previously Reviewed by FDA

Study Duration/Species/Route/Source (date)	BPA Dose	Dose: Effects	NOAEL/Comments
2-week/rat/inhalation/Dow Chemical U.S.A. (1985)	0, 10, 50 or 150 mg/m^3	150 mg/m^3: Decreased body weight gain (males), decreased abdominal fat 50, 150 mg/m^3: anterior nasal inflammation and/or epithelial hyperplasia (both sexes)	10 mg/m^3 (low confidence due to deficiencies noted in review)
90-day/dog/diet/International Research and Development Corporation (1976)	0, 1000, 3000, 9000 ppm	9000 ppm: increased liver weights	3000 ppm* (low confidence due to deficiencies noted in review)
13-week/rat/inhalation/Dow Chemical Co. (1988)	0, 10, 50 or 150 mg/m^3	≥ 10 mg/m^3: Decreased body weight 50, 150 mg/m^3: enlarged cecum, hemolyzed blood present in stomach, perineal and facial soiling, very slight goblet cell hyperplasia in the respiratory epithelium and nasal turbinates.	no NOAEL; LOAEL of 10 mg/m^3 (low confidence due to deficiencies noted in review)

National Toxicology Data (not reviewed by FDA)**

Study Duration/Species/Route/Source (date)	BPA Dose	Reported Dose: Effects	Reported NOAEL/Comments
90 day/F344 rat/dietary/NTP (1982)	0, 250, 500, 1000, 2000, 4000 ppm	≥ 1000 ppm (100 mg/kg bw/d): decreased body weight gain 250 ppm: hyaline masses in bladder lumen (males) All (except 250 ppm females): caecal enlargement	NOAEL of 250 ppm (25 mg/kg bw/d) in females; LOAEL of 250 ppm (25 mg/kg/d) in males
90 day/B6C3F$_1$ mice/diet/NTP (1982)	0, 5000, 10000, 15000, 20000, or 25000 ppm	≥ 15000 ppm (1950 mg/kg bw/d): reduced body weight gain (males) ≥ 5000 ppm (650 mg/kg bw/d): reduced body weight gain (females) ≥ 500 ppm (600 mg/kg bw/d) multinucleated giant hepatocytes with dose related increase in incidence and severity (males)	LOAEL: 5000 ppm (600 mg/kg bw/d in males)

* Due to lack of purity and concentration data, FDA did not estimate a dose – reported elsewhere [European-Union. Risk Assessment Report - 4,4'-isopropylidenediphenol (Bisphenol A). In; 2003] as 74 or 87 mg/kg/day in males and females, respectively.

**Doses estimated in EU Risk Assessment Report on BPA – Final Report, 2003 accessible at http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/bisphenolareport325.pdf

Reproductive Toxicity Studies

GLP Study Data Previously Reviewed by FDA

Source	Species/Route/Experimental Design	Endpoint/Dose/Effects			NOAEL/Comments
RTI International, Research Triangle Park, NC, (Study number 65C-09301.000.003/0209301.000.003); Title: Two-Generation Reproductive Toxicity Evaluation of Bisphenol A Administered in the Feed to CD-1® Swiss Mice Published as: Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Hentges SG, Waechter JM Jr (2008) Two-generation reproductive toxicity study of dietary bisphenol A (BPA) in CD-1 (Swiss) mice. *Tox Sci* 104(2):362-384. FDA Memorandum - Review of Two-Generation Reproductive Toxicity Evaluation of Bisphenol A Administered in the Feed to CD-1® Swiss Mice. Shackelford/Twaroski, 06/24/2007	Mice (CD-1®); via feed; 9 groups of 6 week old mice at doses of 0 (2 groups), 0.018, 0.18, 1.8, 30, 300, or 3500 ppm BPA (equivalent to intakes of 0, 0.003, 0.03, 0.3, 5, 50, or 600 mg/kg bw/day, respectively). 17β-estradiol was used as a positive control and was administered at 0.5 ppm (intake of 0.08 mg/kg bw/day) to a separate group. F0 animals were exposed for eight weeks prior to mating, during the mating period, through gestation, and during the three week lactation period. F1 offspring (28/sex/group) were exposed through premating, mating, gestation and lactation. F0 dams were necropsied after weaning occurred, F1 dams and F2 offspring were necropsied at the time of weaning F2 offspring. F0 and F1 males were necropsied at the end of the gestation of their respective F1 and F2 litters. For males of the F1 group, subchronic exposure (3 months) to BPA continued for one/litter from each dose group prior to necropsy.	Endpoint	Dose	Finding	Systemic: 30 ppm (5 mg/kg bw/day) Reproductive: 300 ppm (50 mg/kg bw/day) Offspring: 300 ppm (50 mg/kg bw/day).
		Reproductive	3500 ppm	F0 males: decreased epididymal sperm concentration; decreased paired epididymal weights (did not achieve statistical significance); F1 males: significantly reduced absolute paired epididymal weights; F0 and F1 females: significantly increased gestational length.	
		Offspring	3500 ppm	F1: reduced pup body weight (PND* 7 – 21); F1 and F2 weanlings: reduced absolute and relative spleen weights;	

Source	Species/Route/Experimental Design	Endpoint/Dose/Effects			NOAEL/Comments
				F1 and F2 male weanlings: increased incidence of undescended testes, seminiferous tubule hypoplasia, and decreased testes weight. F1 male offspring: delayed preputial separation. F1 females: absolute day of acquisition (as measured by vaginal patency) was not statistically significant; day of acquisition was statistically significant accelerated when adjusted by body weight on PND 21 for F1 (only animals measured).	
		Systemic	3500 ppm	F0 and F1 adults and retained F1	

Source	Species/Route/Experimental Design	Endpoint/Dose/Effects			NOAEL/Comments
				adult males: increased liver weights (absolute and relative), increased incidence in minimal to mild centrilobular hepatocyte hypertrophy, increased kidney weights (absolute and relative), increased minimal to mild nephropathy	
			300 ppm	Adult F0 males, retained F1 males and F1 females: increased incidence in minimal to mild centrilobular hepatocyte hypertrophy.	
		Other: 17β-estradiol	0.5 ppm	Reduced fertility index in Fl females; increased stillbirth index in FO and Fl females; reduced	

Source	Species/Route/Experimental Design	Endpoint/Dose/Effects			NOAEL/Comments
				livebirth index in FO and F 1 females; reduced litter sizes in FO and Fl females; increased gestational length in FO and Fl females; reduced anogenital distance in Fl/F2 males on PND 21 (but not on PND 0); delay in preputial separation in Fl males (parameter not measured in F2); decreased testes and epididymal weights in the Fl/F2 male weanlings; increased incidence of seminiferous tubule hypoplasia of the testes and undescended testes in Fl and	

Source	Species/Route/Experimental Design	Endpoint/Dose/Effects			NOAEL/Comments
				F2 weanling males exhibited; acceleration of puberty in Fl females (parameter not measured in F2); increased weights of the uterus plus cervix plus vagina in F0/Fl adults and F1/F2 weanlings; increased incidence (>90%) of vaginal epithelial keratinization and bilateral luminal dilatation of the uterine horns in the Fl and F2 weanling females.	
RTI International, Research Triangle Park, NC. (Study number 65C-07036-000); Three-Generation Reproductive Toxicity Evaluation of Bisphenol A in the Feed of CD® (Sprague-Dawley) Rats. Published as: Tyl RW, Myers CB,	CD®-SD rats; via feed: doses of 0, 0.015, 0.3, 4.5, 75, 750, or 7500 ppm BPA (equivalent to intakes of 0, 0.001, 0.02, 0.3, 5, 50, or 500 mg/kg bw/day, respectively). F0 animals were exposed for 10 weeks prior to mating, during the mating period, through gestation, and during the lactation period until weaning (PND 21). F1 litters were culled to 10 pups (equal sex ratio) at PND4. F1	Endpoint	Dose	Finding	Systemic: 75 ppm (5 mg/kg bw/day) Reproductive: 750 ppm (50 mg/kg bw/day) Offspring: 750 ppm (50 mg/kg bw/day).
		Reproductive	7500 ppm	All females: reduced absolute paired ovarian weights; F0, F1 and F2: reduced relative paired ovarian	

Source	Species/Route/Experimental Design	Endpoint/Dose/Effects			NOAEL/Comments
Marr MC, Thomas BF, Keimowitz AR, Brine DR, Veselica MM, Fail PA, Chang TY, Seely JC, Joiner RL, Butala JH, Dimond SS, Cagen SZ, Shiotsuka RN, Stropp GD, Waechter JM (2002) *Toxicol Sci.* Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. 68(1): 121-146. FDA Memorandum - Review of study entitled "Three-Generation Reproductive Toxicity Evaluation of Bisphenol A in the Feed of CD® (Sprague-Dawley) Rats". Gu/Twaroski, 07/18/2007	and F2 offspring (30/sex/group) were exposed through premating (13-15 weeks), mating, gestation and lactation. F0 males were sacrificed and necropsied after F1 delivery. F3 weanlings were sacrificed after approximately 10 week of continued dietary exposure.			weights; F0: increased paired ovarian primordial follicle counts; F1, F2, F3: reduction in number of implants, total and live pups per litter at birth; F1 males: reduction in epididymal sperm concentration; F3 males: decreased testicular homogenization-resistant spermatid head counts (DSP)	
			0.3 ppm	F3: reduction in number of implants, total and live pups per litter at birth.	
		Offspring	7500 ppm	F1, F2, and F3: decreased pub body weights per litter during lactation; F1, F2 and F3: delayed absolute	

Source	Species/Route/Experimental Design	Endpoint/Dose/Effects			NOAEL/Comments
				age of vaginal patency; F1, F2, F3: delayed absolute age at preputial separation**	
			75 ppm	F2,: decreased pup body weights per litter during lactation;	
			4.5 ppm	F2,: decreased pup body weights per litter during lactation; F2 females: anal genital distance increased*;	
			0.3 ppm	F2 females: anal genital distance increased*;	
			0.015 ppm	F2 females: anal genital distance increased*;	
		Systemic	7500 ppm	F0, F1, F2, and F3: reduced body weight and body weight gain; F0, F1 and F2 females: reduced body weight during gestation and lactation; All: Decreased terminal body	

Source	Species/Route/Experimental Design	Endpoint/Dose/Effects			NOAEL/Comments
				weights F1 and F2 females: increased slight to mild renal tubular degeneration and chronic hepatic inflammation; F0 males: chronic hepatic inflammation	
			750 ppm	F1 females: reduced body weights during lactation; F0 and F2 females: reduced body weights during gestation and lactation; F1 (all) and F2 (males): Decreased terminal body weights;	

*AGD findings were deemed as sporadic based on the lack of dose response and lack of finding in F3 females and; therefore, were not considered treatment related.

** When adjusted for body weights or for the body weights on SD 14, the age at PPS was delayed in F1 generation at 750 and 7500 ppm and F2 generation at 7500 ppm only .

Developmental Toxicity Tables

Studies on the acceleration of puberty in female rodents

(FDA Memorandum – Acceptance of "Bisphenol A – Effects on onset of puberty in female and prostate and urinary tract in male rodents" reviewed by Drs. K. Barry Delclos (HFT-110) and Deborah K. Hansen (HFT-130) at FDA's National Center for Toxicological Research (NCTR).. Twaroski/Gu/Food Master File 580. 05/27/2008.)

Authors	Species/Strain/Source	BPA Doses	Route	N	Exposure period	Results on Day of Vaginal Opening (dose)	Results on Day of First Estrus (dose)	Study Limitations
Howdeshell *et al.*, 1999	Mouse/CF-1/Charles Rive	2.4 µg/kg bw/day	Gavage	21	GD 11-17	↔	not analyzed**	SD, E, ID
Ryan & Vandenbergh, 2006	Mouse/ C57/Bl-6/Charles River	2 and 200 µg/kg bw/day	Gavage	4-7	GD 3-PND 21	Not Evaluated	↓ (200)	E, N, ED, ID
Honma *et al.*, 2002	Mouse/ ICR/Jcl/Not indicated	2 and 20 µg/kg bw/day	Sc injection	10	GD 11-17	↓ (20)	↓ (20)	E, R, ID
Ashby *et al.*, 1999	Mouse/CF-1/Charles River	2 and 20 µg/kg bw/day	Oral	7-8	GD 11-17	↔	Not Evaluated	+, N, ID
Markey *et al.*, 2003	Mouse/CD-1/Charles River	25 and 250 µg/kg bw/day	sc pump	6-10	GD 9-PND 4	↔	Not Evaluated	V, R, ID
Tyl *et al.*, 2008	Mouse/CD-1/Charles River	0.018, 0.18, 1.8, 30, 300 and 3500 ppm	In chow	28	Lifetime	↔*	Not Evaluated	ID
Durando *et al.*, 2007	Rat/Wistar-derived/ University colony (Santa Fe, Argentina)	25 µg/kg bw/day	sc pump	11-14	GD 8-23	↓	Not Evaluated	SD, R, ID
Tyl *et al.*, 2002	Rat/Sprague-Dawley/Charles River	0.15, .3, 4.5, 75, 750 and 7500 ppm	In chow	30	lifetime	↑ (7500)	Not Evaluated	ID, E
Tinwell *et al.*, 2002	Rat/Sprague-Dawley/Harlan and Rat/Alderley Park (Wistar-derived) /AstraZeneca	20, 100, and 50,000 µg/kg bw/day	Gavage	6-7	GD 6-21	↑ (50, AP rats)	↔	N, +, ID
Ema *et al.*, 2001	Rat/Sprague-	0.2, 2, 20 and	Gavage	25	lifetime	↔	Not	ID

Authors	Species/Strain/Source	BPA Doses	Route	N	Exposure period	Results on Day of Vaginal Opening (dose)	Results on Day of First Estrus (dose)	Study Limitations
	Dawley/Charles River (Japan)	200 µg/kg bw/day					Evaluated	
Yoshida *et al.*, 2004	Rat/Crj:Donryu/Charles River (Japan)	6 and 6000 µg/kg bw/day	Gavage	12-19	GD 2-PND 20	↔	Not Evaluated	ED
Kubo *et al.*, 2003	Rat/Wistar/Kyudo	30 and 300 µg/kg bw/day	Drinking water	5-6	GD 0-PND 21	↔	Not Evaluated	E, ED, ID, R, N
Murray *et al.*, 2007	Rat/Wistar-Furth/Harlan	2.5, 25, 250 and 1000 µg/kg bw/day	sc pump	?	GD 9-PND 1	↔	Not Evaluated	N, V, R, ID
Rubin *et al.*, 2001	Rat/Sprague-Dawley/Taconic Farms	100 and 1200 µg/kg bw/day	Drinking water	6	GD 6-PND 21	↔	Not Evaluated	ED, R, ID, N

↓ Indicates a significant acceleration in the age at vaginal opening or first estrus.
↑ Indicates a significant delay in the age at vaginal opening or first estrus.
↔ Indicates no change in the age at vaginal opening or first estrus.
Study Limitations Key: N = small number of animals; SD = single dose; ED = experimental design designation includes, but is not limited to: experimental design lacking in detail or flawed, not accounting for litter effects, dose not calculated by author, assessment not performed blind, lack of positive control (neuro studies), assessment does not include common concomitant endpoints of analysis for endpoint under investigation, statistical analysis reporting issues, lack of histochemistry; V = vehicle concerns; + = positive control performed erratically; E = environmental estrogens not accounted for; R = route of exposure limits interpretations (subcutaneous or drinking water); ID = lack of internal dose (blood levels)

* F1 females: absolute day of acquisition was not statistically significant; day of acquisition was statistically significantly accelerated when adjusted by body weight on PND 21 for F1 (only animals measured).
** reported a reduction in the number of days between vaginal opening and first estrus

Studies on altered prostate and urinary tract development in males

(FDA Memorandum – Acceptance of "Bisphenol A – Effects on onset of puberty in female and prostate and urinary tract in male rodents" reviewed by Drs. K. Barry Delclos (HFT-110) and Deborah K. Hansen (HFT-130) at FDA's National Center for Toxicological Research (NCTR).. Twaroski/Gu/Food Master File 580. 05/27/2008.)

Authors	Species/Strain/ Source	BPA Doses	Route	N	Exposure period	Observations (Prostate, urinary tract)*	Study Limitations
Timms *et al.*, 2005	Mouse/CD-1/Charles River	0, 10 μg/kg bw/day	Oral (fed by micropipette tip)	6 (5 for controls)	GD 14-19	↑ dorsolateral prostate duct volume and number ↓ diameter of urethra near bladder neck	SD, ID, E, N
Ho *et al.*, 2006	Rat/Sprague-Dawley/Zivic-Miller Labs	0, 10 μg/kg bw/day; Testosterone/estradiol (T/E) challenge at PND 90 in half of animals	s.c. injection	10	PND 1, 3, 5	↔ prostate weight ↑ high grade prostatic intraepithelial neoplasia, proliferative and apoptotic indices (only after T/E challenge) Altered gene methylation pattern	R, ID, SD
Ogura *et al.*, 2007 Experiment 3	Mouse/Balb/c/ CLEA(Japan)	0, 20 μg/kg bw/day	Oral (gavage)	3	GD 13-18	↑ CK10 staining in absence of morphological difference by H&E	N, ID, SD
Nagel *et al.*, 1997	Mouse/CF-1/Charles River	0, 2, and 20 μg/kg bw/day	Oral (fed by micropipette tip)	7 (11 controls: 5 unhandled, 6 vehicle-dosed)	GD 11- 17	↑ prostate weight at 6 months, 2 and 20 μg/kg/day	E, ID
Ashby *et al.*, 1999	Mouse/CF-1/Charles River	0, 2, and 20 μg/kg bw/day	Oral (fed by micropipette tip)	5-7	GD 11- 17	No effect on prostate weight	+, N, ID

Authors	Species/Strain/ Source	BPA Doses	Route	N	Exposure period	Observations (Prostate, urinary tract)*	Study Limitations
Kwon *et al.*, 2000	Rat/Sprague-Dawley/Charles River	0, 3.2.x10^3, 3.2x10^4, 3.2x10^5 µg/kg bw/day	Oral (gavage)	8	GD 11 – PND 20	At PND 180, no effect on prostate weight or histology of the ventral prostate (only lobe examined microscopically)	ID, ED, E
Ichihara *et al.*, 2003	Rat/Fisher 344/Charles River (Japan)	0, 50, 7.5x10^3, 3.0x10^4, 3.0x10^5 µg/kg bw/day Challenge animals from each group with carcinogen (DMAB) at 5 weeks age	Oral (gavage)	8 – 14 dams; 21 for DMAB challenge, 12 vehicle challenge	Daily throughout pregnancy and lactation	No effects on prostate in control or carcinogen-treated rats	E, ED, ID
Gupta, 2000 Experiment 1	Mouse/CD-1/Charles River	0, 50 µg/kg bw/day	Oral (administered in corn oil with 10% ethanol)	15	GD 16-18	↑ prostate weight at PND 3, 21, and 60 ↑ prostate size at PND 15 ↑ androgen receptor at PND 21 and 60	SD, ID, ED
Tyl *et al.*, 2002	Rat/Sprague-Dawley/Charles River	0, 0.015, 0.3, 4.5, 75, 750, 7500 ppm (Approximately 0, 20, 300, 5x10^3, 5x10^4, and 5x10^5 µg/kg bw/day)	Mixed in diet; 3 generation exposure	30 males, 30 females per dose group per generation	10 weeks prior to mating of F_0 through postnatal week 14 of the F_3 generation	↓ absolute prostate weight, all generations at 7500 ppm (500 mg/kg/day) ↔ prostate histology	ID, E
Tinwell *et al.*, 2002	Rat/Sprague-Dawley/Harlan and Rat/Alderly Park (Wistar-derived)/AstraZeneca)	0, 20, 50 µg/kg/day and 5x10^4 µg/kg bw/day	Oral (gavage)	6-7	GD 6- 21	No effects on prostate weight (Reduction in daily sperm production in AP rats only at 50 mg/kg)	N, +, ID, ED, E

Authors	Species/Strain/ Source	BPA Doses	Route	N	Exposure period	Observations (Prostate, urinary tract)*	Study Limitations
Tyl *et al.*, 2008	Mouse/CD-1/Charles River	0, 0.018, 0.18, 1.8, 30, 300, 3500 ppm (Approximately 0, 30, 300, $5x10^3$, $5x10^4$, and $5x10^5$ μg/kg bw/day)	Mixed in diet; 2 generation exposure	28 males, 28 females per dose group per generation	8 weeks prior to mating of F_0 through postnatal week 14 of the F_2 generation	No effects on prostate weight or histopathology (↓ testis weight, delayed preputial separation and testicular descent at 3500 ppm (500 mg/kg/day)	ID
Ramos *et al.*, 2001	Rat/Wistar-derived/University colony (Santa Fe, Argentina)	0, 25, and 250 μg/kg bw/day	s.c. (osmotic pump)	4	GD 8 to 23	All effects at 25 and 250 μg/kg/day ↔ proliferation as measured by BrdU ↑ fibroblast to smooth muscle cell ratio ↓ stromal androgen receptor and prostatic acid phosphatase	R, ID, E, V, N

↑, increase; ↓, decrease; ↔, no change. Significantly affected male reproductive endpoints outside the prostate and urinary tract are listed in parentheses.
Study Limitations Key: N = small number of animals; SD = single dose; ED = experimental design designation includes, but is not limited to: experimental design lacking in detail or flawed, not accounting for litter effects, dose not calculated by author, assessment not performed blind, lack of positive control (neuro studies), assessment does not include common concomitant endpoints of analysis for endpoint under investigation, statistical analysis reporting issues, lack of histochemistry; V = vehicle concerns; + = positive control performed erratically; E = environmental estrogens not accounted for; R = route of exposure limits interpretations (subcutaneous or drinking water); ID = lack of internal dose (blood levels)

Studies on Developmental Neurotoxicity Potential

(FDA Memorandum - Acceptance of updated reviews of the developmental neurotoxicity potential performed by Oak Ridge National Laboratory (ORNL, FDA Interagency Agreement #224-00-2615, Task #2007-20) and by Drs. Sherry A. Ferguson and Merle G. Paule at FDA's National Center for Toxicological Research Food Master File 580. 05/28/2008.)

Summary of *in vivo* studies with neurodevelopmental testing following direct oral treatment of developing animals (intact animals only)^

Authors	Species/Strain[a]	Route	Exposure Period	BPA Doses	E and NE[b]	Effects	Study Limitations^^
Della Seta *et al.*, 2005	Rat/Sprague-Dawley	Oral (pipette)	GD 1 – lactation	40 µg/kg bw/d	E - 40 µg/kg bw/d	Altered maternal behavior on PND 3/4 and 8/9	SD, E, ED
Della Seta *et al.*, 2006	Rat/Sprague-Dawley (males only)	Oral (pipette)	PNDs 23-30	40 µg/kg bw/d	E - 40 µg/kg bw/d	Changes in social, non-social, and sexual behavior @PND 45 & >90; ↓ testosterone levels @ PND 37 and 105	SD, ED, E
Ceccarelli *et al.*, 2007	Rat/ Sprague-Dawley	Oral (pipette)	PNDs 23-30	40 µg/kg bw/d	E - 40 µg/kg bw/d	↑ number of ERα labeled cells in brains of males and females; ↓ testosterone levels in males	SD, ED, E
Ishido *et al.*, 2007	Rat/ Wistar (males)	Oral (pipette)	PND 5 – 21	600 µg/pup (ca. 12-60 mg/kg/day)	E – 600 µg/pup	↑ motor activity; ↓ tyrosine hydroxylase immunoreactivity; altered gene expression	SD, N, ED, E
Patisaul *et al.*, *et al.*, 2006, 2007	Rat/Sprague-Dawley (males)	s.c.	PND 1 and 2	250 µg/12 hours ($5x10^4$ µg/kg)	E - 250 µg/12 hours ($5x10^4$ µg/kg)	PND 19 males - ↑ tyrosine hydroxylase in the anteroventral periventricular (AVPV) nucleus of the preoptic area in pups, reverse seen in females (↓).	R, SD, ED, E

Authors	**Species/Strain**[a]	**Route**	**Exposure Period**	**BPA Doses**	**E and NE**[b]	**Effects**	**Study Limitations^^**
Monje *et al.*, 2007	Rat/Wistar (females)	s.c.	PND 1-7; sacrificed PND 8 or 21	500 µg/kg, $2x10^4$ µg/kg	E - 500 µg/kg	Females Sac. PND 8 or 21 = ↑ ERα mRNA and protein in preoptic area; 20 mg/kg ERα mRNA - Females sac. PND 8 ↓; PND 21 ↑; Serum estradiol at PND21 ↔	R, ID, ED, E
Nagao *et al.*, 1999	Rat/Sprague-Dawley	s.c.	PND 1 – 5	300 µg/kg/day	NE – 300 µg/kg/day	12 weeks – ↔ in male sexual behavior or adult volume of the SDN-POA	SD, R, E, ED
Patisaul & Bateman 2008	Rat/Long Evans (males)	s.c.	PND 1 – 4	50 µg/kg	E – 50 µg/kg	↑anxiety-like behavior	SD, R, E, ED
Akingbemi *et al.*, 2004	Rat/Long Evans (males)	Gavage	PND 21 – 35	2.4 µg/kg bw/day	E – 2.4 µg/kg bw/day	↓ LHβ mRNA and ↑ ERβ mRNA in the pituitary	SD, E, ED
Akingbemi *et al.*, 2004	Rat/Long Evans (males)	Gavage	PND 21 – 35	2.4, 10, $1x10^5$ and $2x10^5$ µg/kg bw/day bisphenol A	E – 2.4 µg/kg bw/day	↓ serum T and LH at PND 35 (no effect at higher doses); ↓ E2 at 0.0024 -100 mg/kg bw/day (no effect at 200 mg/kg bw/day)	SD, E, ED
Akingbemi *et al.*, 2004	Rat/Long Evans (males)	Gavage	PND 21-90	2.4 µg/kg bw/day	E – 2.4 µg/kg bw/day	Serum T ↔, serum LH ↑	SD, E, ED

^Excludes data previously summarized in *"Exponent: Literature Review of Neurobehavioral Effects of Bisphenol A."*
PND (Postnatal day), GD (Gestation day), ↑, increase; ↓, decrease; ↔, no change
Study Limitations Key: N = small number of animals; SD = single dose; ED = experimental design designation includes, but is not limited to,: experimental design lacking in detail or flawed, not accounting for litter effects, dose not calculated by author, assessment not performed blind, lack of positive control (neuro studies), assessment does not include common concomitant endpoints of analysis for endpoint under investigation, statistical analysis reporting issues, lack of

histochemistry, evaluation of only one sex; V = vehicle concerns; + = positive control performed erratically; E = environmental estrogens not accounted for; R = route of exposure limits interpretations (subcutaneous or drinking water); ID = lack of internal dose (blood levels)
^^note that none of the studies under neuro measured an internal dose
[a]if only one sex examined for endpoints, sex stated (considered a study limitation); [b] Effective Dose (E) and Non-effective Dose (NE), as applicable

Summary of *in vivo* studies with maternal treatment followed by testing of offspring (intact animals only)^

Reference	Species/Strain[a]	Route	Exposure Period	BPA Doses	E & NE[b]	Effects	Study Limitations^^
Gioiosa *et al.*, 2007	Mouse/CD-1	Oral (pipette)	GD 11 – PND 8	10 µg/kg bw/d	E - 10 µg/kg bw/d	Elimination of sex-related behavioral differences	SD, ED, E
Laviola *et al.*, 2005	Mouse/CD-1	Oral (pipette)	GD 11 – 18	10 µg/kg bw/d	E - 10 µg/kg bw/d	Lack of conditioned response to amphetamine in females	SD, ED, E
Mizuo *et al.*, 2004	Mouse/ddY	Diet	mating to weaning	$4x10^2$, $1x10^5$, and $4x10^5$ µg/kg bw/day*	E - $1x10^5$ µg/kg bw/d NE - $4x10^2$ µg/kg bw/d	Enhanced reward effect and hyperlocomotion induced by morphine	ED, E
Narita *et al.*, 2006	Mouse/ddY (males)	Diet	mating to weaning	6, 60, $6x10^2$, $1x10^5$, and $4x10^5$ µg/kg bw/day**	E - 6 µg/kg/d	Potentiation of central dopamine receptor-dependent neurotransmission	N, ED, E
Narita *et al.*, 2007	Mouse/ddY (males)	Diet	GDs 0-7, 7-14, 14-20; or PNDs 0-20	$4x10^5$ µg/kg bw/day	E - $4x10^5$ µg/kg/day	Enhanced response to morphine from exposures GD 7-14 and PNDs 0-20	SD, ED, N, E
Ryan and Vandenbergh 2006*	Mouse/ C57/Bl6 mice	Oral (pipette)	GD 3 – PND 21	2 or 200 µg/kg bw/d	E – 200 µg/kg bw/d	↑ anxiety in females	N, ED, E
Nakamura *et al.*, 2006, 2007	Mouse/ ICR/Jc1	s.c.	GD 0 to GD10.5, GD12.5, GD14.5 or GD16.5	20 µg/kg/day	E – 20 µg/kg/day	Gene expression and cellular architecture changes in cortex	SD, R, N, ED, E
Rubin *et al.*, 2006	Mouse/CD-1	s.c. - osmotic minipump, 50% DMSO)	GD 8 – PNC16	0.025 and 0.25 µg/kg bw/day	E – 0.025 µg/kg bw/day	Sexual differences in open field activity, rearing , time spent in center, and time stopped were altered in 6-9 week animals; changes in tyrosine hydroxylase neurons in females in the anteroventral periventricular preoptic, but not the arcuate nucleus.	R, V
Miyagawa *et al.*, 2007	Mouse/ C57BL/6J	Diet	mating to weaning,	30 ng/g or 2 mg/g diet	E - 30 ng/g or 2 mg/g	Passive avoidance retention impaired; hippocampal choline	ED, E

Reference	Species/Strain[a]	Route	Exposure Period	BPA Doses	E & NE[b]	Effects	Study Limitations^^
	(males)				diet	acetyltransferase-like immunoreactivity ↓ in hippocampus; No effects on anxiety behavior or motor coordination	
Nishizawa *et al.*, 2003	Mouse/ICR	Oral	6.5 to 11.5, 13.5, 15.5, or 17.5 days PC	2 µg/kg/day	E – 2 µg/kg/day	Mixed results on the expression of retinoid receptors (retinoic acid receptor α and retinoid X receptor α) in the cerebrum and cerebella of mouse embyros	SD, E, ED
Kawai *et al.*, 2007	Mouse/ICR (males)	Oral (pipette)	GD 11-17	2 µg/kg/day	E - 2 µg/kg/day	Males @ 5weeks and 13 weeks - ERα and ERβ ↑; ↓ testosterone during puberty	SD, ED, E
Tadno *et al.*, 2007	Mouse/ddY	diet	mating to weaning	600 or $1.6x10^6$ µg/kg bw/day*	E – 600 µg/kg bw/ay	8-11 weeks: ↓ number of tyrosine hydroxylase-positive neurons in the substantia nigra in females; ↔ in Ca^{2+} binding proteins in the somatosensory cortex	ED, N, E
Nishizawa *et al.*, 2005a	Mouse/ICR	Oral	6.5 – 13.5 or 6.5 - 17.5 PC	0.02, 2, 200, or $2x10^4$ µg/kg bw/day	E - 0.02 µg/kg bw/day	14.5 & 18.5 PC: U shaped dose response for ↑ mRNA (retinoic acid, arylhydrocarbon receptors) in brain; retinoid X receptor mRNA only ↑ at 18.5	ED, E
Nishizawa *et al.*, 2005b	Mouse/ICR	Oral	6.5 – 13.5 or 6.5 - 17.5 PC	0.02, 2, 200, or $2x10^4$ µg/kg bw/day	E - 0.02 µg/kg bw/day	14.5 & 18.5 PC: U shaped dose response, brain mRNA ↑ for arylhydrocarbon receptor and related proteins	ED, E
Xu *et al.*, 2007	Rat/ Sprague-Dawley	drinking water	GD 11 – PND 21	20 and $1x10^4$ µg/kg bw/day	E – 20 µg/kg/d	↑ motor activity, ↓ learning/memory, changes in thyroid hormone levels in males; ↔ in thyroid hormone receptor α/β and RC3/neurogranin in male pups; ↑ steroid hormone receptor coactivator-1 in low dose males at PNDs 5 and 7	R, ED, E, only low dose males examined for mRNA changes
Fujimoto *et*	Rat/ Wistar	drinking	GD 13 –	15 µg/kg bw/d	E - 15	Impaired sexual differentiation of	SD, N, ED, E

Reference	Species/Strain[a]	Route	Exposure Period	BPA Doses	E & NE[b]	Effects	Study Limitations^^
al., 2006		water	parturition		µg/kg bw/d	rearing and struggling behaviors in males	
Porrini *et al.*, 2005	Rat/ Sprague-Dawley (females)	Oral (pipette)	M – PND21	40 µg/kg bw/d	E - 40 µg/kg bw/d	↑ exploration, ↓ play and social behaviors in females	SD, ED, E
Negishi *et al.*, 2004	Rat/F344/N (males)	gavage	GD 3 – PND 20	100 µg/kg bw/d	E – 100 µg/kg bw/d	Altered perception of fear-provoking stimuli and monoaminergic neural pathways in males (only tested)	SD, ED, E
Kwon *et al.*, 2000	Rat/ Sprague-Dawley	Gavage	GD11 – PND20	$3.2x10^3$, $3.2x10^4$, $3.2x10^5$ µg/kg bw/day	NE - $3.2x10^5$ µg/kg bw/day	↔ at doses of 3.2, 32, or 320 mg/kg on volume of SDN-POA (PND 10, females only); ↔ on lardosis	E, ED, +
Facciolo *et al.*, 2002	Rat/ Sprague-Dawley	Oral	premating, GD, PND 10 or PND 23	40, 400 µg/kg bw/day	E – 400 µg/kg bw/day	↓ sst_2 receptors in the limbic region; mixed results regarding interactions of sst_2 with α-containing γ-aminobutyric acid (GABA) receptors	E, ED
Takagi *et al.*, 2004	Rat/ Sprague-Dawley	Dietary	GD15 – PND10	60, 600, or 3000 ppm (3000 ppm calculated by author to be $2.3\text{-}3.8x10^5$ µg/kg bw/day)	NE – all doses	↔ volume of SDN-POA	N, E, ED
Akingbemi *et al.*, 2004	Rat/ Long Evans	Gavage	GD12 – PND 21	2.4 µg/kg bw/day	NE – 2.4 µg/kg bw/day	↔ no change in serum LH or T levels in PND 90 males	SD, E, ED
Honma *et al.*, 2006	Rat/ Sprague-Dawley	Gavage	GD6-PND20	$4x10^3$, $4x10^4$, or $4x10^5$ µg/kg bw/day	E – $4x10^3$ µg/kg bw/day	3 weeks: 3,4-dihydroxyphenylacetic acid ↑ in the brain; higher doses ↑ homovanillic acid, serotonin, and 5-hydroxyindoleacetic acid; 6 weeks –↑ choline in hippocampus and striatum (4 mg/kg only)	N, ED, E
Facciolo *et*	Rat/ Sprague-	Oral	8 days prior	40, 400 µg/kg	E – 40	PND7 or 55 – changes in sst_3 mRNA	ED, E

Reference	Species/Strain[a]	Route	Exposure Period	BPA Doses	E & NE[b]	Effects	Study Limitations^^
al., 2005	Dawley (females)	(pipette)	to mating - lactation	bw/day	µg/kg bw/day	in females	
Zoeller *et al.*, 2005	Rat/ Sprague-Dawley	Oral (wafer)	GD6 – PND 4, 8, 15, or 35	$1x10^3$, $1x10^4$, $5x10^4$ µg/kg bw/day	E – $1x10^3$ mg/kg/day bw/day	PND 15 ↑ T4; RC3/neurogranin expression was ↑ in the dentate gyrus in males	R, ED, E
Funabashi *et al.*, 2004	Rat/ Wistar	drinking water	G – PND 21	$2.5x10^3$ µg/kg/day	E - $2.5x10^3$ µg/kg/day	Sexual difference in corticotrophin-releasing neurons altered by BPA treatment; ↔ in preoptic area	SD, ED, E

^Excludes data previously summarized in *"Exponent: Literature Review of Neurobehavioral Effects of Bisphenol A."*
PND (Postnatal day), GD (Gestation day), PC (post coitum), ↑, increase; ↓, decrease; ↔, no change.
Study Limitations Key: N = small number of animals; SD = single dose; ED = designation includes, but is not limited to: experimental design lacking in detail or flawed, not accounting for litter effects, dose not calculated by author, assessment not performed blind, lack of positive control (neuro studies), assessment does not include common concomitant endpoints of analysis for endpoint under investigation, statistical analysis reporting issues, lack of histochemistry, only one sex examined; V = vehicle concerns; + = positive control performed erratically; E = environmental estrogens not accounted for; R = route of exposure limits interpretations (subcutaneous or drinking water); ID = lack of internal dose (blood levels)
[a]if only one sex examined for endpoints, sex stated (considered a study limitation); [b] Effective Dose (E) and Non-effective Dose (NE), as applicable.
,*Dose calculated by FDA **Dose calculated by NTP CERHR
^^note that none of the studies under neuro measured an internal dose
*Ryan and Vandenbergh 2006: Animals used for behavioral assays were treated ovariectomized one week after weaning.

Appendix 2: Detailed Reviews of Select Manuscripts

As detailed in Appendix 1 and the text of this document, FDA has considered all the publications cited in the NTP draft Brief for the select endpoints discussed as 'some concern'. Several papers indicating positive 'low dose' effects were highlighted in the NTP's peer review presentation[89] or were discussed in the recent Norwegian Scientific Committee on Food Safety assessment. Additional details regarding FDA's assessment of these studies are provided below. As noted, many of these studies have been considered in reviews by other governmental bodies or agencies including the CERHR expert panel[90], the Environment Canada[91], the European Union Risk Assessment Reports on BPA dated 2003 and updated 2008[92] the Norwegian Scientific Committee[93], and the Scientific Panel on Food Additives (EFSA)[94]. As such, and to aid the reader, pertinent excerpters from these reviews are provided as well.

Carcinogenesis

Studies on the Prostate Gland

- Ho SM, Tang WY, Belmonte de Frausto J, Prins GS (2006) *Cancer Res.* Developmental exposure to estradiol and bisphenol A increases susceptibility to

[89] Meeting Presentations – June 11 – 12, 2008 BSC, accessible at http://ntp.niehs.nih.gov/index.cfm?objectid=78A617B9-F1F6-975E-7F3871DF6BC95C22

[90] CERHR final report NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A, dated November 2007 (accessible at http://cerhr.niehs.nih.gov/chemicals/bisphenol/BPAFinalEPVF112607.pdf) and published as Chapin et al. (2008) NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A Birth Defects Research (Part B) 83:157–395.

[91] Government of Canada, Environment Canada draft screening assessment and risk management documents dated April 2008 accessible at http://www.chemicalsubstanceschimiques.gc.ca/challenge-defi/batch-lot_2_e.html#ReleaseofDraft

[92] European Union Risk Assessment Report on 4,4'-isopropylidenediphenol (bisphenol A) 2003 (3rd priority list, volume 37). Updated European Risk Assessment Report (EU RAR) 2008 4,4'-Isopropylidenediphenol (Bisphenol-A) CAS Number: 80-05-7 EINECS Number: 201-245-8 Final Approved Version Awaiting For Publication accessible at http://ecb.jrc.it/documents/Existing-Chemicals/RISK_ASSESSMENT/ADDENDUM/bisphenola_add_325.pdf and

[93] Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food Safety 18 June 2008 Assessment of four studies on developmental neurotoxicity of bisphenol A, accessible at http://www.vkm.no/eway/default.aspx?pid=266&trg=MainLeft_5419&MainLeft_5419=5468:17924::0:5420:1:::0:0

[94] Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-Bis(4-Hydroxyphenyl)Propane (Bisphenol A) Question number EFSA-Q-2005-100 Adopted on 29 November 2006 and European Food Safety Authority, Toxicokinetics of Bisphenol A Scientific Opinion of the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food (AFC) (Question No EFSA-Q-2008-382) Adopted on 9 July 2008 http://www.efsa.eu.int/EFSA/efsa_locale-1178620753812_1211902017492.htm and http://www.efsa.eu.int/EFSA/efsa_locale-1178620753812_1178620772817.htm;

prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. 66(11): 5624-5632.

Ho *et al.* (2006) (also summarized in Prins et al. (2008)) administered 10 µg/kg bw/day BPA to Sprague-Dawley rats via s.c. injection on PND 1, 3, and 5. At PND 90, half of the rats in each group were implanted with silastic tubes containing testosterone and estradiol (Testosterone +E2); rats of the other half were implanted with empty pumps. Animals were necropsied 16 weeks after the implants were placed. Other animals (n= 5-7) were used for gene methylation analyses on PND 10 and 90. These authors reported changes in methylation patterns of cell signaling genes, increased high-grade precancerous lesions, and increased proliferative and apoptotic indices in the prostate glands of BPA-treated rats in the presence of T+E2, but not in the prostate gland of rats that received the control implant. The authors compared these results to the positive control estradiol benzoate. The authors concluded that BPA increases the prostate gland's susceptibility to adult onset of precancerous lesions and hormonal carcinogenesis.

Relevant comments on Ho *et al.:*
FDA: This paper examined effects that are important in the tumor development process (changes in methylation and apoptosis) which would not be screened for in typical regulatory studies. Although this paper provides an interesting protocol for the examination of early exposure to environmental compounds and subsequent challenge with hormones, the relevance of this study to a direct effect of BPA treatment alone and an increased incidence in tumor formation or a clear progression of the findings is unclear. This study is severely limited by the use of only one dose and subcutaneous administration.
CERHR: "This is a carefully performed study by a group with significant expertise in this area of work. The paper has many strengths, from the use of a relatively low dose level of bisphenol A to the search to identify molecular mechanisms, possibly including site-specific promoter methylation, underlying the observations made. Weaknesses include the use of a single dose level with subcutaneous dosing. It could be suggested that carrying the study further in terms of animal age might have produced more dramatic phenotypes and clarified the relevance of PIN resulting from BPA exposure to prostate cancer (potentially enhancing cancer incidence) in this model. Failure to do this could be considered a weakness of the work. This paper is adequate and of limited utility for the evaluation process due to use of subcutaneous route of administration." (181-182)
EU RAR 2008: "Although the study authors claim that PIN is a precancerous lesion leading to prostate cancer, as the animals were sacrificed at 6-7 months of age, this could not be verified and, hence, the toxicological significance of PIN in animals remains unknown. It is also noted that no information was provided on the background variation of PIN in this strain of rats and on the experimental variation of E+T-induced PIN. Overall, therefore, due to the small sample sizes, use of a single dose level (and hence no dose-response information) and lack of information on the background variation of PIN and E+T-induced PIN, it is difficult to establish whether the increased incidence of E+T-induced prostrate lesions was a real, treatment-related effect. Furthermore, because of the subcutaneous route of administration, it is questionable whether the reported findings

are relevant to normal routes of exposures. The kinetics of BPA following subcutaneous administration, including the extent of absorption and its rapid metabolism in the liver to the endocrine inactive conjugate, BPA-glucuronide, are likely to differ from the kinetics of BPA by relevant routes of exposure." (80)

- Timms BG, Howdeshell KL, Barton L, Bradley S, Richter CA, vom Saal FS (2005) *Proc Natl Acad Sci U S A*. Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra. 102(19): 7014-7019.

Timms *et al.* administered 0 (vehicle - tocopherol-stripped corn oil), 0.1 μg/kg bw/day ethinyl estradiol, 0.1 μg/kg bw/day diethylstilbestrol or 10 μg/kg bw/day BPA orally by pipette to pregnant CD-1 mice (Charles River) from GD 14-19. Animals were sacrificed on GD 19 and only one male fetus per litter that developed between a male and a female was used to minimize variations in *in utero* endogenous hormone exposure. Reportedly, all three compounds increased dorsolateral prostate duct volume and number of ducts (about 40% increase) as determined from 3D reconstruction from serial sections and proliferating cell nuclear antigen staining (500-1,000 cells counted in dorsal, lateral and ventral ducts, and urethra – 100%). The urethra near the neck of the bladder was also narrowed in the BPA and ethinyl estradiol groups. The high dose of DES (200 μg/kg) inhibited prostate duct formation.

Relevant comments on Timms *et al.:*
FDA: Strengths of this study include the use of oral exposure and control of endogenous hormones as supplied by littermates. Although the methodology of the study is extremely interesting and unique, the findings of this study are very limited due to the use of only one dose. Additionally, it is unclear if the observations made continue to develop into adverse changes in the animal model. As such, the data are difficult to interpret with regard to long term, chronic exposure and effects on the prostate.
CERHR: "Strengths are the oral route of administration, the low dose level of bisphenol A, the use of diethylstilbestrol and ethinyl estradiol as positive controls, and the sophisticated measures applied to the prostate. Weaknesses are the use of a single dose level and small sample size, although the Panel judged it to be adequate for the methodology. This paper is adequate and of high utility for the evaluation." (197)
EFSA (2006): "The Panel noted that only a single dose level was used in this study and thus a dose-response for BPA was not assessed and prostate weights (absolute or relative) were not given." (34)
EU RAR (2008): Reference is provided (209), but no additional comments.

- Ogura Y, Ishii K, Kanda H, Kanai M, Arima K, Wang Y, Sugimura Y (2007) *Differentiation.* Bisphenol A induces permanent squamous change in mouse prostatic epithelium. 75(8): 745-756.

Ogura *et al.* 2007 utilized Balb/c mice (CLEA, Japan), fed a low phytoestrogen diet (NIH-07 PLD, phytoestrogen level not specified) and housed in polyolefin cages with chip bedding. The containers used to deliver tap water were not specified. The

experiments described included both *in vivo* and *in vitro* studies. The main endpoint evaluated was the expression of cytokeratin 10 (CK10) as a marker of squamous metaplasia of basal epithelial cells of the prostate. This lesion is established to be related to estrogen exposure. Histological evaluation of hematoxylin and eosin (H&E) sections of the prostate and analysis of ER alpha expression levels by real time PCR are also reported. Three experiments are reported: (1) 9-week-old male mice received s.c. implants containing 0, 0.2, 2, 20, 200 mg BPA or 2 mg DES (n=7-9) per group. Prostate glands were evaluated after 3 weeks of exposure. The anterior prostate (AP) and dorsolateral prostate (DLP) expressed CK10 in animals receiving BPA pellets (2 mg and above) or DES pellets. The ventral prostate (VP) expressed CK10 in the DES-treated animals and the high dose (200 mg) BPA-treated animals. The data are reported in terms of intensity of staining and are not quantitative; (2) Explants of adult (8-9 weeks) prostate glands were incubated for 6 days with 1 nM DES or 1 nM or 1 μM BPA. Squamous metaplasia was evident in the DES-treated cultures and in the 1 μM BPA-treated cultures. The 1 nM BPA-treated cultures were histologically normal, but showed CK10 staining. (3) Pregnant mice (n=3) were treated with 20 μg/kg/day BPA or 0.2 μg /kg/day DES by gavage from GD 13-18. Tocopherol-stripped corn oil was the vehicle. Males (2-5 pups per litter) were evaluated at 12 weeks of age. The prostate glands were morphologically normal when assessed by H&E. However, BPA- and DES-treated animals "appeared" to express CK10, with the most intense staining in the AP, intermediate staining in the DLP, and lowest staining in the VP. This order of intensity corresponds to the relative levels of ER alpha expression as measured in the adult prostate.

Relevant comments on Ogura *et al.*:
FDA: This study presents interesting findings; however, the ER staining studies are neither quantitative nor do they readily lend themselves to risk assessment. They are interesting from a hazard identification point of view as they provide evidence for effects of BPA on the prostate gland, particularly after developmental exposure. Accordingly, this study examined whether exposures during development may have the potential to lead to effects later in life and these changes are not evident by standard evaluation techniques (H&E histology, organ weight). Confidence in this study's findings is severely limited by the small number of animals and the use of subcutaneous exposure. Since these data generated *in vitro* their *in vivo* extrapolation is speculative as the degree of metabolism of BPA over the *in vitro* culture period is also not known.

- *FDA conclusions regarding prostate gland data:*

Although these studies demonstrate very interesting findings that are important with regard to modes of action involved in tumor formation (apoptosis, proliferation, methylation, etc.), the studies are very limited by the use of only one dose of BPA (Ho *et al.* and Timms *et al*.), the route of administration (Ho *et al.* and Ogura *et al*.) and the nature of the findings (these studies do not demonstrate findings that are clearly relatable to adverse findings in humans and do not demonstrate progression to tumors). With regard to Ogura *et al.* specifically, this study used a small number of animals and did not report the rate of release of BPA from the implanted pellets or the internal dose achieved by either exposure route (s.c. or oral) for comparisons of routes of exposure. The results

are of interest because they provide evidence for effects of BPA on the prostate gland, particularly after developmental exposure, which has the potential to lead to effects and are not evident by standard evaluation (H&E histology, organ weight). However, based on the data currently available, these findings are difficult to interpret as progression of the findings is not evident. Additionally, FDA is limited in its ability to evaluate the findings based on the data presented (highlighted slides and narrative). Another confounder is that some of these studies did not control for environmental estrogens. Noteworthy, the two year bioassay conducted by NTP did not indicate an increased incidence in prostate tumors; however, FDA recognizes that this study did not include an *in utero* exposure period[95]. Given the totality of the information, these studies are interesting, but are limited in utility by the type of information reported and the methods used.

FDA notes the Environment Canada's draft assessment, comments on these studies as a whole, stating that "the limited evidence is insufficient to demonstrate that early bisphenol A exposure, acting independently, could lead to neoplastic events." (58)

Studies on the Mammary Gland

- Durando M, L. K, Piva J, Sonnenschein C, Soto AM, Luque E, Muñoz-de-Toro M (2007) *Environ Health Perspect.* Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats. 11580-86.

Durando *et al.* (2007) examined the effect of prenatal BPA exposure on susceptibility to mammary tumors in Wistar rats. Rats were housed in stainless steel cages, provided water from glass bottles and fed commercially available chow. On GD 8–23, groups of 11–14 dams were dosed s.c. using an osmotic mini-pump with a DMSO vehicle or 25 µg/kg bw/day BPA. Female offspring were sacrificed on PND 30, 50, 110, and 180. Two hours prior to sacrifice, rats were injected with bromodeoxyuridine (BrdU) to determine proliferative index. Abdominal-inguinal mammary glands were dissected out bilaterally. Whole mounts were used for H&E staining or immunohistochemistry. BPA exposure had no significant adverse effects on pregnancy outcome or the sex ratio, and there were no effects on AGD on PND 1 or 5. Vaginal opening was accelerated by 5 days by BPA. Body weight was not determined at the time of vaginal opening, but it was not affected by BPA treatment at any of the postnatal time points examined. With regard to the mammary gland, the authors concluded that the treatment of BPA was associated with an increased proliferation/apoptosis ratio in epithelial and stromal compartments. Additionally, animals sacrificed at PND 110 or 180 had an increased number of

[95] Carcinogenesis Bioassay of Bisphenol A in F344 rats and B6C3F1 mice - Feed Study, NTP Technical Report 215 Reviewed in FDA Review Memorandum -Acceptance of Final TDERs for review of NTP's Carcinogenesis Bioassay of Bisphenol A in F344 rats and B6C3Fl mice (Feed Study) (NTP TR 215). Shackelford/Food Additive Master file 580. 07/24/2007 and FDA Memorandum - CAC Meeting Dates: 04/24/2008, 05/09/2008 CFSAN Cancer Assessment Committee (CAC), Full CAC Review – Bisphenol A (BPA)

hyperplastic ducts and augmented stromal nuclear density. These findings were also associated with an increased number of mast cells.

Relevant comments on Durando *et al.*:
FDA: The use of only one dose of BPA, the limitations regarding reporting (only select slides are shown), and the lack of progression of reported changes are weaknesses of the study. A major weakness is the use of a non-oral exposure route and that the BPA blood levels were not determined. Complicating this issue is the lack of an indication of the concentration of DMSO used in the mini-pump; pure DMSO is not recommended by the pump manufacturer and could cause its failure.
CERHR: "Weaknesses include route of administration and the high single dose is a weakness as is the use of pure DMSO. This study is inadequate for inclusion due to the use of 99.9% DMSO as a vehicle to administer bisphenol A via sc osmotic pump." (139)[96]
EU RAR 2008: "The study authors concluded that in rats prenatal exposure to a low dose (0.025 mg/kg bw/day) of BPA perturbs mammary gland histoarchitecture and increases its carcinogenic susceptibility to a chemical carcinogen (NMU) administered 50 days after the end of BPA exposure. However, due to the small sample size, lack of clarity on statistical analysis and use of a single dose level, it is difficult to establish whether the effects reported were due to chance or were real, treatment-related effects. Furthermore, because of the subcutaneous route of administration, it is questionable whether the reported findings are relevant to normal routes of exposures." (82)

- Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM (2007) *Reprod Toxicol.* Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. 23(3): 383-390.

The effect of prenatal BPA exposure on *in situ* induction of mammary tumors in Wistar-Furth rats was examined. Cages and bedding tested negative for estrogenicity, and water was supplied in glass bottles. The chow was reported to contain 20 fmol/g estrogen equivalents. From GD 9 through PND 1, rats received BPA at 0 (50% DMSO), 2.5, 25, 250, or 1000 μg/kg bw/day. Group sizes were not indicated. Dosing solutions were delivered by implanted osmotic mini-pumps. Litters were adjusted to 8 pups on PND 2. AGD was measured on PND 4, and female offspring were monitored for body weight and vaginal opening after weaning. It is unclear if the litter or the individual pup was considered as the experimental unit, but the authors attempted to "maximize the number of maternal units represented in each group." Female offspring were sacrificed on PND 50 or PND 95 and the 4th and 5th inguinal mammary glands were fixed and processed for paraffin embedding and whole mount analysis. Exposure to BPA had no adverse effects on the number of live pups or the sex ratio at PND 1. Age at vaginal opening was not affected by any dose of BPA. The incidence of hyperplastic ducts in BPA treated females was increased 3-4 fold over controls at PND 50. No dose response was

[96] FDA notes that the interpretation of the study methodologies by the committee was aided by personal communication.

observed. At PND 95, the difference was insignificant in all but the 2.5 µg/kg bw/day treated group. Cribriform-like structures were also reported in the 250 and 1000 µg/kg bw/day treated groups at PND 50 and PND 95. Additionally, the authors concluded that Ki67 and ERα staining were increased in the ductal hyperplastic lesions as compared to normal ducts.

Relevant comments on Murray *et al.*:
FDA: FDA notes that multiple doses were evaluated, care was taken to decrease exposure to environmental estrogens, and the authors used four doses covering a wide range. However, there was no indication of the number of dams treated in each group and the sample size, as noted in the results section, is small (n=4 – 6 for the cribriform-like structures). Additionally, it was not indicated whether the litter or the individual pup was considered the experimental unit. Furthermore, it is unclear if the observations are progressive, the route of administration is non-oral, and according to the manufacturer, 50% DMSO can be used in the Alzet mini-pumps. Lastly, blood levels of BPA were not determined.
CERHR: "Relevance of endpoints is a strength, as is the use of multiple dose levels. Weaknesses include an unstated number of dams (and by inference, a small number of these, and thus, because of dam-related effects, a small overall n), the uncertainty of the response rate of histopathology in the controls, and the use of 50% DMSO as vehicle. This study was inadequate due to small sample size, route of administration, and lack of clarity on statistical analysis. " (138)[97]
EU RAR 2008: "… again, due to the small sample size, lack of clarity on the statistical analysis, absence of a dose-response relationship and uncertainty about the incidence of the cribriform-like lesions in the controls it is difficult to establish whether the effects reported were due to chance or were real, treatment-related effects. In addition, because of the uncertainty about the significance of the cribriform structures, it is unclear whether real neoplasia actually occurred. Furthermore, because of the subcutaneous route of administration, it is questionable whether the reported findings are relevant to normal routes of exposures." (83)

- Markey CM, Luque EH, Munoz De Toro M, Sonnenschein C, Soto AM (2001) *Biol Reprod.* In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. 65(4): 1215-1223 [Erratum: *Biol Reprod* 2004;1271:1753].

CD-1 mice were exposed *in utero* to two doses of BPA, 25 and 250 µg/kg bw/day. Mice were fed RMH 3000 rodent diet, which was evaluated for estrogenic content and water was supplied via glass bottles. Starting on GD9, dams (6-10 per treatment) were implanted with s.c. minipumps either containing DMSO (vehicle, appears to be 100%) or BPA. Dose (not accessed internally) was stated to have decreased as pregnancy progressed due to the increased weight of the dam. Female pups (6-10 per treatment) were sacrificed at 10 days, 1 month or 6 months. Prior to sacrifice, BrdU was injected.

[97] Ibid.

At sacrifice, mammary glands (fourth inguinal) were dissected bilaterally, the right gland was sectioned and stained with Carmine Alum whereas the left gland was stained with BrdU or H&E. Results indicated that the total length of the ductal tree was not affected by treatment whereas the elongation of the duct was affected having increased in the low dose and having been retarded at the high dose of BPA. Reported results indicated that BPA treatment resulted in a significant increase of all ductal and alveolar structures as compared to control groups (% of ducts, terminal ducts, terminal end buds (only at low dose) and alveolar buds). The results also indicated changes in the incorporation of BrdU in the epithelial cells and stroma of the mammary gland in BPA treated animals as compared to controls with regard to puberty and development. Secretory product in the lumina of epithelial structures was also affected by BPA treatment, but only at the low dose.

Relevant comments on Markey *et al.*:
FDA: Study strength includes the control of environmental estrogens. Although the study examined interesting endpoints with regard to development of mammary ducts during puberty in combination with BPA treatment, the study is severely limited by the use of s.c. minipump administration (non-oral route) and DMSO as a vehicle. The use of minipumps and the weight changes in dams also limits our confidence in any quantitative measure of the two doses administered. It appears that litter was evaluated statistically, but it is unclear how animals were chosen with regard to litter effects.
CERHR: "The examination of the mammary gland, a system not often studied, is a strength. A critical weakness is the uncertainty of the DMSO concentration as a vehicle and therefore pump performance. An additional weakness is that the proliferative changes reported in mammary tissues in virgin mice have not been satisfactorily established as precursors of breast cancer. This paper is inadequate for the evaluation process given exposure uncertainties." (206)
EU RAR (2008): Reference is provided, but no additional comments. (200)
EFSA (2006): Review of this study was part of a series from this laboratory: "The panel noted absence of dose-response for many changes reported or the evaluation of samples for only one dose level." (68-69)

- Moral R, Wang R, Russo IH, Lamartiniere CA, Pereira J, Russo J (2008) *J Endocrinol.* Effect of prenatal exposure to the endocrine disruptor bisphenol A on mammary gland morphology and gene expression signature. 196(1): 101-112.

Moral *et al.* (2008): Pregnant Sprague-Dawley CD rats were gavaged with 0 (vehicle - sesame oil), 25 or 250 µg/kg/day BPA on days 10 – 21 post-conception. Female off-spring were nursed using surrogate dams and sacrificed at PND 21, 35, 50 or 100 days. Mammary gland analyses were conducted using whole mounts, proliferative index, and real-time RT-PCR. Changes were observed in terminal end buds only at the high dose as compared to the low dose BPA (not controls) at 21 days (not observed at 35, 50 or 100 days). No changes were observed with regard to alveolar buds. Numbers of terminal ducts were increased in the high dose group, at days 21 and 100 only. At 35 days of age, the number of lobules type 1 (defined as undifferentiated lobules having a high

concentration of stem cells) was significantly higher in the high dose group as compared to low and control groups. There were no changes in the proliferative index at any dose and genetic changes depended on both dose and time of sacrifice.

Relevant comments on Moral *et al.*:
FDA: Strengths of this study included the use of multiple doses and the oral route of administration. Limitations of this study included: 1) no mention other than diet as to the remaining estrogenic environmental factors; 2) a positive control was not utilized in the study, instead the BPA data were compared to data results on estrogenic compounds from other studies; 3) the study authors did not comment on whether litter effects were controlled in the study and 4) the fact that the authors cite data not shown.

- *FDA conclusions regarding mammary gland data:*

Although these studies demonstrate very interesting findings that are important with regard to modes of action involved in tumor formation (hyperplastic ducts, proliferation, and changes in ductal morphology or development), the studies are very limited by the fact that all but one, Moral *et al*. (2008), used s.c. injections as the route of exposures. Further complicating this is the use of DMSO as a solvent in the implanted minipump studies, which limits the confidence in the accuracy of dosing, especially when multiple doses are stated but an internal dose was not measured. Only Moral *et al.* used the oral route of administration and this study was strengthened by the use of multiple doses. However, this study is severely weakened deficiencies in methodology (lack of control for estrogens, the control for litter effects was not documented and the authors rely on data not shown). All of the studies are limited by the nature of the findings (these studies do not demonstrate findings that are clearly relatable to adverse findings in humans and do not demonstrate progression to tumors). The results of these studies are of interest because they provide evidence for effects of BPA on the mammary gland, particularly after developmental exposure, which has the potential to lead to effects which are not evident by standard evaluation (H&E histology, organ weight). However, FDA is limited in its evaluation of the findings based on the data presented. Additionally, based on the data currently available, these findings are difficult to interpret as progression of the findings is not evident. Noteworthy, the two year bioassay conducted by NTP did not indicate an increased incidence in mammary gland tumors[98]. Although the NTP study was more robust in design as compared to reported studies, FDA recognizes that this study did not include an *in utero* exposure period. Given the totality of the information, these studies are interesting, but are limited in utility by the type of information reported and the methods used.

FDA notes the Environment Canada's draft assessment, comments on these studies as a whole, stating that "the limited evidence is insufficient to demonstrate that early bisphenol A exposure, acting independently, could lead to neoplastic events." (58)

[98] Ibid.

Acceleration of Puberty in Female Rodents

- Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, vom Saal FS (1999) *Nature.* Exposure to bisphenol A advances puberty. 401(6755): 763-764.

Howdeshell, KL *et al.* (1999) examined the effects of prenatal BPA exposure on the age of puberty in female CF-1 mice; results were reported in a very brief communication. Pregnant mice were given either oil vehicle or BPA at 2.4 μg/kg bw/day on GD 11 – 17 (day of vaginal plug not defined). On GD 19, pups were removed by cesarean section, and intrauterine position was determined (position near male/female pups). Pups were fostered to untreated mothers and were weaned on PND 22. Female pups were monitored for the day of vaginal opening and the day of first estrus. Data were analyzed on a litter basis. Results were presented for all pups from each dose group (for body weight) or in relation to intrauterine position (for time between vaginal opening and first estrus). BPA significantly increased body weight at weaning. This effect was greater if the fetus was positioned next to other female fetuses *in utero*. There was no effect of BPA on the day of vaginal opening, but the period between vaginal opening and first estrus was accelerated by BPA only in female fetuses positioned next to other female fetuses *in utero*. The authors concluded that prenatal exposure to BPA altered postnatal growth and reproductive function in female mice, but that natural variation in endogenous hormone levels may influence the response to BPA.

Relevant comments on Howdeshell *et al.*:
FDA: Strengths of the study include the oral route of exposure and a large n of 21 per group. Both the time of vaginal opening and the day of first estrus, neither of which were affected by BPA treatment, were measured. Weaknesses include only a single dose of BPA was used, so no dose-response could be determined; there was also a lack of experimental details including no description of animal care so it is unclear if there might have been exposure to environmental estrogens in either food, water or bedding; uncontrolled environmental factors that might have affected these endpoints.
CERHR: "Strengths are the oral route of exposure and the use of a low dose level of bisphenol A. The omission of a description of husbandry conditions and lack of clarity of statistical procedures are weaknesses. Use of only a single dose is a weakness. Further, the use of time from vaginal opening to first estrus is not a standard endpoint for assessing puberty in mice and is of questionable biological significance. This paper is adequate for the evaluation process but utility is limited due to uncertainties in data analyses." (194)

- Honma S, Suzuki A, Buchanan DL, Katsu Y, Watanabe H, Iguchi T (2002) *Reprod Toxicol.* Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. 16(2): 117-122.

Honma *et al*. (2002) examined the effects of prenatal BPA exposure on the reproductive system of female ICR/Jcl mice. Groups of 10 pregnant mice were injected s.c. with BPA in sesame oil at 0, 2 or 20 μg/kg bw/day on GD 11–17. Additional groups of mice were

injected with diethylstilbestrol at 0.02, 0.2 or 2 μg/kg bw/day. At birth, pups were sexed, counted, and weighed, and litter size was adjusted to 8 pups. After weaning, females were monitored daily for vaginal opening and subsequently for the time of first estrus (defined as only cornified cells in the vaginal lavage). Female offspring were mated with untreated males from 90 to 120 days of age, and F_2 pups were counted and sexed at birth. The litter was considered the experimental unit in statistical analyses. Based on the data presented, there were no adverse effects on pregnancy outcome. The age of vaginal opening and time of first estrus were accelerated in the high dose group females by about 1 day or less than 1 day, respectively. Body weight at vaginal opening was lower in both BPA dose groups. Among F_1 females that were mated, there were no significant effects on number of pups/litter or the sex ratio of F_2 pups. Females exposed to any of the three DES doses also demonstrated acceleration in the age at vaginal opening and age at first estrus with no effects on fertility. The authors concluded that prenatal exposure to low doses of BPA results in early vaginal opening in mice but did not affect female reproductive function.

Relevant comments on Honma *et al.*:
FDA: Strengths of this study included three doses of DES as a positive control and the use of low doses of BPA. Sample size was adequate (10 litters per group), and the litter was used as the experimental unit in the statistical analysis. Weaknesses include a lack of description of possible estrogen exposure in the food, water or bedding materials. Additionally, the s.c. route of exposure was used; this could lead to higher plasma levels of biologically active BPA. Blood levels of BPA and/or its metabolites were not determined in this paper. Also noteworthy is the small incremental change in mean response as compared to controls (~1 day for either endpoint).
CERHR: "Strengths are that this study represents one of the few studies that appropriately examines the onset of puberty in the mouse as an endpoint, it uses low dose levels of bisphenol A, relatively large sample sizes, and effectively uses a positive control at 3 dose levels. The lack of AGD measurement at birth and difficulty of measurement at PND 60 are weaknesses. The Expert Panel was unable to confirm the statistical significance of the effects shown in Table II of the manuscript. The study is adequate for inclusion but of limited utility due to statistical questions about body weight and AGD and subcutaneous route of exposure." (209)

- Ryan BC, Vandenbergh JG (2006) *Horm Behav.* Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice. 50(1): 85-93.

Ryan and Vandenbergh (2006) examined the effects of perinatal exposure to BPA and the onset of puberty as endpoints in C57BL/6 mice. Mice were maintained in polycarbonate cages (that were checked frequently for condition) with chip bedding; however, estrogenicity of the food, water and bedding were not determined. Females were mated, and the day that a vaginal plug was identified was considered GD 1. Beginning on GD 3, dams were gavaged with BPA at 0 (tocopherol-stripped corn oil), 2 or 200 μg/kg bw/day, or ethinyl estradiol at 5 μg/kg bw/day. Animals were dosed daily from GD 3 to PND 21,

when pups were weaned. Female mice were checked for vaginal opening, and vaginal smears were taken daily subsequently. Puberty was defined as the first day on which cornified cells were detected in the vaginal lavage. The results indicated that puberty was advanced by exposure to ethinyl estradiol and the high dose of BPA (200 µg/kg bw/day). The authors concluded that BPA and ethinyl estradiol accelerated puberty in female mice.

Relevant comments on Ryan and Vandenbergh *et al.*:
FDA: FDA notes that a positive control group, ethinyl estradiol, was included and performed adequately in this study. Weaknesses include the failure to determine estrogenicity of the animal environment; the chow used in this study (Purina rodent chow 5001) is known to have a high soy content. A particular weakness of the study is the small number of females examined in each group for the determination of the time of puberty (n = 4 - 7), and it was not described how these females were selected. There were apparently 16 litters in each treatment group, so far less than one female from each litter was examined for this endpoint. It also appears that the individual was used as the experimental unit for statistical analysis of this endpoint.
CERHR: "Selection of established measurements of sexually dimorphic behaviors and replication of previous work by Howdeshell *et al.*, 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.* This study is adequate and of high utility for the evaluation process with the exception of the pubertal data." (Relevant statement is italicized for emphasis since multiple endpoints are discussed.) (222)
Norwegian Scientific Committee: "A positive control, two dose levels of BPA and some parameters on reproductive toxicity were included in the study design. However, the reproductive parameters were assessed at weaning and not at delivery, which is an incomplete assessment. The test animals were ovariectomised females only which excludes evaluation of possible sex differences in response to BPA or EE exposures. Additionally, even if the use of ovariectomised mice removes the potential confounding factors of cyclicity on behaviour, it also eliminates the evaluation of possible hormonal interactions of the test substance that may influence on behaviour. *With regard to puberty onset, the number of females per group was limited*, 5-4-5-7 for the control, low dose BPA, high dose BPA and the EE groups, respectively, and it is not known whether the animals in each group represent different litters. The result is thus questioned." (Relevant statement is italicized for emphasis since multiple endpoints are discussed.) (16)

- Ashby J, Tinwell H, Haseman J (1999) *Regul Toxicol Pharmacol.* Lack of effects for low dose levels of bisphenol A and diethylstilbestrol on the prostate gland of CF1 mice exposed in utero. 30(2 Pt 1): 156-166.

Ashby *et al.* (1999) evaluated the effects of prenatal BPA exposure on the reproductive system of CF-1 mice. Although the chow was not tested for estrogenicity, it was noted that the chow used during pregnancy and lactation contained 18.5% soy, and the chow used at all other times contained 6.5% soy. On GD 11–17, groups of 8 mice were dosed with BPA at 0 (tocopherol-stripped corn oil), 2 or 20 µg/kg bw/day (n = 6); a positive

control group (n = 5) was included in the study design and was dosed with diethyl stilbestrol (DES) at 0.2 µg/kg bw/day. A naïve group of 5 dams was not weighed or dosed. The dosing solution was administered orally by being slowly expelled from a pipette placed in the animals' mouths and allowing them to lick the solution. All female offspring were checked daily for vaginal opening after weaning and were weighed at various intervals. Care was taken to reduce any stress to the animals and included administering test agents by drip feeding, minimal handling of pups, and minimal environmental noise. Data were analyzed using the litter as the experimental unit. There were no adverse effects on pregnancy outcome including litter size or the sex ratio. In female offspring from the BPA groups, there were no significant effects on body weight or organ weights when compared to the vehicle control group. Age and weight at vaginal opening were also unaffected in groups exposed to BPA. Vaginal opening was delayed in the diethylstilbestrol-treated group and in the naïve control group compared to the vehicle control group.

Relevant comments on Ashby *et al*.:
FDA: FDA notes that this study used low doses of BPA, and the litter was used as the experimental unit for most of the statistical analyses. Weaknesses of the study included the fairly small group sizes and the unexpected effect on vaginal opening in the positive control group and the naïve control group. Vaginal opening was delayed in the naïve control group by about 3 days, and DES delayed vaginal opening by over 3.5 days, rather than causing acceleration. The dose of DES used (0.2 µg/kg bw/day) is apparently borderline for producing effects on the reproductive tract and was probably not a good choice.
CERHR: "Strengths are the rather close replication of the designs of the studies by vom Saal et al. and Nagel et al. with diet as the only major difference, the use of both solo and group housed mice, and the support of the conclusions by the NTP Statistics Subpanel. The use of small samples is an understandable weakness given that this study was designed to be a replicate study. The lack of response of the positive control DES group is problematic. This paper is inadequate for the evaluation process due to absence of response of the positive control group and small sample sizes." (193)
EU RAR 2003: "…the results of this study are in agreement with those reported by Cagen et al. (1999b) that low doses of bisphenol-A do not increase prostate weight or reduce sperm efficiency in CF1 mice." (230)

- Markey CM, Coombs MA, Sonnenschein C, Soto AM (2003) *Evol Dev.* Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs. 5(1): 67-75.

Markey *et al.* (2003) examined the effects of perinatal BPA exposure on reproductive development in CD-1 mice. Care was taken to decrease exposure from environmental estrogens by testing the cages, bedding, and chow and using only glass water bottles. From GD 9 through PND 4, groups of 6–10 mice were exposed to BPA at 0 (DMSO), 25 or 250 µg/kg bw/day via a subcutaneously implanted osmotic mini-pump. Age at vaginal opening was determined and classified as either partial or complete. It appears that the

individual pup was evaluated as the experimental unit. Although there were trends toward a younger age for partial vaginal opening as well as for the time between partial and complete vaginal opening, these differences were not statistically significant. Based on the data presented, it is not clear how many animals were evaluated for this endpoint or how those animals were selected.

Relevant comments on Markey *et al.*:
FDA: Strengths of the study include the authors' use of environmentally relevant doses of BPA and the care used to decrease exposure to environmental estrogens. Weaknesses of the study include a non-oral route of administration and the use of pure DMSO as the vehicle in the osmotic mini-pump. This vehicle is not recommended by the manufacturer and could have caused pump failure leading to inaccurate BPA dosing. Blood levels were not determined in this work.
Other: See above under *Mammary Gland*

- Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Hentges SG, Waechter JM Jr (2008) Two-generation reproductive toxicity study of dietary bisphenol A (BPA) in CD-1 (Swiss) mice. *Tox Sci* 104(2):362-384.

Tyl *et al.* (2008) is reviewed in the text of the assessment, but included herein as a discussion of puberty. Briefly, a two generation study in CD-1 mice was conducted using dietary doses of 0.018, 0.18, 1.8, 30, 300 and 3500 ppm BPA; these doses resulted in estimated intakes of approximately 0.003, 0.03, 0.3, 5, 50 or 600 mg BPA/kg bw/day. A positive control group was fed 17β-estradiol at 0.5 ppm with an estimated intake of about 0.08 mg/kg bw/day. Groups of 28 mice were fed the diets for 8 weeks before breeding and throughout breeding, gestation and lactation. Concentration, stability, and homogeneity of BPA and E2 in feed were verified, and animal body weights and food intake were monitored throughout the study. Animals were housed in cages with chip bedding with glass water bottles. The chow was analyzed by the manufacturer and contained isoflavones at 394.2 ppm. F1 litters were culled to 10 pups on PND 4, with equal numbers of each sex when possible. At weaning on PND 21, 28 F1 offspring/sex/group were randomly selected and exposed to BPA in the diet according to the same protocol as F0 mice. Those selected offspring were monitored for vaginal opening and preputial separation and later mated. At weaning, an additional 1 male/litter was randomly retained with BPA exposure continuing for an additional 3 months; preputial separation was also determined in those males. Pregnant F1 females were followed through gestation, birth and lactation. At weaning, all F2 animals and F1 parents were sacrificed and necropsied. There were no adverse effects among F0 animals regarding mating, fertility, number of live pups/litter or birth weight of F1 pups. Preputial separation was significantly delayed at 3500 ppm BPA whether considering the absolute time or the time adjusted for body weight at the time of acquisition. If the time of preputial separation was adjusted for body weight on PND 30, this difference was not statistically different. Estradiol delayed preputial separation (absolute time as well as time adjusted for body weight at time of acquisition or PND 30). Females exposed to 3500

ppm BPA weighed less than control animals. As indicated in Table 9 of the published study absolute day of acquisition was not statistically significant at 3500 ppm. Day of acquisition was statistically significantly accelerated when adjusted by body weight on PND 21 for F1 (only animals measured). Again, no findings were reported at the lower doses.

Relevant comments on Tyl *et al.*:
FDA: FDA concludes that this is a well-conducted, thorough study done under GLP conditions. Concentration and stability of dosing solutions were verified, exposure to environmental estrogens was controlled, two vehicle control groups were used to help define the intrinsic variability in the endpoints evaluated in the study, six doses of BPA were used covering a wide dose range, oral administration was used, group sizes were large, a large number of endpoints was evaluated, the litter was used as the experimental unit, and fertility of exposed animals was evaluated. Weaknesses of the study included the lack of blood levels of BPA for comparison to other reported non-oral results (informational weakness) and the time of first estrus was not evaluated. Additionally, markers of puberty were not determined among F2 offpsring.
CERHR: "Strengths include the large number and range of doses examined, the rigor with which the study was performed (including evaluation of phytoestrogen content of feed), the large sample size in each group, the number of additional animals per litter that were retained and examined, the use of a concurrent estrogenic positive control group, and the thoroughness of the histological evaluation. Strengths include the large number and range of doses examined, the rigor with which the study was performed (including evaluation of phytoestrogen content of feed), the large sample size in each group, the number of additional animals per litter that were retained and examined, the use of a concurrent estrogenic positive control group, and the thoroughness of the histological evaluation." (224)
EU RAR (2008): "As we consider this investigation by Tyl *et al*. (2007) as the gold-standard, definitive study of the reproductive toxicity of BPA (for the endpoints examined), all the other recent publications investigating the same standard reproductive and developmental endpoints have not been evaluated in detail in this report." (86)
Environment Canada: No specific comments with regard to this study:endpoint combination; however, the following statement is made: "The NOAELs from the multigeneration reproductive toxicity studies in Sprague- Dawley rats and CD-1 mice of 5 mg/kg-bw per day for systemic effects (reduced body weight gain in rats and minimal to mild hepatocyte hypertrophy in adult male and female mice) and 50 mg/kg bw per day for reproductive and developmental toxicity (Tyl et al. 2002; 2007) are considered an appropriate departure point for characterizing risk to human health from exposure to bisphenol A." (70)

- *FDA conclusions regarding puberty data:*

As indicated in the assessment and the appendices, numerous weaknesses were noted in published studies evaluating changes in puberty following BPA treatment. Additionally, a concern regarding the proper positive control and dose for this endpoint has been noted. Of the studies highlighted above, two mouse studies (Ryan and Vandenbergh, 2006 and

Honma *et al.*, 2002) reportedly observed acceleration of the day of first estrus; however, it is noteworthy that the reported effects in the Honma *et al*. study were of questionable significance (~1 day). Additionally the Honma *et al.* study used s.c. administration which is of questionable relevance for oral exposure assessment. An additional study, Howdeshell *et al.*, 1999, reported a reduction in the number of days between vaginal opening and first estrus; however, neither the age of vaginal opening nor the age at first estrus were accelerated. Accordingly, this study, though interesting, did not report a potential acceleration in puberty. The study by Tyl *et al.* (2008), which FDA considers the highest utility, did not observe an effect on puberty following BPA treatment with low doses; however, day of acquisition was statistically significant accelerated when adjusted by body weight in the highest dose administered (3500 ppm) on PND 21 for F1 (only animals measured). Until effects on puberty are repeated in an appropriately controlled study, FDA considers the current data of limited use for a safety assessment.

Although the EC assessment did not provide an assessment of each study and used the Tyl et al. 2008 NOAEL for reproductive and developmental findings, they did comment on the concern regarding divergent results to low doses of BPA and the characterization of the degree for which these would be considered "adverse" and useful for human health risk assessment. (62)

Neurotoxicity

- Negishi T, Kawasaki K, Suzaki S, Maeda H, Ishii Y, Kyuwa S, Kuroda Y, Yoshikawa Y (2004) *Environ Health Perspect.* Behavioral alterations in response to fear-provoking stimuli and tranylcypromine induced by perinatal exposure to bisphenol A and nonylphenol in male rats. 112(11): 1159-1164.

Pregnant F344/N rats were administered 0.1 mg BPA/kg bw/day or corn oil vehicle by oral gavage beginning on GD 3 until PND 20. Offspring were weaned on PND 21 and the males (n = 8-10/group) were subjected to a series of behavioral tests as adults. Female offspring were not tested. Neurobehavioral tests included open-field behavior at 8 weeks of age, spontaneous motor activity at 12 weeks, passive avoidance at 13 weeks, elevated plus-maze test at 14 weeks, and active avoidance at 15 weeks. At 22-24 weeks of age, the males underwent a monoaminedisruption test by injection with *trans*-2-phenylcyclopropyl amine hydrochloride followed by measurement of spontaneous activity and open-field behavior. Results indicated that maternal and male offspring body weight and organ weight and litter parameters were not affected by treatment. For BPA-exposed male offspring, results of open-field, spontaneous motor activity, and elevated plus-maze tests were similar to the controls. In the passive avoidance test during the retention trial, the BPA group showed significant hesitation (increased latency) to enter the dark compartment. In the active avoidance test, the treated group had significantly fewer avoidance responses during the first, second, and third (of five) sessions compared with the controls. The frequency of failure of avoidance was significantly higher in the BPA group. BPA treated animals failed to show an increase in motor activity in response to *trans*-2-phenylcyclopropyl amine hydrochloride. Results were interpreted by the study

authors to indicate that BPA exposure to dams during gestation and lactation irreversibly affected perception of fear provoking stimuli and monoaminergic neural pathways in male offspring.

Relevant comments on Negishi *et al*.:
FDA: FDA noted a number of positive attributes for this study, including oral exposure, acceptable number of replicates and protocol for assignment to treatment groups, behavioral tests were well-defined, litter was the statistical unit, and additional concomitant toxicity endpoints were examined (body weight, parturition, maternal oral weight at weaning, general development). Limitations affecting interpretation included use of only a single dose and single sex (males), a lack of positive control, use of a single pup and multiple time period (ages) for multiple behavioral measurements, lack of discussion on the differential findings in the monoamine disruption test which may indicate a highly specific effect of BPA on the monoaminergic system (BPA treatment prevented the tranylcypromine induced increase in locomotor (horizontal) activity but BPA had no suppressant effect on the tranylcypromine-induced decrease in rearing behavior), lack of concomitant neurochemical or endocrine measures to relate to measured findings, and a lack of information regarding environmental estrogens.
CERHR: "The use of a single dose level is a weakness. Strengths include the variety of endpoints used to provide data, which point to effects that are not gross structural changes but relatively subtle behavioral effects. These data are adequate and of high utility for the evaluation process." (163)
EU RAR (2008): With regard to the summarized data: "Overall, there does not appear to be a consistent pattern across species and gender in the results of the tests for anxiety." (117) "Single BPA treatment group." "Behavioural testing conducted according to acceptable techniques. Analysis of results used appropriate statistical unit." (112)
Norwegian Scientific Committee: "The study design did not include a positive control or dose-response of BPA, but some parameters on reproductive toxicity. Test animals were males only, which excludes evaluation of possible sex differences in response to BPA or NP exposures. The animals went through a set of different tests and bad experience in one test may influence on the performance in the following ones. Results were analysed by ANOVA and for the active avoidance test adjustment for repeated measures was included. There is however concern about the lack of information about how the data were recorded (e.g. manually, blinded to the tester) in the elevated plus maze and the passive and active avoidance tests. Developmental exposure to BPA did not influence on the animals' level of activity or on the tolerability for anxiety in general, but in situations with extreme stress the tolerability seems to be raised. The interpretation that this may be related to alterations in the monoaminergic system is questioned because alterations in locomotion that is evident only after pharmacological manipulations must be interpreted with caution." (14)
EFSA (2006): Study was reviewed and comments were made regarding a lack of positive control. (40)
Environment Canada: Specific comments were not available; however, draft assessment states in a discussion of Negishi *et al.* : "Together, these findings illustrate the variable

responses observed across gender and species in anxiety-related behavioural endpoints following a range of bisphenol A exposures." (66)

- Laviola G, Gioiosa L, Adriani W, Palanza P (2005) *Brain Res Bull.* D-amphetamine-related reinforcing effects are reduced in mice exposed prenatally to estrogenic endocrine disruptors. 65(3): 235-240.

Pregnant CD-1 mice were orally administered 10 µg/kg bw/day of BPA or the tocopherol-stripped corn oil vehicle using a micropipette on GDs 11-18. The mice had been trained to drink the oil from the micropipette. At 60 days of age, offspring were subjected to behavioral testing which consisted of changes in the reinforcing effects of amphetamine (0, 1, or 2 mg/kg, i.p.) using the conditioned place preference paradigm. The expected dose-dependent increase in locomotor activity was observed in both sexes following amphetamine administration. Prenatal exposure to BPA did not affect the initial response to amphetamine. The conditioned response to amphetamine was not affected in males by BPA exposure. In contrast, females failed to show the conditioned response to the rewarding property of amphetamine following prenatal exposure to BPA.

Relevant comments on Laviola *et al.*:
FDA: FDA noted several positive attributes to this study including oral administration, acceptable number of replicates (n = 10-12), examination of both sexes, and the methods/criteria for the conditioned place preference behavioral test were clearly described. Efforts were made to minimize confounding variables in the testing procedure (e.g., testing of experimental groups was counterbalanced across time and test chambers were cleaned after each animal to minimize residual odor cues). Limitations of the study included use of a single dose, lack of a positive control, lack of concomitant hormonal analyses or neurochemical assessments of the functional status of the dopaminergic system in the central nervous system with which to correlate the treatment related behavioral effects of prenatal BPA exposure (the availability of such correlative information would have been of value in helping to determine the biological relevance of the prenatal BPA effects on adult amphetamine-induced conditioned place preference), mixed results with regard to the central dopamine system in females without explanation (diminished amphetamine-induced conditioned place presenence in BPA exposed female offspring whereas no effect on motor activity) and lack of information regarding environmental estrogen exposure. Due to the limitations of this study, particularly in its experimental design, and the need for clarification of the divergent findings regarding BPA's effects on amphetamine-induced changes in behaviors associated with the brain dopamine systems, this paper is of limited utility in determining an assessment for oral exposure to BPA.
CERHR: "Strengths of this study include robust and appropriate design and analysis, adequate sample size, and oral dosing. The use of only 1 dose level is a weakness. This study is adequate and of high utility in the evaluation." (205)
Environment Canada: (combined comments on Laviola et al. and Gioiosa et al.) "…these studies, though, were limited to a single exposure level precluding the evaluation of dose-response. The lowest dose leading to bisphenol A-induced organizational effects in the

brain was 10 µg/kg-bw per day in CD-1 mice. It should be recognized that studies were conducted using the same outbred strain of mice (CD-1), the same experimental dosing protocol (single dose, no positive control) and by the same group of researchers in one research institute." (65). "Pharmacological challenge studies are suggestive of potential organizational alterations of the neural system following perinatal bisphenol A exposure. The details of additional studies are not elaborated on as the doses that resulted in significant effects on the neurochemical systems were above the established NOAEL of 50 mg/kg-bw per day for reproductive/developmental effects; altered behaviour was observed following administration of 250 or 400 mg/kg bw per day." (67) and "While collectively these studies provide evidence that exposure to bisphenol A during gestation and early postnatal life may be affecting neural development and some aspects of behaviour in rodents, the overall weight of evidence was considered limited from the perspective of rigour (e.g., study design limitations such as conduct of behavioural assessments at a single time point); power (e.g., limited number of animals per test group), corroboration/consistency (limited consistency of studies) and biological plausibility (e.g., certain studies involve use of a single dose, lack of dose response relationship). These limitations make it difficult to determine actual significance of findings to human health risk assessment." (71)
EU RAR (2008): "Single BPA treatment group" and "Behavioral testing conduced according to acceptable techniques. Analysis of results used appropriate statistical unit." (110)

- Gioiosa L, Fissore E, Ghirardelli G, Parmigiani S, Palanza P (2007) *Horm Behav.* Developmental exposure to low-dose estrogenic endocrine disruptors alters sex differences in exploration and emotional responses in mice. 52(3): 307-316.

Pregnant CD-1 mice were orally administered 10 µg/kg bw/day of BPA or the tocopherol-stripped corn oil vehicle using a micropipette beginning on GD 11 and continuing through PND 8. The mice had been trained to drink the oil from the micropipette. Offspring were subjected to behavioral testing at 30 days of age in the novelty-seeking test and at 70 days of age in the free-exploratory open-field and elevated plus maze tests. In all tests in both prepubertal and adult offspring, BPA exposure eliminated sex-related behavioral differences observed with control animals. Generally, the lack of sex-related differences was due to the fact that the behavior of the treated females was more similar to that of control males than to that of control females. Thus, characteristic differences between male and female mice in non-reproductive behaviors were not observed following prior exposure to BPA.

Relevant comments on Gioiosa *et al.*:
FDA: FDA noted several positive features to this study including the oral route, an acceptable number of replicates, examination of both sexes at adolescent (PND 30) and adult (PND 70) periods, methods/criteria for behavioral testing were clearly described and confounding effects due to litters were eliminated (only 1 male/female per litter were used). Limitations noted in the study were the use of a single dose, a lack of positive control, lack of concomitant morphologic evaluations (via histology), hormonal analyses,

or neurochemical assessments with which to correlate the treatment related behavioral effects of perinatal BPA exposure, novelty seeking and free-exploratory open-field tasks are relatively unique and there were no positive controls used in the study to demonstrate the validity, sensitivity, or reliability of these latter test measures, the magnitude of the responses in dimorphic measurement were relatively small such that interpretation would be helped by historical information from the performing laboratory, and details regarding environmental contaminants (PC cages and bottles) were unclear.
CERHR: Not reviewed – unavailable
Environment Canada: (combined comments on Laviola et al. and Gioiosa et al.) "…these studies, though, were limited to a single exposure level precluding the evaluation of dose-response. The lowest dose leading to bisphenol A-induced organizational effects in the brain was 10 μg/kg-bw per day in CD-1 mice. It should be recognized that studies were conducted using the same outbred strain of mice (CD-1), the same experimental dosing protocol (single dose, no positive control) and by the same group of researchers in one research institute." (65); and "While collectively these studies provide evidence that exposure to bisphenol A during gestation and early postnatal life may be affecting neural development and some aspects of behaviour in rodents, the overall weight of evidence was considered limited from the perspective of rigour (e.g., study design limitations such as conduct of behavioural assessments at a single time point); power (e.g., limited number of animals per test group), corroboration/consistency (limited consistency of studies) and biological plausibility (e.g., certain studies involve use of a single dose, lack of dose response relationship). These limitations make it difficult to determine actual significance of findings to human health risk assessment." (71)
EU RAR (2008): "Single BPA treatment group" and "Behavioral testing conduced according to acceptable techniques. Analysis of results used appropriate statistical unit." (110)

- Ryan BC and Vandenbergh JG (2006) *Horm Behav.* Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice. 50(1): 85-93.

Ryan and Vandenberg (2006, described under *Acceleration of puberty in female rodents*) examined short-term spatial memory and anxiety. One week after weaning on PND 21, female offspring were ovariectomized; after a two week recovery period, each animal was subjected to behavioral testing. Two anxiety tests were conducted: the elevated-plus maze and the light/dark preference chamber. All animals were tested in both apparatuses (n = 14). In the elevated-plus maze test, animals from the high-dose BPA group spent slightly less time in the open arms than the controls but statistical significance was not attained. Animals from the estradiol group spent significantly less time in the open arms than did the controls. In the light/dark preference chamber, animals from both the high-dose BPA and estradiol groups spent significantly less time in the lighted section than the controls. The authors stated that the results were consistent with an increased level of anxiety. Short-term spatial memory was assessed by the radial-arm maze and the Barnes maze. Each animal was tested in both assays (n = 16). Overall performance in both mazes by animals from the BPA groups did not differ significantly from that of the controls.

The estradiol treated animals had significantly fewer errors in both mazes than did the controls.

Relevant comments on Ryan and Vandenberg *et al.*:
FDA: With regard to the neural studies, FDA noted several positive features of this study including the use of two doses via the oral route of exposure, use of a positive control, general toxicity measurements (bw, litter size), concomitant endocrine endpoints evaluated in the study (puberty, estrus cyclicity, AGD), and use of ovariectomized female to eliminate confounders due to cycling. Limitations of the study included lack of details on the number of replicates, lack of culling of litters, unusually small average size litters, lack of planned statistical analysis, confounding environmental estrogens, measurements of toxicity were made in at the time of weaning (lack of information about pup mortality or transient changes in pup body weight that could have occurred during the first three weeks of neonatal life which is considered more meaningful information), the same female offspring were used for both tests of "anxiety" and another set of animals was used for both tests of spatial memory, raising questions of 'test-test' interactions and differences between treated and control mice, the author's discussion of the light/dark preference test overreaches the observations to a conclusion of anxiety, especially as it relates to humans, there was no indication that the observer(s) scoring the behaviors was (were) blind to the experimental treatment of the test animals, and there were no concomitant endocrine or morphochemical measures with which to correlate the reported reproductive and behavioral effects of BPA.
CERHR: "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. This study is adequate and of high utility for the evaluation process with the exception of the pubertal data." (222)
EU RAR (2008): "…the studies by Farabollini *et al*. (1999) and Ryan and Vandenbergh (2006) provide evidence of increased anxiety in rats (males and females, hole board test) and mice (females), respectively, at doses levels of 0.04-0.4 mg/kg/day, but evidence of decreased anxiety in male rats (elevated plus maze) was also seen in the study of Farabollini *et al.* (1999) and no evidence of an effect on anxiety in males was reported by Negishi *et al*. (2004) at similar dose levels. Overall, there does not appear to be a consistent pattern across species and gender in the results of the tests for anxiety." (117)
Norwegian Scientific Committee: "A positive control, two dose levels of BPA and some parameters on reproductive toxicity were included in the study design. However, the reproductive parameters were assessed at weaning and not at delivery, which is an incomplete assessment. The test animals were ovariectomised females only which excludes evaluation of possible sex differences in response to BPA or EE exposures. Additionally, even if the use of ovariectomised mice removes the potential confounding factors of cyclicity on behaviour, it also eliminates the evaluation of possible hormonal interactions of the test substance that may influence on behaviour." And "Effects interpreted as anxiety-related behaviour was only shown in one (light/dark) of two tasks and only in mice exposed to the highest BPA dose. There is concern about the lack of

information about how the data were recorded (e.g. manually or automatically) in all the behavioural tests." (16)

- Ceccarelli I, Della Seta D, Fiorenzani P, Farabollini F, Aloisi AM (2007) *Neurotoxicol Teratol.* Estrogenic chemicals at puberty change ERalpha in the hypothalamus of male and female rats. 29(1): 108-115.

Male and female Sprague-Dawley rats (n = 14/sex) were orally administered 40 µg BPA/kg bw/day or the peanut oil vehicle using a micropipette on PND 23-30, inclusive. A concurrent positive control group was treated with 0.4 µg ethinyl estradiol/kg bw/day. The rats had been trained to drink the oil from the micropipette. Half of the animals were sacrificed on PND 37 and the remainder on PND 90. At sacrifice, blood was collected for hormone assays and the animals were perfusion fixed. Coronal sections of the brain were incubated with estrogen receptor ERα rabbit polyclonal antibody. ERα immunoreactive cells were counted in selected hypothalamic areas including the arcuate nucleus, ventromedial nucleus, and medial preoptic area. On PND 37, an increased number of ERα labeled cells was observed in the arcuate nucleus from BPA treated males and females and in the ventromedial nucleus of females compared to the controls. On PND 90, BPA treated females had a higher number of labeled cells in the medial preoptic area than the treated males but not compared to the female controls. Plasma testosterone levels were significantly decreased in BPA treated males on PND 37; no other treatment-related differences in hormone levels were found (data presented graphically).

Relevant comments on Ceccarelli *et al*.:
FDA: FDA noted the following positive attributes of this study: oral administration, use of a positive control, examination of both sexes at two different periods of development (PND 37 and 90), defined procedures and criteria for ERα analysis, blinded analysis, and concomitant analysis of hormonal blood level (testosterone and estradiol). Limitations of the study included single doses for both BPA treatment and positive control, lack of details regarding how littermates were treated and the assignment of animals to treatment group, lack of control of potential dietary/environmental estrogens, and abrupt changes in the housing environment (social conditions and light/dark cycle) were made immediately after dosing which could have had uncontrolled confounding effects on the study. Due to the limitations of this study, particularly in its experimental design, this paper is of limited utility in determining an assessment for oral exposure to BPA.
CERHR: "This interesting and novel manuscript examined the potential for the ethinyl estradiol positive control and bisphenol A administered prior to puberty, but after the most sensitive period (i.e., PND 3–10), to modulate ER and steroid hormones during puberty and sexual maturity. It appears that the authors tried to remove the potential for bias by blinded quantification of ER-positive neurons. The oral route of exposure was relevant. These data must be linked functionally to the results of Della-Seta et al., 2006 (*369*). A weakness is that hormonal measurements were taken at single time points. These data are adequate and of high utility for the evaluation process." (170)

Environment Canada: "Although these effects may be considered biomarkers of exposure to bisphenol A, and potential precursor events to adverse effects, the biological relevance of these effects for purposes of human health risk assessment is not known." (63)
EU RAR 2008:"Single BPA treatment group." "A mechanistic study of limited value for hazard assessment. Analysis of results used appropriate statistical unit" (110)

- Della Seta D, Minder I, Belloni V, Aloisi AM, Dessi-Fulgheri F, Farabollini F (2006) *Horm Behav.* Pubertal exposure to estrogenic chemicals affects behavior in juvenile and adult male rats. 50(2): 301-307.

Male Sprague-Dawley rats (n = 7-10) were orally administered 40 μg/kg bw/day of BPA or the peanut oil vehicle using a micropipette on PND 23-30, inclusive. A concurrent positive control group was treated with 0.4 μg ethinyl estradiol/kg bw/day. The rats had been trained to drink the oil from the micropipette. On PND 45, animals were tested for social and non-social behavior to an object placed in the cage (4 animals/cage) and on PND >90 they were tested for sexual behavior. Animals not used for behavioral testing were sacrificed on PND 37 or 105 and blood collected for hormone determination. Body weight was recorded every two days. Body weight was not affected by treatment. In juvenile animals (PND 45), significantly lower frequencies ($p = 0.01$) of the behaviors grouped under elements directed to the object placed in the cage (biting, sniffing, climbing) were found in animals treated with BPA and ethinyl estradiol. Sexual behavior was clearly affected in animals treated with ethinyl estradiol as noted by increased frequency of intromission, decreased latencies for mount and intromission, decreased duration of genital sniff, and an increase in the refractory period. A similar trend for most endpoints was found in BPA treated animals with statistical significance attained only for intromission latency. Plasma testosterone levels in the BPA treated animals were significantly lower than the control and ethinyl estradiol treated animals at PND 37 and 105. No differences in plasma estradiol levels were found between groups at any timepoint.

Relevant comments on Della Seta *et al.*:
FDA: FDA noted the following positive features of the study: experimental rationale was clearly explained, compounds were administered orally, a rationale was provided for the dose of BPA administered, and a positive control was included. Limitations of the study included use of only a single dose of BPA and the positive control compound, lack of details regarding assignment of animals or use of littermates, potential dietary/environmental estrogen contamination, abrupt changes in the housing environment (social conditions and light/dark cycle) were made immediately after dosing which could have had uncontrolled confounding effects on the study, details regarding tests for adult socio-sexual behaviors were limited as were the criteria for definitions (such as 'receptive'), lack of sexual activity in all three groups however (Discussion section contradicts Results in stating BPA and positive control related), and overstatement of results with regard to altered patterns of sexual behavior for BPA (only one (decrease in intromission latency) of six measures were affected by treatment with BPA; all six were affected by treatment with ethinyl estradiol). In the absence of

appropriate dose-response information and valid correlative behavioral data it is difficult to interpret the biological significance of any of the treatment related changes reported in this study or to extrapolate their significance to humans. Due to the limitations of this study, particularly in its experimental design and the inexplicable low proportion of adult animals achieving ejaculation within 30 min of testing, this paper is of limited utility in determining an assessment for oral exposure to BPA.
CERHR: This study was well-conceived and executed. Appropriate dosing periods, design, and testing methods and timeframes were used to capture developmental effects of pubertal bisphenol A exposure of a short-term (juvenile period) and long term (into adulthood) nature. Sample sizes were adequate. This paper is adequate and of high utility for use in the evaluation process. (169)[99]
Environment Canada: "In addition, in Sprague-Dawley rats, pubertal exposure to oral doses of 40 µg/kg-bw per day altered behaviour of male rats (Della Seta et al. 2006). These studies, again conducted by a common group of researchers, provide convincing evidence of bisphenol A-induced effects at 40 µg/kg-bw per day and illustrate the implementation of a comprehensive approach. Replication of the aforementioned results by independent researchers is needed." (65)
EU RAR 2008: "Some marginal differences in play and sexual behavior. Possibly inappropriate statistical methods. Single BPA treatment group. Behavioural testing conducted according to acceptable techniques. (110) and "A number of studies, notably most of the behavioural studies from the Italian team (…Della Seta *et al*. 2006…), used just one exposure level of BPA and so there is no opportunity to evaluate observed differences between control and treated groups in the light of a dose response assessment. Consequently, confidence in the validity of claims of a causal effect of BPA exposure is reduced." (115)
EFSA (2006) - Cited but not discussed.

- Palanza PL, Howdeshell KL, Parmigiani S, vom Saal FS (2002) *Environ Health Perspect.* Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. 110(Suppl 3): 415-422.

Palanza *et al* (2002) examined the effects on perinatal BPA exposure in two generations of mice. Briefly, CD-1 pregnant mice were micropipette fed vehicle (n=14) or 10 µg/kg bw/day BPA (n=9) from GD 14-18. Adult offspring (2-2.5 months of age) were timed mated and fed the same treatment using the same protocol. This resulted in four groups: oil-oil (n=20), BPA-oil (n=15), oil-BPA (n=15), and BPA-BPA (n=15). Mice were fed Purina 5008 (soy-based) chow during pregnancy and lactation and Purina 5001 (soy-based) after weaning; water was supplied in glass bottles. Maternal behavior was examined on PNDs 2-15 and included time in nest, nursing, licking of pups, nest building, eating/drinking, grooming, activity, resting, and forced nursing. Additionally, nest-related behavior and out-of-nest behavior were also evaluated based on the data collected. Behavior examination occurred during the dark period using a 25-W red light and included examination of each dam once every 4 minutes for a total of 30

[99] Ibid.

observations. Postnatal development was evaluated at multiple time points and included pups per litter, ratio of males to females, body weight (PNDs 3, 5, 7, 9, and 15), and, for a subset of litters, cliff-drop and righting reflexes on PNDs 3, 5, 7 and 9 were measured. No effect was noted on maternal body weight, pups per litter alive on day of birth, the sex ratio of pups, body weight at birth, and cliff-drop aversion behavior. Righting reflex tended to take longer in BPA-oil dams offspring as compared to control (oil-oil) on PNDs 3 and 5. Observations included alterations (as compared to controls) in percent incidence of nursing, resting alone, nest related, nest building, grooming and out of nest behavior for BPA-oil and oil-BPA groups. Percent activity was only affected in the oil-BPA group. However, only the resting alone measurement was affected by BPA-BPA treatment; all other BPA-BPA treatment measurements were comparable to controls. No effects were noted on eating, in-nest licking or forced nursing.

Relevant comments on Palanza *et al.*:
FDA: FDA notes that this study used oral exposure and a positive control, a large number of dams (15-20/dose/group), all culled pups from 8 litters/treatment group were evaluated for body weight and sensory/motor behaviors, and litter was used as the statistical unit. Limitations in the study include the use of only one dose for both BPA and the positive control, the interpretability of the study design and results to continuous exposure, lack of blind measurement and no comment on equal testing of behavior parameters across treatment level, and the authors' interpretation of the small changes in behavior as being adverse as with regard to the measurements of individual indexes. For example, effects were cited on changes in the incidence of nursing; however, there were no associated effects on measured developmental parameters of offspring indicating that no adverse effect on development resulted from this changed behavior of the dam. Noteworthy only one of the observed behaviors, resting alone, was affected following BPA-BPA treatment, as this treatment regime would be most applicable to human exposure, the applicability of the other treatment groups are unclear. The authors do discuss the lack of findings in BPA-BPA animals as possibly related to a shift in homeostatic mechanisms. The authors concluded a change occurred in righting reflex, but this was time dependent, and was unaffected at the last measurement (PND9). Although this study used a large number of animals, the study design, use of a single dose of BPA, observations of findings primarily in animals which did not consistently receive BPA treatment, and the lack of observation of concomitant adverse outcomes, limit its utility in determining an assessment for oral exposure to BPA.
CERHR: "Strengths are the oral route of administration, the low dose level of bisphenol A, and the exploration of effects on complex maternal behaviors. It is unusual that pre- and postnatal exposure had effects but not the combination of pre- and postnatal exposure, and failure to explain this finding is a weakness. The use of a diet high in soy isoflavones is an additional weakness. This paper is adequate and of high utility for the evaluation process." (198)
EFSA (2006): The study is summarized on page 40, but no discussion of study is found in the document.
Environment Canada: "… these studies, though, were limited to a single exposure level precluding the evaluation of dose-response. The lowest dose leading to bisphenol A-

induced organizational effects in the brain was 10 µg/kg-bw per day in CD-1 mice. It should be recognized that studies were conducted using the same outbred strain of mice (CD-1), the same experimental dosing protocol (single dose, no positive control) and by the same group of researchers in one research institute." (65)
EU RAR 2008: "The lack of consistency between the effects seen the groups exposed either prenatally or as an adult and the group exposed during both periods suggests that these intergroup differences were unlikely to have been caused by BPA exposure." (316); "No convincing evidence of an effect on nursing behaviour. Possibly inappropriate statistical methods. Small group size. Behavioural testing conducted according to acceptable techniques." (329); "A number of studies, notably most of the behavioural studies from the Italian team (…Palanza *et al*. 2002…), used just one exposure level of BPA and so there is no opportunity to evaluate observed differences between control and treated groups in the light of a dose response assessment. Consequently, confidence in the validity of claims of a causal effect of BPA exposure is reduced." (115); and "In mice, Palanza *et al*. (2002) found no convincing evidence of an effect on maternal nursing behaviour in females exposed during the prenatal period and/or as adults at 0.01 mg/kg/day." (117)

- Adriani W, Seta DD, Dessi-Fulgheri F, Farabollini F, Laviola G (2003) *Environ Health Perspect.* Altered profiles of spontaneous novelty seeking, impulsive behavior, and response to D-amphetamine in rats perinatally exposed to bisphenol A. 111(4): 395-401.

Adriani *et al.* (2003)[100] examined the long-term effects of perinatal exposure to BPA on later complex behavior in adult rats of both sexes. Female SD rats, 9 per dose, were orally administered BPA (0.04 mg/kg) or vehicle (arachis oil) by micropipette from mating to weaning (PND 25). One male and one female offspring (9/sex/dose) were selected and tested for novelty preference during adolescence (PND 30-45) and for impulsivity and response to amphetamine (4~5/sex/dose) after reaching adulthood (PND > 70). The authors concluded that perinatal exposure to BPA increased novelty-induced stress (less decreased activity with time) during adolescence in both sexes compared to the controls (novelty preference test results). BPA also produced a reduction of time spent in the novel environment in adolescent females, meaning increased neophobia. Males were unaffected by treatment. With regard to the impulsivity test (a reduced ability to tolerate a delay of gratification), the authors concluded that perinatal exposure to BPA increased preference for the large and delayed rewarding or large reinforcement (LAD) during the entire experiment, indicating decreased impulsivity, in BPA-treated animals in both sexes. Moreover, as the delay time increased for the preferred LAD, BPA-treated males resulted in reduced inadequate responding (a response results in no punishment or rewarding) compared to the controls, indicating reduced impulsive behavior. No such effect was observed in females. Open-field test and response to amphetamine (AMPH): the authors concluded that perinatal exposure to BPA

[100] In Adriani *et al*., FDA notes that the results are inconsistent between the figures and text. This was corrected in the erratum published in 2005.

significantly reduced both AMPH-induced elevated crossing and rearing activities in males (n=4~5). Females were unaffected.

Relevant comments on Adriani *et al.:*
FDA: FDA notes that the study used oral exposure and that treatment covered mating, pregnancy and lactation. The study is limited by the fact only a single dose was utilized, the study lacked a positive control, and although nine pregnant female rats were used in the experiment, only one male and one female pup are selected from each litter for later complex behavior tests and the selection process is unclear. Additionally, the study lacked any additional concurrent measurements of toxicity, neurochemical or endocrine endpoints and some effects in alteration of behavior responses, such as reduced response to inadequate responding, are not considered as an adverse effect in function. Therefore, this study is limited in its utility in determining an assessment for oral exposure to BPA.
CERHR: "This study used protocols that are well established by this group. The use of only a single exposure level of bisphenol A is a weakness, with the proviso that the dose used is directly comparable to other studies. The degrees of freedom reported for behavioral measures suggest inflation of sample size due to failure to account for multiple time sampling. The paper is inadequate for evaluation due to inappropriate statistical procedures." (167-168)[101]
EFSA 2006: "In summary, BPA at low doses given during gestation and/or lactation is reported to cause effects on some of the behavioural endpoints assessed. Overall, however, there were no consistent treatment-related effects in the behavioural endpoints and apparently contradictory observations were published. For example, neophobia was found as an effect in one study (Adriani *et al.*, 2003) in females and not in males. In other studies (Negishi *et al.*, 2003), no effect was found in the open field (which should show an effect if neophobia is present) in male offspring, and BPA was also reported to abolish and invert the sexual differentiation in the open field (Kubo *et al.*, 2003). Moreover, the Panel noted the absence of positive controls, use of test paradigms which are not widely used, lack of assessment of dose-response in several studies, partial lack of information on blinding of investigators to status of animals, and insufficient information on food consumption in some studies. The neurobehavioural database reveals that there are no consistent adverse effects of perinatal exposures to doses of BPA below 50 mg/kg/day. The reported influence of low doses of BPA on the sex difference in morphometric measurements of the locus coeruleus should be considered as a preliminary finding that needs to be repeated in a larger study, with the litter as the experimental unit, blinded evaluation and comparison to historical control data. (42)
Environment Canada: "These studies, again conducted by a common group of researchers, provide convincing evidence of bisphenol A-induced effects at 40 μg/kg-bw per day and illustrate the implementation of a comprehensive approach. Replication of the aforementioned results by independent researchers is needed." (65); "These studies, using standard testing paradigms, provide evidence for altered stimulated responses following bisphenol A administration and are outlined in Appendix D." (67); and "At 40

[101] FDA notes that the interpretation of the study methodologies by the committee was aided by personal communication.

µg/kg-bw per day, a small number of studies in rats have reported behavioural effects including gender-specific changes in sexual performance, effects on active and passive maternal behaviour, and altered novelty seeking and impulsive behaviour in both sexes in adults whose mothers were administered bisphenol A during gestation and lactation (Farabollini et al. 2002; Della Seta et al. 2005; Adriani et al. 2003)." (71)
EU RAR 2008: "Small group size. Single BPA treatment group. Behavioural testing conducted according to acceptable techniques. Analysis of results used appropriate statistical unit." (110) and "A number of studies, notably most of the behavioural studies from the Italian team (... Adriani *et al*. 2003…), used just one exposure level of BPA and so there is no opportunity to evaluate observed differences between control and treated groups in the light of a dose response assessment. Consequently, confidence in the validity of claims of a causal effect of BPA exposure is reduced. However, it is noted that the reduced novelty seeking seen in females (Adriani *et al*. 2003) was not confirmed by changes in open field behaviour in females in the same study or in other studies." (115)
Norwegian Scientific Committee: "No positive control, no dose-response to BPA, and no parameters on reproductive toxicity was included in the study design. Concerning the dosing of BPA, it is not known whether the concentration given is per kg oil or per kg body weight of rats. The authors state that the administered dose is "within the range of human exposure". Based on this, VKM will assume that the dose is given as mg/kg bw/day. No control of cyclicity in females was included in the study, and thus not adjusted for in the statistical analysis. It is known that motor activity varies with the cyclic period in females with a peak phase of activity that corresponds to the cornification phase of estrus. Statistics: Results were analysed by 3-4 ways ANOVA. A repeated measure design was presumably added to the ANOVA when repeated measures from the same rat were utilized." (13)

- Carr R, Bertasi F, Betancourt A, Bowers S, Gandy BS, Ryan P, Willard S (2003) *J Toxicol Environ Health A*. Effect of neonatal rat bisphenol A exposure on performance in the Morris water maze. 66(21): 2077-2088.

Carr *et al.* (2003) examined the effects of postnatal exposure to BPA in male and female rats on spatial cognitive function using the Morris water maze. Fischer 344 parents were placed on a casein based rodent chow (Purina Test Diet 8117) two weeks prior to breeding. Female rats were kept on this diet during pregnancy and lactation period. The pups were also kept on this diet from PND 22 throughout the duration of the study. The pups (10/sex/dose from different litters for each dose group) were orally gavaged with safflower oil (vehicle); 72 µg/kg bw/day E2; 100 µg/kg bw/day BPA (low); and 250 µg/kg bw/day BPA (high) in a volume of 0.5 ml/kg from PND 1 (the day of birth as PND 0) to PND 14. On PND 34, the pups were tested for 7 days; first 4 days were acquisition phase (spatial learning and memory) and followed by a 3-day probe trial. Body weights were unaffected in E2 or BPA treated animals. Low BPA and E2 treatments diminished the gender-dependent pattern of acquisition of maze performance (effects were observed on males as in general the males perform better than females at this task). Treatment with 250 µg/kg bw/day BPA appeared to impair the retention of spatial information. The

authors concluded that neither postnatal exposure to E2 nor BPA negatively affected acquisition of Morris water maze performance (compared to the controls in same sex). However, the normal gender-dependent differences in Morris hidden platform acquisition performance in prepubertal rats were disrupted by low BPA and E2, but not high BPA. High BPA decreased the retention of the spatial information in probe trial performance.

Relevant comments on Carr *et al.*:
FDA: FDA notes that the study used the oral route of exposure, a positive control and two dose levels were evaluated and that exposure to environmental estrogens in diet was considered in this study. Additionally, the study examined body weight as an indicator of toxicity. However, some observed changes were not statistically significant. The significant change was shown in one dose level but without a dose-response pattern. More significantly, cross-contamination of pups with BPA or E2 may have been possible as pups were treated with different compounds and/or dosages but stayed in the same litter. Additionally, the study lacked any additional concurrent measurements of toxicity, neurochemical or endocrine endpoints. FDA concludes that this study is limited in its utility in determining an assessment for oral exposure to BPA.
CERHR: "Strengths are the additional behavioral dimensions captured by this paper and the use of a positive control. The analyses appeared appropriate. The within litter dosing design raises concerns about cross-contamination which would decrease differences between groups and challenge interpretation of results of non-standard dose-response curves. Analyses did not account for the repeated measures design, thus inflating degrees of freedom. A weakness is the limited number of endpoints investigated. This study is considered inadequate because of the limitations noted." (169)
Environment Canada: A discussion is provided following the citation of Carr *et al.* 2003 "While collectively these studies provide evidence that exposure to bisphenol A during gestation and early postnatal life may be affecting neural development and some aspects of behaviour in rodents, the overall weight of evidence was considered limited from the perspective of rigour (e.g., study design limitations such as conduct of behavioural assessments at a single time point); power (e.g., limited number of animals per test group), corroboration/consistency (limited consistency of studies) and biological plausibility (e.g., certain studies involve use of a single dose, lack of dose response relationship). These limitations make it difficult to determine actual significance of findings to human health risk assessment." (71)
Norwegian Scientific Committee: "Although positive control and two dose levels of BPA were included in the study design, the exposure regimen in which all dose groups were represented in each litter leave behind huge uncertainties about the results. Presumably only 10 litters were used totally. Test animals in different treatment groups were littermates. There was no verification of pup exposure, e.g. chemical analysis of blood or tissue residues included in this study. Thus, the cause of the behavioural differences which appeared is unclear. Less emphasis is therefore placed on this study." (14)

- *FDA conclusions regarding neurotoxicity data:*

As summarized in the assessment and in Appendices 1 and 2, many of the studies reviewed appear to suggest that developmental BPA treatment can cause alterations in

brain development and behavior; however, the limitations noted for individual studies as stated above, ranged from mild to severe. The majority of the studies appeared focused on mechanism testing, rather than safety assessment, and many of the study authors did not clearly define the criteria used in the analysis and had a tendency to inappropriately anthropomorphize behaviors or make exaggerated conclusions regarding the relevance of the results shown. The endpoints examined in these studies (behavioral changes related to stress, pharmacological challenges, and sexual dimorphism) represent an emerging area in developmental neurotoxicity for which validated protocols are currently unavailable. Noteworthy, the studies commented on above by the Norwegian Scientific Committee are studies that were examined as meeting the criteria outlined in the draft OECD protocol for neurotoxicity[102]; however, these studies were limited in their findings or protocols such that they are insufficient for defining a NOAEL for this endpoint. Major limitations of many of the studies reviewed in this area included a lack of concurrent examination of endpoints used for validating findings (histomorphologic evaluations, hormonal analyses, or neurochemical assessments with which to correlate the treatment-related behavioral effects of perinatal BPA exposure and vice versa) or examining only one sex. Studies demonstrating BPA-related changes at the molecular level with regard to receptor distribution are interesting from an investigational point of view, but do not readily lend themselves to regulatory decision making. These data collectively suggest that more research, using validated studies with feeding protocols modeling human exposure are necessary prior to establishing a NOAEL for this endpoint for use in regulatory safety assessments.

[102] OECD Guideline For The Testing Of Chemicals Draft Proposal For A New Guideline 426 Developmental Neurotoxicity Study accessed at http://www.oecd.org/dataoecd/20/52/37622194.pdf

Appendix 3: References

Although the text of the assessment does not explicitly cite primary literature and other sources of information, the referenced FDA memoranda include citations of all reviewed information. These memoranda and their cited references are listed below. Additionally, during the course of drafting and Intra- and Interagency review of this document, several inconsistencies to the referenced FDA documents were noted. For completeness of the record and to avoid confusion, these are clarified in FDA Memorandum Twaroski/FAP 8T4773, 08/12/2008.

FDA memoranda - Memorandum to the File. Update on cumulative exposure to BPA for infants from epoxy-based container coatings and polycarbonate (PC) bottles in contact with infant formula. Verbal request dated 4/29/08. Bailey/Twaroski, 06/02/2008.

Chemistry memorandum dated 8/13/01 (A. Bailey to M. Cheeseman).

Chemistry memorandum on FAP 9Z4681 dated 5/2/00 (K. Paquette to J. Smith).

Memorandum to the File dated 3/13/96 (A. Bailey to G. Diachenko).

Biles, J., McNeal, T., Begley, T., Hollifield, H. (1997) Determination of Bisphenol-A in Reusable Polycarbonate Food-Contact Plastics and Migration to Food-Simulating Liquids, *Journal of Agricultural and Food Chemistry* 45(9): 3541-3544.

Biles, J., McNeal, T., Begley. (1997) Determination of Bisphenol-A Migrating from Epoxy Can Coatings to Liquid Infant Formula Concentrates. *Journal of Agricultural and Food Chemistry* 45(9): 4697-4700.

"Baby Alert: New Findings About Plastics," *Consumer Reports*, May 1999, pp. 28-29.

Takao Y, Lee HC, Ishibashi, Y, Kohra S, Tominaga, N, Arizono K, (1999) Fast Screening Method for Bisphenol A in Environmental Water and in Food by Solid-Phase Microextraction (SPME) *Journal of Health Science* 45:39.

Cited by NET as unpublished results by Iguchi of Yokohama City University, Japan.

NTP Draft Brief published on April 14, 2008 by the U.S. National Institutes of Health. (NIH)

Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials In Contact with Food on a request from the Commission related to 2,2-bis(4-hydroxyphenyl)propane (Bisphenol A), Question number EFSA-Q-2005-100, *The EFSA Journal*, 2006, 428:1-75.

Simoneau C, Roeder G, and Anklam E. (2000) Migration of bisphenol-A from baby bottles: effect of experimental conditions and European survey. 2nd International Symposium on Food Packaging: Ensuring the Safety and Quality of Foods (ILSI conference), Vienna, Austria.

Cited in the report as: Hanai Y, (1997) Bisphenol-A Eluted from Nursing Bottles. Unpublished Data, Environmental Science Research Center, Yokohama National University.

Earls A, Clay C, Braybrook J, Preliminary Investigation into the Migration of Bisphenol-A from Commercially-Available Polycarbonate Baby Feeding Bottles. LGC Technical Report LGC/DTI/2000/005.

Brede C, Fjeldal P, Skjevrak I, and Herikstad H. (2003) Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing., *Food Additives and Contaminants* 20:684-9.

Tan B and Mustafa AM. (2003) Leaching of bisphenol A from new and old babies bottles, and new babies feeding teats. *Asia Pacific Journal of Public Health,* 15:118-23.

A study of the migration of bisphenol A from polycarbonate feeding bottles into food simulants, Central Science Laboratory Test Report L6BB-1008 for the Boots Group, 2003 (available at www.boots-plc.com/environment/library/250.pdf).

European Commission (2002), "Final opinion of the Scientific Committee on Food on Bisphenol A," April 17, 2002, SCF/CS/PM/3936 (http://ec.europa.eu/food/fs/sc/scf/out128_en.pdf).

European Union Risk Assessment Report. Bisphenol A, CAS No: 80-05-7. Institute for Health and Consumer Protection, European Chemicals Bureau, European Commission Joint Research Centre, 3rd Priority List, Luxembourg: Office for Official Publications of the European Communities.

Kuo H and Ding W. (2004) Trace determination of bisphenol A and phytoestrogens in infant formula powders by gas chromatography-mass spectrometry. *Journal Chromatography A,* Vol. 1027: 67-74.

A Survey of Bisphenol A in US Canned Foods, dated 3/5/07 (available on the EWG website).

Goodson A, Summerfield W, and Cooper I, (2002) Survey of bisphenol A and bisphenol F in canned foods. *Food Additives and Contaminants* 19:796-802.

Personal communication between A. Bailey and T. Begley (HFS-706), May 2008.

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Bisphenol A: A reproduction and fertility assessment in CD-l mice when administered via subcutaneous silastic implants (NTP 84-105)

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