Memorandum

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From: Bisphenol A (BPA) Joint Emerging Science Working Group
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Subject: Updated Review of the 'Low-Dose' Literature (Data) on Bisphenol A (CAS RN 80-05-7) and Response to Charge Questions Regarding the Risk Assessment on Bisphenol A

To: FDA Chemical and Environmental Science Council (CESC)
Office of the Commissioner
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BPA Interim Review

Introduction

In September 2008, US Food and Drug Administration (FDA) requested a review of the draft document entitled, *Draft Assessment of Bisphenol A for use in Food Contact Applications,* by a subcommittee of the FDA Science Board. FDA presented the findings of this review at a public meeting on September 16th, 2008. Subsequently, the FDA Science Board met and released its comments on the draft assessment (accessible at http://www.fda.gov/OHRMS/DOCKETS/ac/08/briefing/2008-4386b1-05.pdf).

Comments received related to the need to more clearly define the criteria used in reviewing studies on Bisphenol A (BPA; CAS RN. 80-05-7) and to consider the utility of studies reviewed by the National Toxicology Program (NTP) and their expert panel [Center for the Evaluation of Risks to Human Reproduction (CERHR)] (Chapin et al.,

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2008;NTP-CERHR, 2008) for which a conclusion of 'adequate' utility was reached for endpoints of 'some concern'. As summarized in the CERHR expert panel report, the concern for adult toxicity at low doses was designated as "negligible"[2]. Conversely, the CERHR expert panel and others have concluded that "some concern" exists for developmental toxicity with regard to neural and behavioral effects at low doses typically transferred to humans from food contact articles. The NTP draft Brief extends their "some concern" finding to the prostate gland[3], 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[4].

In response to the Science Board's recommendations, FDA's Center for Food Safety and Applied Nutrition (CFSAN) detailed the criteria utilized in the review and reassessed the studies identified by the Science Board along with other as relevant studies that became available after the NTP report was finalized. These reviews were detailed in two memoranda released in the docket in 2010 (FDA, 2009a;FDA, 2009b). (The first memorandum (dated 08/31/2009) was also peer reviewed by five non-FDA government scientists prior to its release in the docket.)

As noted in these memoranda, CFSAN considered a study useful for hazard identification if it met the criteria detailed by the CERHR as an adequate study.[5] CFSAN considered a study suitable for use in a quantitative risk assessment if additional criteria were met. These additional risk assessment criteria were: route of administration (preferred route oral), adequate sample size ($n \geq 10$ animals/sex/group) and appropriate statistical analysis (consideration of toxicological response); end point measure (validated finding[6]);

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2 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.

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

4 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.

5 CERHR Developmental Toxicity criteria (Chapin et al., 2008): "First, effects related to litter of origin needed to be accounted for in design and statistical procedures. Second, animals needed to be dosed via the dam or directly under individual housing conditions. Concern that multiple exposures within a cage to different animals could cause cross-animal contamination across cage-mates led to the determination that this design was not acceptable. Third, a minimum of 6 animals per treatment condition needed to be used to provide minimal confidence in results. Fourth, if similar tests were conducted at multiple ages, the statistical analyses needed to account for repeated measurement in order not to inflate degrees of freedom." In addition, the CERHR panel weighted more heavily studies using oral exposure and considered results of concurrently employed positive controls. Lastly, as clarified in comments received by a CERHR panel reviewer (E. Gray, email 10/20/2009) no consideration was given to type of effect, lack of effect, or relevance to humans and both oral and SC studies were adequate, but oral studies were considered of higher utility, while SC could be supportive or followed up with an oral study. Noteworthy, the NTP considered several subcutaneous studies pivotal; accordingly, though these studies were deemed 'inadequate' by CERHR in their evaluation, these were also considered as meeting the first level of criteria.

6 As elaborated upon in the cited memorandum, *quantitative* safety assessments rely on in vivo data. Endpoints are limited to validated studies examining physical, behavioral, and pathological observations indicative of a toxicological dysfunction. Protocols not validated for regulatory use concerning mechanistic and in vitro data may not be considered sufficiently interpretable as a toxicity (adverse)

plausibility (relevance to humans/finding in complex system based on totality of information[7]); dose response (inclusion of an adequate number of doses); sex (evaluation of both as appropriate); repeatability (independently observed finding or corroborated by other information); and appropriate control for environmental contamination. The criteria for risk assessment are described further in the referenced memoranda and were used in a weight of evidence approach to determine the utility of the data for performance of a regulatory risk assessment. As noted in the 11/10/2009 review, FDA/CFSAN concluded that the available data, though indicating a need to further evaluate a number of endpoints identified in the hazard identification analyses, were not sufficient to adjust the current no observed adverse effect level (NOAEL) of 5 mg/kg bw/day as derived from two rodent multigenerational studies (Tyl et al., 2002;Tyl et al., 2008). Although CFSAN did not reach a conclusion that suggested a lower NOAEL, the data that were reviewed raised questions concerning the risk assessment with regard to developmental exposures and neurodevelopment and effects on the prostate (predisposition to cancer).

As part of FDA's on-going assessment of BPA, FDA has convened a new panel in January 2011 to review recent data on BPA as part of the BPA Joint Emerging Science Working Group (of the FDA Chemical and Environmental Science Council, CESC) through the Office of the Commissioner. As part of this group, expertise has been drawn from all FDA Centers to assist in the review of updated literature on BPA. Specifically, this group has been tasked with addressing the following questions:

- what hazards should be added or removed from FDA's continuing review/research evaluation;
- what dose/response level for a specific effect/endpoint should be changed and to what level; and
- how new exposure data or improved assessments should be incorporated into risk assessment.

The function of this group is strictly limited to performing a review of the updated data for the purposes of informing the risk assessment for BPA. This process includes updating the hazard identification and quantitative risk assessment (dose response assessment) for BPA.

Methods

The methods employed by the group were similar to those used in the previous 'low dose review', but took into consideration feedback from five non-FDA government reviewers

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endpoint in a quantitative safety assessment but can be included to increase reliability in determination of mode of action, intra/interspecies variability, or for hazard identification. Validated in vitro protocols, such as certain genetic toxicity assays and endocrine activity assays, are also limited to hazard identification and may be used to trigger the need for additional dose response studies applicable to risk characterization. Interpretation of in vitro data as relevant to a safety assessment can be augmented through the use of pharmacokinetic data.

7 As elaborated upon in the cited memorandum: The observation in the animal study must be considered in the context as to: whether there exists a concern for the human correlate (does the endpoint of concern or proposed mechanism of toxicity occur in humans?), the proposed mechanism of action, the cumulative knowledge base, or other findings reported in the analyzed study.

regarding publications and criteria[8]. As such, a weight-of-evidence review was conducted in a three tiered fashion: (1) literature search inclusion criteria, (2) hazard identification inclusion criteria, and (3) risk assessment inclusion criteria. The hazard identification and risk assessment criteria discussed below were used for toxicology/physiology studies. A separate discussion is included for the epidemiology and pharmacokinetic studies.

Literature Search

FDA's previous review of the literature included papers available in-press as of 11/2009. For the current review, PubMed was searched for publications (including those in-press) using the term "bisphenol" from 11/2009 – 01/31/2011. Studies were limited to English language reports, human epidemiology studies and animal studies with direct dosing to mammals (*in vivo*, direct dosing) which included doses of ≤ 5 mg/kg bw/day. Since FDA's last review of the pharmacokinetic literature occurred in 2008, the search for data on this topic was inclusive of any relevant data published on this subject since the previous review. In addition, FDA's previous review posed the following question to reviewers: '*should any studies either be removed from or added for consideration?*' The response of the five non-FDA reviewers was also used in determining which literature to include in the present review[9]. As a second search mechanism, a Dialog search was conducted from 2009 – 2011 using MEDLINE, Embase SciSearch (another name for Web of Science), and Pascal. The CAS RN (80-05-7) or Bisphenol A and various limiting terms[10] were used in this search. In addition, reviews of studies on BPA epidemiology were expanded to include all of 2009 – 2011, keeping in mind that the NTP/CERHR review, along with our previous review of two epidemiology studies (Braun et al., 2009;Lang et al., 2008a) served as a starting point. FDA also would have considered any non-published data submitted directly to FDA but there were none identified via this method.

Hazard Identification (HI)

For the current review, FDA utilized the approach of the previous review, considering the criteria defined by CERHR as adequate/definitive for studies useful for hazard identification (see above and references).

Risk Assessment (RA)

FDA reviewed the comments received from five non-FDA government reviewers and discussed and developed an updated approach to the criteria for risk assessment. These criteria consider the same factors and reasoning [guideline study foundations of Redbook,

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8 The reviewers were asked to comment on the following: Are the studies reviewed as adequate appropriately selected? If not, should any studies either be removed from or added for consideration? Do you agree with the assessments of the studies listed? If not, how would that affect the assessment? If you would suggest any changes in what studies are included, how would that affect the assessment? Do you agree with the summary assessment? Do you agree with the summary, including regarding the reviewed studies and their use and effect for hazard identification and NOAEL determination? Please comment.

9 Several articles noted by reviewers were either review articles, high dose, in vitro or were reviewed in (FDA, 2009a); as such, the relevant suggestions were limited to two publications (Adewale et al., 2009;Somm et al., 2009).

10 Limiting terms included: not vitro, not fish or fishes; mammal? or animal? or human, epidemiology

Organisation for Economic Co-operation and Development (OECD), US Environmental Protection Agency (EPA)] that are detailed in the 'low dose' review memorandum (please see 'low dose review' memorandum for expanded discussion). Accordingly, the updated criteria applied to the current assessment are as follows:

- Route of Administration: studies using direct dosing (oral, subcutaneous (SC), intraperitoneal (IP), intravenous (IV) as well as intramuscular (IM) administration) were considered. Here, factors such as dosing errors, acceptable vehicle and dosing procedures were considered;
- Sample Size and Statistical Analysis: $n \geq 10$ for rodent studies; toxicological response and/or statistically significant result;
- End Point Measure (Validity): the endpoint measured is considered validated by the regulatory community or the experimental protocol utilized has scientific agreement as to its acceptability and there was confidence in the result; relevance of the finding to humans and how the finding relates to other data available in the scientific area of research were considered.
- Dose-Response: some knowledge of dose-response should be presented, with added weight given to studies that investigated a sufficiently wide range of doses including both 'low' and high doses;
- Sex: both sexes should be tested when appropriate for informing the validity or meaning of the data/endpoint measured;
- Repeatability: the results of the study were compared to findings in other laboratories or to complementary endpoints;
- Environmental Contamination: some characterization or consideration of the phytoestrogen content of the diet, any other contamination (such as polycarbonate cages, etc.); these factors should be measured if possible to allow some insight into their contribution to findings.

Additionally, in assessing the relevance of data for use in weight-of–evidence considerations, findings from studies employing non-oral exposures were to be considered for their relevance to hazard identification and risk assessment based on the ability of pharmacokinetic data to inform dose translations. In addition, to the extent possible, data were to be grouped among corroborating experiments to determine if they affected the strengths or weaknesses of findings. Although studies were reviewed individually, collective findings were to be considered for their ability to indicate themes or identify potential hazards.

As noted above, the updated literature review was carried out with the intent to identify any new information that could inform the hazard identification and/or risk assessment (dose response assessment) of BPA. A number of studies identified in the updated literature review reported biological changes/observations that are currently of unknown relevance toxicologically. As part of the multistep review process, these studies were assessed for quality even though the impact of their findings is currently unknown. Moreover, as these were generally considered to be mechanistic studies, where links to adverse effects or pathways leading to toxicity are unknown, they were not considered as meeting the criteria for identification as "hazard". However, FDA scientists recognize that the importance and/or classification of these types of findings may change over time

or may be informative with regard to potential modes of action of BPA. Thus, these studies were classified as relevant for mode of action (MOA) as opposed to HI.

Pharmacokinetic Criteria

Several key elements were considered in the review of BPA pharmacokinetic (PK) studies. These included:

- Analytical methodology sufficiently validated and reported with respect to background (blanks) levels; limit of detection/quantification; accuracy; and precision within the range of concentrations used for the study (i.e., intra- and inter-day variability).
- Measurement of both the conjugated and unconjugated (aglycone or "free") forms of BPA; quality control discussion of deconjugation enzymes (i.e., β-glucuronidase and sulfatase activities).
- Preferred dosing with isotopically labeled BPA to eliminate uncertainties surrounding use of native (i.e., unlabeled) BPA.
- Quality of methods used with the highest weight given to mass spectrometric methods, particularly liquid-chromatography–tandem mass spectrometry (LC/MS/MS), as it provides best signal/noise performance and requires minimal sample preparation (i.e., derivatization reactions).
- Use of isotope dilution quantification (i.e., use of isotopically-labeled internal standards) of at least 3 atomic mass units is preferred because of higher performance.
- Adequate demonstration of quality control in sample preparation and analysis is required (i.e., laboratory reagent and sample collection blanks, matrix spikes at relevant concentrations, authentic standards).
- For determination of pharmacokinetic parameters, samples obtained from individual animals (and humans) are considered more powerful statistically than those derived from pooled/averaged determinations.

Each study was reviewed for its ability to provide novel pharmacokinetic data or inform FDA's on-going efforts to develop physiologically-based pharmacokinetic (PBPK) models for BPA PK.

Epidemiology Criteria

For epidemiology studies, several key elements were considered during their review. These included:

- Utility of study design [cross-sectional, case-control, cohort (prospective)], with more weight given to prospective studies; studies of sufficient size (consideration of uncertainty regarding size and representativeness of study sample and generalizability of results); use of multi-geographical approaches; adherence to proper statistical analyses.
- Measurement of BPA exposure and outcome metrics, with measurement uncertainty due to diurnal, seasonal, and individual variability, as well as possible environmental contamination, impact from lab plastics, interference by other biological compounds, and other factors being weighted in the analyses.
- Appropriate treatment of the data with regard to non-detectables [limit of detection values (LOD)]; adjustment of urinary concentrations (creatinine or

specific gravity, with the use of specific gravity preferred); consideration given to the potential misclassification of LOD values in the confidence of the finding; and consideration of current state-of-the-science with regard to measurement of BPA in different biological matrices.

- Other factors: potential biologically plausible reverse causation; unconsidered confounders, risk factors and effect modifiers; and ascertainment of the correspondence between the measurement time and the relevant exposure window were also to be considered in interpretation of the results.

Each study was reviewed for its ability to inform the HI and RA process for BPA. Links to animal data were used to understand generalizability or justification for the use of specific endpoints.

Literature Review

Based on the updated literature search on BPA, six discrete areas were identified for review: neurotoxicity (including behavior); reproductive and developmental toxicity; carcinogenicity; pharmacokinetics; epidemiology; and other. A summary of the findings of the review and their impact on the current assessment of BPA and a summary of each publication reviewed are included below. In addition, critical aspects of each study are included in tabulated form in the Attachments.

Pharmacokinetic (PK) Studies

Summary

The present evaluation of the PK literature is an update to CFSAN's previous assessment of BPA pharmacokinetics completed in 2008 (FDA, 2007b;FDA, 2007a). As such, 18 publications regarding PK and biomonitoring data suitable for informing the PK of BPA in laboratory animals and humans were reviewed. BPA PK studies can provide important information to help interpret animal toxicity studies when animals are dosed by different routes of administration. First pass metabolism occurs in the gastrointestinal tract and liver after oral administration of BPA, resulting in lowered systemic bioavailability of agylcone (biologically active) BPA. Pharmacokinetic studies in rodents and non-human primates coupled with limited human studies have provided important information for extrapolation of exposure data from laboratory animals to humans. On-going research into BPA dosimetry in the fetus and nursing neonate for both rodents and non-human primates will help to inform human BPA exposure assessments during development.

In order to assess the ability of new research to inform the existing knowledge base of BPA PK, the analytical methods used to measure BPA and its metabolites in biological tissues were critically evaluated because: 1) native BPA is a common laboratory contaminant that can lead to the reporting of erroneous BPA exposure data; 2) analytical methods have advanced over the last few years, with lower limits of detection and quantification; 3) the measurement of both the conjugated and aglycone forms of BPA is required for complete pharmacokinetic evaluations.

As noted in FDA's previous assessment, adjustments in the bioavailable dose are generally required when extrapolating from one route of exposure to another due to first pass metabolism of BPA resulting in the formation of high levels of conjugated BPA (glucuronide or sulfate). For orally ingested BPA (e.g., a 100 µg/kg bw dose), presystemic metabolism of BPA in the small intestine and liver in both young and adult monkeys and adult rats results in similar pharmacokinetic profiles in plasma; however, newborn rats have a much lower capacity for detoxification of BPA through glucuronidation and, therefore, exhibit internal doses of aglycone (active) BPA that are approximately 10-fold higher than those seen in the adult. This suggests that doses producing effects in the neonatal rat cannot be compared directly with those in primates of any age. The choice of which rodent model to use is also likely to be important because the metabolic profiles of infant mice are reportedly different from those of adult mice. A direct comparison of the developmental internal dosimetry (bioavailability) profiles for the BPA aglycone in rats and mice is an outstanding data need. Data from the reviewed papers, along with those from other relevant literature, will be used in the construction of animal and human PBPK models. PBPK models will play an important role in predicting tissue BPA dosimetry for different reproductive states, species, ages, sexes and routes of exposure and eventually in the assessments of human BPA exposure.

Individual Study Reviews

Each study was evaluated with regard to the analytical methods employed based on the parameters identified above under "*Methods*" and the details of this analysis are provided in Table 1. In addition, a summary of each study and its utility with regard to informing the knowledge base on BPA PK or a future PBPK model is presented below.

Human Data

Transfer of bisphenol A across the human placentae (Balakrishnan et al., 2010).

Human placentae obtained from healthy term singleton pregnancies (n = 7) were utilized in a dual recirculating model of *ex vivo* placental perfusion. Placentae were perfused with BPA (10 ng/mL or 44 nM) added to the maternal perfusate for 180 min. The transfer percentage for BPA was 27.0 ± 1.88%, and the transfer index for BPA was 1.1 ± 0.09 after 180 min of perfusion. It was determined that 3.2 ± 1.6% of BPA on the fetal side was in the conjugated form. The analytical methods to measure BPA were adequate.

This study using human tissue ex vivo shows that a relatively high static concentration of unconjugated BPA perfused to the maternal side of the placenta can transfer to the fetal side via passive diffusion. The degree of conjugation on the fetal side was highly variable, with some placentae preparations showing extensive conjugation while others showed none. These results showing placental transfer largely confirm studies in intact rodents but do so under highly artificial conditions (i.e., no maternal metabolism) such that artificially high concentrations of unconjugated BPA provide the driving force for diffusion. Despite the high BPA 'maternal' concentrations, the net flux of BPA into the fetus will be explored using a BPA PBPK model with the possible use of asymmetric

permeability constants to describe the transfer of BPA to the fetus. This study provides qualitative information on phase II metabolism of BPA in the fetus.

Determination of Bisphenol A and its chlorinated derivatives in placental tissue samples by liquid chromatography–tandem mass spectrometry (Jimenez-Diaz et al., 2010)

Human placentae (n = 49) were analyzed for unconjugated BPA and its chlorinated derivatives. Conjugated forms were not analyzed. The analytical methods to measure BPA were adequate but sample collection/storage was uncontrolled.

This study measured unconjugated BPA in 20% of the placental samples collected. No measurement of BPA conjugates was performed so it is not possible to assess the potential contribution of ubiquitous BPA contamination to individual samples. The chlorinated analogs of BPA, derived from putative chlorination of BPA in water-treatment facilities, were also detected in approximately 50% of placenta. The undetectable levels of unconjugated BPA in 80% of samples gives some indication of method reliability for false-positives, but artifactual contamination of the other 20% cannot be ruled out. The difficulty in controlling surgical procedures and the lack of "field blanks" make interpretation of these findings difficult, as is the case for other human tissue biomonitoring studies. These data are considered possibly useful for PBPK modeling.

Placental transport and in vitro effects of Bisphenol (Morck et al., 2010).

In this study, the BEWo cell line was used to study the in vitro effects of BPA and an apparent EC50 of 100-125 μM was reported. Human placentae obtained from term pregnancies (n = 6) were utilized in a dual recirculating model of ex vivo placental perfusion. Placentae were perfused ex vivo with ^{14}C-BPA (500 nM) added to the maternal perfusate for 150 min. A fetal/maternal radioactivity ratio of 1 was achieved after 90 min of perfusion. BPA (19%) was transferred across the placental barrier, with 14 % found in the perfused cotyledon and 13% in surrounding placental tissue. Metabolism to conjugates was not determined. Percutaneous absorption across full thickness human skin was studied using OECD guidelines 428 with a starting concentration of 17.5 mM ^{14}C-BPA for 48 h. Recovery of BPA into the receiving chamber was 13% and the epidermis and dermis contained an additional 7 and 17%, respectively. The radiochemical methods were adequate, but not adequate for discerning both free BPA and its metabolites.

The study focused on the permeability and accumulation of BPA in different tissue constructs. The relatively high concentrations of BPA used as the driving force for both the percutaneous and placental transfer limit the relevance of these findings to realistic human exposures, but do provide mechanistic evidence for the feasibility of transfer of BPA across the placentae. The lack of information about possible metabolism also limits the utility of the study data. This study supports assumptions about type of equations to use in developing PBPK models to describe the transfer of BPA from mother's blood to the fetal blood via the placenta.

Determination of free and total bisphenol A in urine and infants (Volkel et al., 2011).

Ninety one urine samples from 47 healthy infants 1-5 months of age were analyzed for free and total BPA. The authors report that background contamination (0.01 to 0.03 µg/L) occurred in every sample. The authors considered this information in reporting LOD and limit of quantification (LOQ). The LOQ was 0.45 µg/L. BPA was detected above the LOD of 0.15 µg/L in 9% of samples and quantified in 3% of the samples. Total BPA concentrations ranged from <LOD to 17.9 µg/L. The analytical methods to measure BPA were adequate.

This paper provides information on the exposure of infants to BPA as measured by urinary excretion of total BPA which will allow for intake of infants to be compared to adults. The authors recommend caution in interpreting the free BPA levels in urine because of the demonstrated impact of contamination. Since the ratio of free to total BPA was irregular (near a value of 1), either contamination or breakdown of sample may have occurred. These data can be used in a PBPK model to understand exposures to infants.

Hydroxylated polybrominated diphenyl ethers and bisphenol A in pregnant women and their matching fetuses: Placental transfer and potential risks (Wan et al., 2010).

Pregnant women (n = 26), recruited from three hospitals in South Korea were enrolled in the study. Blood was collected during the last trimester with two exceptions of earlier blood draws. Fetal cord blood was collected at delivery (n=28 with twins). Analytical methods are reported elsewhere. The analytical methods to measure BPA were adequate.

These data provide information on the potential exposure to BPA as measured in fetal cord blood and maternal blood well before delivery. However, based on the data presented, it is difficult to distinguish contamination (artifact) from true measurements. These data may be useful in the development of a PBPK model for BPA.

GC–MS analysis of bisphenol A in human placental and fetal liver samples (Zhang et al., 2011).

Human placentae and liver were analyzed for unconjugated BPA (n = 21) and for conjugated BPA (n= 9 placentae and 10 livers). This study measured unconjugated BPA in 86% of the placental samples collected and 78% contained conjugated BPA; similarly, in fetal liver, 57% contained unconjugated BPA and 80% contained conjugated BPA. A number of samples contained greater unconjugated than conjugated levels, which is consistent with contamination by ubiquitous environmental BPA. The issue of background contamination was largely ignored because all “background” samples used for method validation (including chicken heart and liver from chickens, bovines, and swine) contained measurable levels of unconjugated BPA but were not analyzed for BPA conjugates. The values determined in the “Background” samples contained BPA in a similar range (1.8-17 ng/g) to those reported in fetal tissues (1.2-64 ng/g). The measurable levels of unconjugated BPA in 86% of placenta samples and in 57% of liver

samples suggest the possibility of contamination and hydrolysis of conjugates during sample preparation, particularly for those samples showing higher unconjugated than total BPA levels. The analytical methods used to measure BPA were adequate.

The difficulty of controlling surgical procedures and the lack of "field blanks" make interpretation of these findings difficult, and, as such, they need to be treated with caution in terms of utility for PBPK modeling.

Non-Human Primate Data

Pharmacokinetics of Bisphenol A in neonatal and adult rhesus monkeys (Doerge et al., 2010b).

This study reports pharmacokinetics for a relatively low dose (100 µg/kg bw) of orally and intravenously administered deuterated BPA to monkeys of varying age starting on post-natal day (PND) 5 (n = 5-6) and in adults (n = 4). The analytical methods to measure BPA were adequate. Individual PK profiles were collected from neonatal and adult monkeys using both oral and IV routes for absolute bioavailability determinations. In addition, repeated measures of neonatal pharmacokinetics for individual monkeys were obtained on PNDs 5, 35, 70, and 77 to determine individual developmental PK profiles.

This study has high utility for human extrapolation of PK parameters for unconjugated and conjugated forms of BPA, particularly for infants since no such human PK studies are likely to be attempted for ethical reasons. The use of a dose within 2-orders of magnitude of possible human environmental exposures minimizes dose extrapolation issues. The completeness of the data set (developmental, oral vs. IV) makes these data useful for PBPK modeling of infant and adult human internal exposures. The determination of absolute bioavailability in neonatal and adult monkeys of approximately 1% was interpreted as evidence for extensive Phase II metabolism in the GI tract, as observed in rats. The absence of evidence for enterohepatic recirculation of either conjugated or unconjugated BPA is distinct from that observed in rats (see (Doerge et al., 2010a)). The finding of similar capacity for Phase II metabolism of BPA in both infant and adult monkeys suggests lower exposures to the unconjugated form of BPA in newborn primates, relative to newborn rats. This is a critical study due to the experimental demonstration of the differences between neonates and adults and between rats vs primates. In addition, this study can be used for determining specific BPA PBPK model parameters for the adult and infant monkey for extrapolation to infant and adult humans (using scaling).

Similarity of Bisphenol A Pharmacokinetics in Rhesus Monkeys and Mice: Relevance for Human Exposure (Taylor et al., 2011).

Pharmacokinetics of unconjugated and conjugated BPA were evaluated in female rhesus monkeys after oral administration of deuterated BPA (400 µg/kg bw) either as a single dose or repeated doses over 7 days. The pharmacokinetics of unconjugated ^{3}H-BPA in CD-1 mouse serum were also evaluated after oral administration of a single dose of 400

µg/kg bw. The total radioactivity remaining in mouse serum 24 h after oral dosing with 2, 20, 400, and 100,000 µg/kg bw ^{3}H-BPA was also evaluated. The pharmacokinetics of unconjugated and conjugated BPA in female CD-1 mice were also evaluated after oral dosing with 100,000 µg/kg bw native BPA. The analytical methods were adequate, but not well-documented.

The elimination half-lives, C_{max}, and $AUC_{0-\infty}$ values for BPA aglycone and total BPA reported for rhesus monkeys in this study were within an order of magnitude of those previously reported for monkeys (Doerge et al., 2010b;Tominaga et al., 2006). This degree of agreement is perhaps surprising, given the significant differences between the studies in experimental design, analytical methodology used, and method validation information provided in each study. The authors concluded that the pharmacokinetics for the BPA aglycone and total BPA in adult monkeys (as well as female humans) and mice were similar with no significant bioaccumulation demonstrated. These data will be used in the development of a monkey PBPK model for BPA.

Rodent Data

Lactational transfer of bisphenol A in Sprague-Dawley rats (Doerge et al., 2010c).

This study reports on the lactational transfer of BPA after exposure to a relatively low dose (100 µg/kg bw) of orally administered d6-BPA to rats (n = 5 litters). Concentrations of conjugated and aglycone d6-BPA were quantified in milk on PND 7, and in maternal and pup serum on PND 10, all at 1 h following administration via gavage of the dam. Aglycone BPA was detected in all maternal serum and milk samples but not in pup serum. The ratio of aglycone BPA in milk relative to maternal serum was 1.3. Conjugated BPA was detected in all pup and maternal samples. Internal doses to the pups were estimated to be 300-fold lower than in maternal serum and 500-fold lower than those produced by direct oral dosing to PND 10 rat pups. The analytical methods to measure BPA were adequate.

This study has high utility for determining internal dosimetry in neonatal rats from lactational exposures to BPA. These results are paradoxical in that reports in the literature suggest that significant toxicological effects result exclusively from lactational transfer of BPA. Alternatively, in studies that utilize lactational exposure to BPA and observe minimal effects, the findings, or lack thereof, may be attributable to inadequate internal exposures during the critical postnatal period. A BPA model of the lactating rat will be useful for quantifying the lactational transfer of BPA and predicting the resulting free BPA levels in the nursing pups for comparison with the observance of toxicological endpoints.

Pharmacokinetics of bisphenol A in neonatal and adult Sprague-Dawley rats (Doerge et al., 2010a).

This study reports pharmacokinetic parameters for a relatively low dose (100 µg/kg bw) of orally and intravenously administered d6-BPA to rats of varying ages from as early as

PND 3 up to and including adulthood (n = 5-7). The analytical methods to measure BPA were adequate.

This study has high utility for comparison of rat PK parameters for unconjugated and conjugated forms of BPA with those from primates (monkey and human). The use of a dose within 2-orders of magnitude from possible human environmental exposures minimizes dose extrapolation issues. The completeness of the data set (developmental, oral vs. IV) makes these data useful for PBPK modeling of neonatal and adult rodent internal exposures. Linearity of AUC values for doses ranging from 50-200 µg/kg bw validates the use of the selected dose for subsequent pharmacokinetic studies (100 µg/kg bw) for extrapolation to lower human exposures (< 1 µg/kg bw). Clear evidence for enterohepatic recirculation was obtained from the IV administration studies where large re-entry peaks of conjugated, but not unconjugated, BPA were observed. The relatively underdeveloped Phase II metabolic capabilities of neonatal rats, as opposed to the essentially fully developed capacity in monkeys (see (Doerge et al., 2010b)), suggests significantly higher internal exposures to the unconjugated form of BPA in rats relative to primates. These data will be useful for developing a maturing rat BPA PBPK model for comparing internal dosimetry of BPA with the immature human at various ages.

Placental Transfer of Conjugated Bisphenol A and Subsequent Reactivation in the Rat Fetus (Nishikawa et al., 2010).

Pregnant Sprague-Dawley (SD) rat intact uterus preparation (n = 4) was surgically exposed and perfused with modified Krebs-Ringer's buffer (mKRB) containing BPA glucuronide (2 µM) for 20 min and mKRB only for 70 mins. The perfusate was collected at 5 min intervals. The levels of BPA-glucuronide were measured in fetuses (0.09% of total perfused BPA-glucuronide) and levels of unconjugated BPA were measured in fetus (0.005%) and amniotic fluid (0.03%). The analytical methods for measuring BPA were adequate.

The experimental procedure employed--perfused pregnant rat uterus--is technically demanding but useful for developing mechanistic data. A relatively high concentration of BPA-glucuronide was perfused and a sensitive detection method showed that the fetus contained small amounts BPA-glucuronide and much lower amounts of the unconjugated form (near detection limit). Amniotic fluid contained greater amounts of unconjugated BPA but no BPA-glucuronide. This study suggests that while BPA-glucuronide from the maternal circulation does cross the placenta, minimal unconjugated BPA is found in the fetus. This study is useful for helping to formulate PBPK modeling assumptions about the movement of BPA metabolites from mother's blood across the placenta and into the fetus.

Excretion of bisphenol A into rat milk (Okabayashi and Watanabe, 2010).

Twelve week old SD rats (strain Jcl:SD),; Clea, Japan) were mated. Following delivery, pups were culled to 4 males and 4 females on PND4 and a target dose of 100 mg/kg bw was provided to the lactating dams. Dams were separated from the pups 8 hr before

dosing and oxytocin was used to induce milk letdown. BPA was dissolved in dimethylsulfoxide (DMSO; 1 ml/kg bw). After oral administration, dams were milked at 2, 4, 8 or 24 hrs. Five rats, not dosed with BPA were milked and BPA measured. The authors did not detect BPA in the control milk samples. BPA milk concentrations versus time data were presented as means ±SEs. Concentrations ranged from 462 µg/L at 2 hr to about 23 µg/L at 24 hr. The number of rats dosed and/or milked was not provided. The analytical methods to measure BPA were adequate.

This paper is informative since milk concentration versus time course data sets are rare. A validated pharmacokinetic or PBPK model could make use of these data to estimate the fraction of administered dose that would be transferred via milk or a simple method could be employed to estimate the percentage of maternal dose/nursing pup using assumptions about temporal milk yield (volume) per pup. In general, materials with a short half life transfer less into milk than long lived materials, even for moderately lipophilic chemicals. As is already known, this study serves to reiterate the need to characterize the experimental environment by demonstrating BPA contamination of the rat's drinking water from polycarbonate bottles.

<u>Serum bisphenol A pharmacokinetics and prostate neoplastic responses following oral and subcutaneous exposures in neonatal Sprague-Dawley rats (Prins et al., 2011).</u>

Twenty timed pregnant (gestation day (GD)14) SD rats (Hsd:SD), purchased from Harlan Industries yielded 180 male rat pups. Attempts were made to limit exposure to BPA in materials such as cages (new polysulfone solid-bottom cages were utilized), drinking water bottles, etc. Ninety PND3 male pups were individually dosed orally with 10 µg/kg BPA and another 90 PND3 male pups by subcutaneous injection. Powdered BPA, obtained from NTP, was dissolved in 95% ethanol and corn oil. Ethanol concentration was 0.02% of the final vehicle volume. Blood (n=30 per time point) was taken from pups at 0.5, 2 and 3 hours. No details were given on how they managed the sacrifice schedule for this large number of animals. The blood from individual pups was then pooled into groups of n= 3 or 5 per time point and route of administration. The pooled samples (n=3-5 per time point per route of administration) were then analyzed for BPA. Two 0.5 ml aliquots of blood or serum were used in the analysis of BPA and its conjugated metabolites, respectively. The method of blood collection is somewhat unclear as the methods section states that blood was collected after decapitation; however Fig. 1 shows a photograph of a PND3 pup undergoing cardiac puncture to obtain blood. Descriptive kinetics for free BPA and its conjugates are given and compared to another group of rat pups that were similarly dosed and allowed to undergo evaluation of prostate histology. As newborns, male rat pups (n=15-25 per group) were treated using 3 different regimens: no BPA, oral BPA and SC injection of BPA. Although BPA dosimetry differences were found between the oral and SC routes of administration, no dose-dependent changes in prostate responses were observed. The BPA analysis was performed at the Wadsworth Center in Albany, NY. The analytical methods to measure BPA were adequate.

The authors report Cmax and calculated AUC values for only 3 time points after dosing. Although comparing these parameters to each other for this study is valuable, the utility

of these data for use in other dose-response studies is uncertain. Other rat studies indicate that peak concentrations can occur 10-20 min after oral dosing, thus, peak serum concentrations might have occurred before the first sampling time in this study and this would alter both the Cmax and AUC calculations reported here. Nonetheless, using the author's reported dosimetrics, the SC route produced an AUC for free BPA that was 4-fold greater than that observed after oral dosing suggesting that this route of administration would yield a steeper dose-response curve. Of interest is the reported finding that rats pretreated with BPA by either the oral or SC route [3 days of dosing (PNDs 1,3,5)] exhibited similar prostate preneoplastic responses after treatment for 16 weeks with testosterone and estradiol-17B (a critical evaluation of this endpoint is discussed below in the *Carcinogenesis Studies* section). These data are valuable for the development or validation of a rat pup PBPK model for BPA and its conjugate.

Quantification of deuterated bisphenol A in serum, tissue and excreta from adult Sprague-Dawley rats using liquid chromatography with tandem mass spectrometry (Twaddle et al., 2010).

The authors provide details of analytical methods for the measurement of BPA in biological tissues (serum, solid tissue, urine and feces). This methodology was used for the analysis of biological samples for pharmacokinetic studies in both rats and monkeys. Both native and labeled BPA were used in the assays. A serious problem exists in the quantification of low levels of native BPA in any matrix. BPA is extracted from materials used in the preparation of the samples for analysis. This leads to false positive values/artifacts. This is an analytical methods validation paper.

This paper can be considered a standard analytical reference for evaluation of other methods employed in measuring BPA in biological fluids. As such, this paper highlights the difficulties in interpreting the reported low concentrations of native BPA in tissues or serum. As noted above, one concern in using these and other data is careful evaluation and reporting of contamination which may otherwise be reported as false positives (artifact) caused by the sample preparation procedures. Selected data sets from this study will help support a rodent PBPK model for BPA.

Distribution of 14C-bisphenol A in pregnant and newborn mice (Tanaka et al., 2010).

Pregnant mice were administered 14C-BPA (0.46 MBq, no mass-based dose given) by IP injection during gestation (GD 13) and total body radioactivity measured by autoradiography. Kinetics for distribution and elimination of total BPA were compared in maternal tissues, including brain, intestine, liver, kidney, and fetus. The observation of total BPA in brain and fetus was interpreted as evidence of elimination specific barriers to penetration into these organs. No evidence was observed for accumulation in any maternal tissue or fetus and elimination profiles appeared similar. No radioactivity was observed in pups born 4 days after BPA injection.

This study provides some qualitative information that may be useful for PBPK modeling of maternal tissue distribution and the kinetics of elimination for BPA in maternal tissues and the fetus.

In vitro Species Comparison Data

Species difference of metabolic clearance of bisphenol A using cryopreserved hepatocytes from rats, monkeys and humans (Kurebayashi et al., 2010).

In vitro BPA metabolism was studied using cryopreserved hepatocytes from rats monkeys and humans using a high concentration of BPA (20 µM). BPA-glucuronide was identified as the major metabolite, along with BPA sulfate. The total CL_H value for the hepatic formation of BPA-glucuronide plus BPA-sulfate (L/h/kg bw) estimated by well-stirred model with low f_B value produced the following order of rats (3.0) > monkeys (0.68) > humans (0.27). The analytical methods to measure BPA were adequate.

This study shows the metabolic capacity of hepatocytes for the Phase II conjugation of BPA and the authors applied theoretical scaling factors for liver and body sizes of different species to estimate inter-species differences. Although this approach is informative regarding liver capacity to metabolize BPA, interpreting the results requires additional knowledge of the major role of intestinal Phase II metabolism of BPA (or other important physiological functions) available through studies using whole animals. Within the context of a PBPK model, these data are useful in comparing the relative ability of different species to metabolize BPA in the liver and evaluating the contribution of the GI tract to metabolize BPA.

Differences between Human and Rat Intestinal and Hepatic Bisphenol A Glucuronidation and the Influence of Alamethicin on In Vitro Kinetic Measurements (Mazur et al., 2010).

In vitro metabolism to BPA-glucuronide was studied using frozen hepatic and intestinal microsomes from rats and humans using high concentrations of BPA (2.5-65 µM). The study compared BPA metabolism data from humans and rats, as well as from different genders. The analytical methods to measure BPA were adequate.

This study shows the metabolic capacity of hepatic microsomes for the Phase II conjugation of BPA and, using scaling factors for liver and body sizes of different species, provides information on inter-species differences. Based on microsomal protein content, it was demonstrated that human intestinal microsomes catalyzed the conversion of BPA to BPA-glucuronide at lower rates than hepatic preparations. As such, this study demonstrates the role of the gut and the liver in the glucuronidation of BPA using an in vitro approach. However, the results obtained must be coupled with additional information because the system employed in these studies lacks the coupled nature of hepato-intestinal Phase II metabolism and excretion of BPA which is only available using whole animals. Nonetheless, this study is very informative, qualitatively, about the ability of the GI tract and liver to metabolize BPA and supports the PBPK modeling efforts to quantify the GI tract metabolism of BPA. GI tract microsomes are inferior to GI tract slices for conducting metabolism studies.

High concentrations of commonly used drugs can inhibit the in vitro glucuronidation of bisphenol A and nonylphenol in rats (Verner et al., 2010).

In vitro metabolism of BPA (25-600 μM) to BPA-G was studied with fresh hepatocytes and hepatic microsomes from SD rats. In addition, the ability of commonly used therapeutic drugs to inhibit the glucuronidation of BPA was also investigated using concentrations of those drugs above the maximal levels achieved in patients. The analytical methods to measure BPA were adequate.

Concentration-dependent inhibition of BPA-G formation was observed for a number of tested drugs in hepatocytes and in hepatic microsomes, naproxen and carbamazepine (Kiapp 848 and 1023 μM, respectively) competitively inhibited BPA glucuronidation; however, these concentrations exceeded the reported maximal levels achieved in patients as reported in the literature (374 and 38 μM, respectively). This study is qualitatively informative on the ability of therapeutic drugs to potentially alter detoxification of BPA in liver, supporting the PBPK modeling efforts to quantify possible interactions affecting hepatic metabolism of BPA.

Neurobiology Studies

Summary

Of the studies reviewed, several reported hypothalamic changes in rodents (mice and rats) and, in one study, in sheep as a result of BPA treatment during pre- and/or postnatal development. Three rodent studies reported BPA effects on hippocampal NMDA receptors and/or learning performance. Two of those studies used mice and employed oral treatment and the data indicated learning and memory effects at the higher doses tested, a finding seemingly supported by decreases in hippocampal NMDA receptors or receptor subunits and/or estrogen receptor (ER) β. In a similar study using oral treatment in rats by one of the same groups, dose-related decreases in hippocampal NMDA receptor subunits were also observed, thus, adding to the weight of evidence for hippocampal NMDA receptor involvement in BPA effects. While the data are insufficient to elevate these endpoints to the level of HI at this time, they clearly argue for an increased interest in these metrics and suggest that additional effort be directed towards their further study. Data from studies employing subcutaneous treatment suggested possible hormone and receptor changes at high doses in female sheep; hypothalamic hormone, protein, and receptor changes in rats; and modulation of mouse social behaviors.

Three studies from the same laboratory at North Carolina State University focused on hypothalamic alterations in Long-Evans rats. These studies used subcutaneous treatment (50 or 50,000 μg/kg BPA) during early postnatal development. Strengths of these studies included use of a phytoestrogen-free feed and a strong methodological approach. In general, an increased number of effects were noted in the higher BPA dose group.

However, use of cross-fostering as a method to eliminate litter effects precluded use of these studies for HI and RA.

While not specific to the BPA focus here, an overarching issue is the difficulty in translating stages of rodent brain development to comparable stages of human development. A neonatal rodent is more similar to a late gestational human fetus and early postnatal treatment in rodent studies more closely models prenatal exposure in humans. Thus, early postnatal BPA treatment in rodents is more comparable to late gestational exposure of the human fetus: matching developmental timepoints across species can be especially difficult.

Individual Study Reviews

Oral Exposure

The majority of these studies provided interesting observations regarding potential mechanisms of action for BPA treatment during development, particularly for sexually differentiated aspects of neural development. However, no study provided data that met criteria for either HI or RA.

Evidence to suggest glutamic acid involvement in Bisphenol A effect at the hypothalamic level in prepubertal male rats (Cardoso et al., 2010).

This study assessed hypothalamic levels of GnRH and glutamic acid and serum levels of LH, FSH, and testosterone in BPA-treated male rats. Dams (and potentially pups) were treated via drinking water from GD 0 to Lactational Day (LD) 21. Dosage (≈2.5 mg/kg/day) was estimated based on BPA concentration, daily water consumption, and dam body weight. Although not specified, pups were likely directly exposed at older ages given that free access to water was provided. Concentrations of GnRH and glutamic acid in the hypothalamus and serum levels of LH, FSH and testosterone were significantly decreased by BPA treatment. **This study cannot be used for HI or RA** due to the low N (4 dams/group) and the lack of control for environmental exposure to estrogen (i.e., use of a soy-based diet). The number of assessed male offspring/group is 10; adequately accounting for litter effects would make the effective N equal to the number of dams (4/group). Although a potential mechanism of action was identified, the impact of the effects of alterations of neurotransmitter levels and/or serum hormones is unclear.

Effects of prenatal and postnatal exposure to a low dose of bisphenol A on behavior and memory in rats (Goncalves et al., 2010).

This study assessed the effects of BPA treatment during different developmental periods on learning/memory and activity in male and female rats. Dams were treated orally with 40 µg/kg/day of BPA for either 20 days beginning on the day after copulation (prenatal only), 21 days beginning on the day of parturition (lactational only), or 41 days beginning on the day after copulation (prenatal plus lactational). At adulthood, offspring were

assessed for short- and long-term memory, locomotor activity, and spatial learning and memory. Significant BPA treatment effects were described for each assessment. A strength of this study is the use of a soy-free chow. **This study cannot be used for HI or RA** due to the low N (e.g., the prenatal plus lactational group contained 3 dams) and inappropriate statistical analyses. Specifically, litter effects were not taken into account as the analyses were done using data from individual pups (i.e., the litter was not the statistical unit). Further, F values were either incompletely or incorrectly reported (e.g., an ANOVA main effect of sex on velocity in the water maze is reported with numerator degrees of freedom = 5, instead of 1). There is the possibility of environmental estrogen exposure as caging and water bottle type are not described.

Pre- and post-natal Bisphenol A treatment results in persistent deficits in the sexual behavior of male rats, but not female rats, in adulthood (Jones et al., 2011).

This study assessed the effects of pre- and post-natal BPA treatment on sexual behavior in male and female rats. Pregnant female rats consumed BPA in corn oil from a syringe from GD 7 to LD 14 at 5, 50, 500 or 5,000 µg/kg/day. Although the use of multiple doses of BPA was a strength of this study, **it cannot be used for HI or RA** due to a serious litter confound in the experimental design and statistical analyses (number of litters/treatment group ranged from 2-6). For example, although there were only two dams in the 500 µg/kg/day group, data were collected from 7 female offspring. Other problems included inappropriate assumptions regarding estrous cycling (i.e., it was assumed that the estrous phase could be easily detected using behavioral methods) and culling of litters in different treatment groups to different sizes, since litter size alone can affect various developmental milestones. Finally, use of a normal rodent chow which likely contains phytoestrogens is a limitation.

Developmental and metabolic brain alterations in rats exposed to bisphenol A during gestation and lactation (Kunz et al., 2011).

The aim of this study was to investigate the effects of BPA treatment during gestation and lactation on cerebral metabolism and brain development in rats. Dams were treated via drinking water (GD6-lactational day 20) at an estimated dose of 70 µg/kg/day. Strengths of this study included appropriately accounting for potential environmental exposure to estrogens via diet, caging and water bottles and the concordance of the data obtained via measurement with a novel method with that reported in the literature as measured via high performance liquid chromatography (HPLC). BPA treatment significantly increased hippocampal glutamate concentrations in PND 20 male and female offspring and marginally increased glutamate/aspartate ratios. Hippocampal NeuN-positive neuron density was decreased and density of GFAP-positive cells in the cingulum was increased in PND 20 male and female offspring. However, **this study cannot be used for HI or RA** due to an unclear/low N: the n was reported as 7-8 for BPA-treated animals and 4-6 for controls, but the number of litters represented by these numbers was not reported. Additionally, the physiological and human relevance of changes in the ratio of Glu/Asp and their importance for human health are unclear. Given

that there are likely sex differences in some of the endpoints measured here, the statistical analyses should have included sex as a factor.

Effects of perinatal administration of Bisphenol A on the neuronal nitric oxide synthase expressing system in the hypothalamus and limbic system of CD1 mice (Martini et al., 2010).

The aim of this study was to investigate the effects of gestational and lactational BPA exposure on nitric oxide synthase in areas of the mouse brain believed to be involved in the control of sexual behavior. Pregnant CD1 mice were treated orally with 10, 20 or 40 µg/kg/day BPA or vehicle from GD11 to lactational day 8. Litters were culled to 10 pups (5/sex). Six offspring (3/sex) were used for each endpoint with only 1/sex/litter utilized, although some sample sections were lost prior to analysis resulting in an N of 5 for some analyses. Effects of BPA treatment on the number of nNOS cells in the medial preoptic nucleus and the ventrolateral subdivision of the bed nucleus of the stria terminalis were reported. Strengths of this study include the use of multiple BPA doses, the reporting of gestational and lactational body weights as well as litter parameters, and the use of the litter as the statistical unit. However, **this study cannot be used for HI or RA** due to the use of a soy-based isoflavone rich diet and the low number of subjects/group (5-6/sex/group). Further, the use of post-hoc tests to identify differences between males and females within each treatment group without an overall statistically significant interaction of treatment with sex is not justified (as is the case for the results for the MPOM) unless these comparisons were planned a priori. Finally, the functional significance of changes in nNOS cells is uncertain.

Corticosterone-regulated actions in the rat brain are affected by perinatal exposure to low dose of bisphenol A (Poimenova et al., 2010).

The study objective was to evaluate the effects of pre- and post-natal BPA treatment on corticosterone levels, hippocampal glucocorticoid receptors (GR), mineralocorticoid receptors (MR), and performance of a behavioral task (Y-maze). Pregnant Wistar rats were fed BPA on a small piece of food throughout pregnancy and lactation with 40 µg/kg/day or vehicle (n=4/treatment group) and all offspring (34 treated and 36 control) were used as individual subjects for statistical analyses, rather than as representatives of litters. Behavioral results were reported as decreases in exploration (possibly due to increased anxiety) in BPA-treated females. Hormone/receptor level alterations suggested that BPA may be involved in modulation of a specific stress or learning response, rather than specific behaviors. Although this is an interesting mechanism of action study, the lack of statistical or study design control for litter, inappropriate statistical analyses, potential environmental estrogen exposure via diet, caging and water bottle type, and lack of information on animal selection for the various assays, render **this study as not useful for HI or RA**.

Fetal and neonatal exposure to three typical environmental chemicals with different mechanisms of action: Mixed exposure to phenol, phthalate, and dioxin cancels the effects of sole exposure on mouse midbrain dopaminergic nuclei (Tanida et al., 2009).

The purpose of this study was to investigate the effects of prenatal and neonatal BPA treatment and two endocrine disrupters on the midbrain dopaminergic system of male mice. Pregnant ICR mice and their resulting male pups were treated orally (no mention of how this oral treatment was effected) with 5 mg/kg/day BPA (GDs 8-17 and direct to pups on PNDs 3-7), 1 mg/kg/day DEHP (GDs 8-17 and direct to pups on PNDs 3-7), 8 ng/kg TCDD (GD 8 only), or a mixture (BPA, DEHP, and TCDD at the same doses and days as for the single compound groups). Statistically significant decreases in brain weight at 6 weeks of age and in TH-immunoreactive neurons in the midbrain in the ventral tegmental area and the substantia nigra at various ages were described for BPA-treated mice. There were no changes in the number of Fos-immunoreactive cells in any midbrain region. Brain weight and relative brain weight at 2 weeks of age were increased in the combination treatment group. **This study cannot be used for HI or RA** due to the lack of control for possible environmental estrogen exposure via diet, caging or water bottle type; inadequate control for litter effects (10-15% of groups were litter mates and this was not accounted for statistically); purposeful exclusion of subjects due to body weights outside the normal range (although normal range is not defined); and unnecessary stratification (use of only male pups for an outcome relevant to both sexes).

<u>Prenatal and postnatal exposure to Bisphenol A induces anxiolytic behaviors and cognitive deficits in mice (Tian et al., 2010).</u>

The aim of this study was to examine behaviors associated with anxiety and memory, and dopamine and NMDA receptor levels in male and female offspring of ICR mice treated with 0.1 or 0.5 mg/kg/day of BPA (n=2 dams/dose). BPA was dissolved in DMSO and dams were orally treated from GD 7 - 21. Offspring may also have been treated directly from PNDs 22-36, but the methods are unclear. Type of feed, caging and water bottles used are not described. Behavioral tests (open field, elevated plus maze, Y-maze alternation and novel object recognition) indicated decreased 'anxiety-like' behaviors (increased distance traveled or time spent in central open field area for the 0.1 mg/kg/day group and in open arms of the elevated plus maze for the 0.5 mg/kg/day group) and impaired working memory (decreased alternation behavior in the Y-maze for both BPA treatment groups). Potential ceiling effects make interpretation of the novel object results uncertain, as the group that was reportedly impaired in this test (0.1 mg/kg/day) was also reported to have significantly increased activity in the central area. In receptor binding assays, increased binding of caudate D2 receptors at 0.5 mg/kg/day and decreased caudate DAT binding and frontal cortex NMDA binding at both doses were reported, though not always in a dose-dependent manner. NMDA receptor binding in CA1, CA3 and the dentate gyrus of the hippocampus was decreased at both doses as well. **This study cannot be used for HI or RA** due to lack of control for potential environmental estrogen exposure (via diet, caging, water bottles, etc.) and lack of control for litter effects (and resulting low n of 2/group). In addition, prior to statistical analyses, data were combined for male and female offspring for assays known to demonstrate sexually dimorphic endpoints. There was an additional lack of accounting for repeated measures in the open field/novel object recognition test and lack of detail in the methods description making assessment of outcomes problematic.

Perinatal exposure to bisphenol-A impairs learning-memory by concomitant down-regulation of *N*-methyl-*D*-aspartate receptors of hippocampus in male offspring mice (Xu et al., 2010b).

The study objective was a behavioral and immunohistochemical analysis of the effects of developmental BPA treatment on behavioral tasks (Morris water maze and step-down passive avoidance) and hippocampal NMDA receptor subunit and ERβ expression. Female ICR mice were orally ("oral injection") administered BPA at 0.05, 0.5, 5 or 50 mg/kg bw/day in sesame oil from GD 7 through lactational day 21. Male offspring were assessed at PND 21 or 56 for body weight and learning and memory using water maze and passive avoidance tasks. At PNDs 21 or 56, NMDA receptor subunits and ERβ were measured in the hippocampus. The lowest dose (0.05 mg/kg bw/day) decreased male offspring body weight at both ages; other doses (0.5 and 50 mg/kg bw/day) had effects at only one age. Results from the behavioral assessments indicated learning and memory effects at the higher doses at both ages, a finding seemingly supported by decreases in hippocampal NMDA receptor subunits and ERβ receptors. Strengths of this study include measuring hippocampal receptor expression to correlate with treatment-related behavioral effects for biological significance, use of a soy-free diet, and multiple BPA doses. However, **this study cannot be used for HI or RA** since it is not clear if the litter was the statistical unit of analysis. In addition, most endpoints were collected at two ages, but age was not a factor in the statistical analyses. Further, data are reported for males only and there is a lack of information on how offspring were selected for measurement. There is a lack of control for potential environmental estrogen exposure via caging and water bottle type as these are not described.

Perinatal exposure to bisphenol-A changes *N*-methyl-*D*-aspartate receptor expression in the hippocampus of male rat offspring (Xu et al., 2010a).

The objective of this study was to evaluate whether gestational and lactational BPA treatment alters male offspring hippocampal levels of NMDA receptors and ERβ, or aromatase P450 protein expression at five different ages (four preweaning ages and postnatal day 56). Pregnant SD rats (8-9/group) were gavaged with 0.05, 0.5, 5.0, 50.0, or 200.0 mg/kg/day BPA in sesame oil from GD 7 to lactational day 21. Male offspring were sacrificed at PNDs 4, 7, 14, 21 or 56 and protein expression was determined via gel electrophoresis and immunoblot for: hippocampal NMDA receptor subunits (NR1, N2A, NR2B), ERβ, and P450 aromatase. Litter size and offspring body weight at the five ages were reported as unaffected by treatment. There is no mention of dam gestational or lactational body weight. Dose-related decreases in all NMDA receptor subunits were reported, with statistically significant effects reported for the lowest dose at one or more ages. Significant decreases in ERβ receptor were reported at the three highest doses at the younger ages, as well as significant increases in P450 aromatase primarily at the two higher doses at most ages assessed. **This study cannot be used for HI or RA** as it is not clear how offspring were selected for measurement or if litter was the statistical unit of analysis. Although 8-9 dams/group are reported, there is no description of whether data were collected from siblings at the same age, at different ages, or via some another

method. The potential for environmental estrogen exposure is unknown since feed, caging and water bottle type were not reported. Finally, the statistical analyses should have included age as a factor--rather than conducting one-way ANOVAs at each age--in order to allow for a more complete description of age-related effects.

Subcutaneous Exposure

The majority of these studies provided interesting information as to potential mechanisms of action for BPA during development, particularly sexually-differentiated aspects of neural development. However, none were categorized as useful for HI or RA, mainly due to the potential for estrogenic environmental exposures and lack of statistical control for litter effects.

Neonatal bisphenol-A exposure alters rat reproductive development and ovarian morphology without impairing activation of gonadotropin-releasing hormone neurons (Adewale et al., 2009).

The purpose of this study was to investigate the effects of neonatal treatment with BPA on hypothalamic organization in female Long Evans rats. Female rats were cross-fostered at birth and injected subcutaneously on PNDs 0, 1, 2, and 3 with 0.05 mL vehicle (10% EtOH, 90% sesame oil), 25 μg estradiol benzoate, 1 mg/kg PPT [an ESR1 (ERα) agonist], 50 μg/kg BPA, or 50 mg/kg BPA. The lower dose of BPA advanced day of vaginal opening, caused mild qualitative changes in ovarian morphology with a trend toward fewer ovarian corpora lutea and may have had a late effect on the estrous cycle (there was no statistical analysis of cycle data). Day of vaginal opening was not significantly altered in the high dose BPA group; however, only 33% of the subjects exhibited normal estrous cycles 15 weeks after vaginal opening (no statistical analysis of cycle data) and there were significantly fewer corpora lutea in the ovaries. Prior to sacrifice, the rats were ovariectomized and hormonally-primed for assessments of sexual behavior. Neither dose of BPA significantly altered the lordosis quotient, although there was a trend for decreased lordosis behavior in the low dose BPA group. There was no significant change in hormonal stimulation-induced co-localization of GnRH and FOS in the organum vasculosum of the lamina terminalis (OVLT) region of the hypothalamus of either BPA group suggesting no alterations in GnRH neurons in response to steroid stimulation. The strengths of this study include the responsible laboratory's historical experience in these measures and the use of a low phytoestrogen feed. However, **this study cannot be used for HI or RA** due to the low subject numbers ($n<10$), a lack of statistical control for potential litter effects (cross-fostering can minimize, but does not eliminate, litter effects) and a lack of control for potential environmental exposure to BPA from caging and/or water bottles.

The impact of neonatal bisphenol-A on sexually dimorphic hypothalamic nuclei in the female rat (Adewale et al., 2011).

As in the previous study by this group (Adewale et al., 2009), the aim of this study was to investigate the effect of BPA treatment during the neonatal period on sex-specific

hypothalamic organization in female Long Evans rats. Female rats from 10 dams were cross-fostered at birth (maximum of 12 per litter, no more than 2 true siblings in each litter) and injected subcutaneously on PNDs 0, 1, 2 and 3 with 0.05 mL vehicle (10% EtOH, 90% sesame oil), 25 μg estradiol benzoate, 1 mg/kg PPT [an ESR1 (ERα) agonist], 50 μg/kg BPA, or 50 mg/kg BPA. A significant increase in body weight was reported for the high-dose BPA group at PND 99 but not at PND 31. Both BPA doses increased the number, but not activity (defined as FOS activity), of oxytocin producing neurons in the rostral PVN. No other hypothalamic endpoint (i.e., 5-HT fiber density in the VMN, number of ERα cells in the VMN, MPOA, and arcuate nucleus) was significantly affected. This study also re-analyzed the sexual behavior data from Adewale et al. (Adewale et al., 2009) to quantify the number of hops and darts (measures of proceptive behavior). The frequency of these behaviors did not differ between BPA-treated females and control females. Strengths of this study include use of a low phytoestrogen diet, inclusion of useful reference groups (EB and PPT) and the measurement of body weight. However, **this study cannot be used for HI or RA** due to low subject numbers (n<10) and lack of adequate control of litter effects (cross-fostering alone is not adequate). In addition, caging and water bottle type were not described, thus, environmental estrogen exposure was unknown.

Developmental programming: Impact of fetal exposure to endocrine-disrupting chemicals on gonadotropin-releasing hormone and estrogen receptor mRNA in sheep hypothalamus (Mahoney and Padmanabhan, 2010).

The objective of this study was to assess the effect(s) of prenatal BPA treatment on estrogen receptor expression in the medial preoptic area (mPOA) and GnRH expression in the hypothalamus of female sheep. Pregnant ewes were injected subcutaneously with 5 mg/kg/day BPA or cottonseed oil (vehicle) on days 30-90 of an approximately 147 day gestation period. Female offspring (n=3-7, depending on endpoint) were euthanized at 21 months of age. The hypothalamus was labeled to detect GnRH and levels of ESR1 (ERα) and ESR2 (ERβ) were measured in the medial preoptic area (mPOA). BPA-treated sheep had lower levels of GnRH expression (defined as amount of GnRH mRNA and the size of GnRH neurons), but increased levels of ESR1 and decreased levels of ESR2 expression in the mPOA. These hypothalamic changes correlated with a previously reported decreased LH surge in female offspring treated prenatally with BPA (animals were from the same cohort). One strength of this study is its use of a large animal model: the authors also cite evidence in their introduction that sheep have ovarian cycles and steroid and GnRH secretion patterns similar to human females. However, sheep appear to be seasonal breeders in that their puberty and sexual activity are controlled by the photoperiod with estrus becoming more frequent with shorter days (Bocquier et al., 1997;Yellon and Foster, 1986;Thiery et al., 2003). This may have been controlled in the control and BPA-treated offspring via prostaglandin administration to synchronize estrus, but data provided are insufficient to conclude as such. Other strengths include previously described BPA blood levels in pregnant ewes from studies using this same treatment and dosing regimen and a methodical approach to studying the effects of BPA on the HPG axis. **This study cannot be used for HI or RA** due to the small numbers of subjects/group (controls=5, BPA-treated=3-7) and a lack of control for potential

environmental estrogen exposure (a previous report of this same cohort describes the feed as consisting partially of alfalfa hay).

Exposure of neonatal female rats to bisphenol A disrupts hypothalamic LHRH pre-mRNA processing and estrogen receptor alpha expression in nuclei controlling estrous cyclicity (Monje et al., 2010).

The objective of this study was to assess the effect(s) of early postnatal BPA exposure on the regulation of luteinizing hormone releasing-hormone (LHRH) pre-mRNA processing, the expression of ESR1 (ERα) and the progesterone receptor (PR) and their co-factors steroid receptor coactivator 1 (SRC-1) and the repressor of estrogen receptor activity (REA) in hypothalamic nuclei of adult female Wistar rats. Female pups were injected subcutaneously on PNDs 1, 3, 5 and 7 with 50 or 20,000 µg/kg BPA in corn oil. The lower BPA dose increased the percent of time in either proestrous or estrous, increased expression of the mature version of LHRH mRNA, decreased expression of the immature LHRH mRNA, increased expression of ESR1 in the AVPV, but decreased expression of the same in the arcuate nucleus. In addition, the lower dose of BPA decreased expression of the progesterone receptor and SRC-1 in the AVPV. The higher BPA dose decreased expression of the mature and immature versions of LHRH mRNA and decreased serum LH levels. Further, this higher dose decreased expression of ESR1 in the arcuate nucleus but increased expression of the same in the AVPV. The higher dose was without effect on expression of SRC-1 and REA in the AVPV or arcuate nucleus. Strengths of this study include a nested pilot study which determined the expression of the mature and immature versions of LHRH mRNA at two different times in control animals in order to best design the subsequent study of BPA. In addition, stainless steel cages and glass water bottles minimized potential environmental estrogen exposure. However, **this study cannot be used for HI or RA** due to the lack of control for litter effects (cross-fostering does not avoid litter effects) and the small numbers of subjects/group (n=5-6 for the LHRH mRNA and receptor and transcription cofactor endpoints). An additional limitation of the study is the use of a standard phytoestrogen-containing chow.

Prenatal and lactational exposure to low-doses of bisphenol-A alters brain monoamine concentration in adult mice (Nakamura et al., 2010).

The aim of this study was to investigate whether prenatal and lactational BPA treatment affects neurotransmitter levels in male and female ICR/Jcl mice. BPA (20 µg/kg) or sesame oil (n=8/group) was injected subcutaneously daily from E0 (day of vaginal plug detection) to lactational day 21. At 3 and 12 weeks of age (the text states 12 weeks of age although the table states 14-15 weeks of age), levels of dopamine, DOPAC, 5-HT, and 5-HIAA (as well as the ratios of DOPAC/dopamine and 5-HIAA/5-HT) were measured via HPLC in somatosensory cortex, caudate/putamen, lateral hypothalamus-preoptic area, thalamus, dorsal raphe nucleus, and substantia nigra. There were 13 statistically significant effects of BPA treatment and each brain region contained at least one statistically significant effect. No effects differed by sex. With the exception of the DOPAC/dopamine ratio, all neurotransmitters (and the 5-HIAA/5-HT ratio) were altered in at least one region by BPA treatment. **This study cannot be used for HI or RA** due

to inadequate details provided about pup selection for analysis (potentially introducing litter effects), lack of control for potential environmental estrogen exposure via diet, caging or water bottle, and small subject numbers/group.

Prenatal and lactational exposure to low-doses of bisphenol A alters adult mice behavior (Nakamura et al., 2011).

The objective of this study was to determine the effects of prenatal and lactational BPA treatment on weanling and adult mouse body weight, locomotor activity, anxiety-related behavior, and spatial learning/memory (last evaluation only in adults) in male and female ICR/Jcl mice. Dams were injected subcutaneously daily with either 20 µg/kg/day of BPA or sesame oil vehicle beginning on the day after breeding (GD0) through weaning of pups (PND 21). The body weight of PND 21 male and female offspring treated with BPA was decreased; however, this body weight effect disappeared at adulthood. Open field activity indicated decreased distance moved (activity) at adulthood (but not at weaning). Elevated plus maze activity indicated decreased distance moved (activity) at weaning (but not at adulthood). There were no treatment-related effects observed for water maze performance. The number of litters (8/group) and the number of subjects tested (15-20/sex/group) indicates that the litter was not utilized as the statistical unit, introducing a litter confound. There is no information on type of diet, caging, or water bottles and thus, there is the potential for environmental estrogen exposure. **This study cannot be used for HI or RA** due to lack of control for potential environmental estrogen exposure and the failure to statistically control for litter.

Impact of neonatal exposure to the ERα agonist PPT, bisphenol-A or phytoestrogens on hypothalamic kisspeptin fiber density in male and female rats (Patisaul et al., 2009).

The purpose of this study was to assess the effect(s) of neonatal treatment with PPT or BPA on KISS fiber density in the anterior ventral periventricular nucleus (AVPV) and arcuate nucleus (ARC) of female Long Evans rats and further determine if neonatal treatment with BPA, genistein or equol could disrupt KISS fiber density in the AVPV or ARC nuclei of male Long Evans rats. As such, two separate studies are reported. In both, cross-fostered rats were injected subcutaneously daily for 4 days (PNDs 0-3) with 50 µg/kg or 50 mg/kg BPA; EB 25 µg; PPT 1 mg/kg or equol 10 mg/kg in 10% EtOH, 90% sesame oil. For each endpoint, there were 6-11/group from 20 cross-fostered litters. No significant effects were reported at the 50 µg/kg BPA dose in males or females whereas the higher BPA dose decreased KISS immunoreactivity in the ARC of females (there was no 50 mg/kg/day BPA dose group for males). Strengths of the study include use of a low phytoestrogen feed and careful methodological study of sexually dimorphic endpoints. Limitations of the study include the use of cross-fostering in an attempt to account for litter effects (cross-fostering can decrease, but does not eliminate, the litter confound), potential environmental estrogen exposure from caging or water bottle type, and small numbers of subjects/group. **This study cannot be used for HI or RA.**

Pubertal exposure to bisphenol A disrupts behavior in adult C57BL/6J mice (Yu et al., 2011).

The objective of this study was to determine how pubertal BPA treatment affects locomotion, exploration, anxiety and sociability in adult C57BL/6J mice. Each of 30 litters were culled to 5/sex and housed in groups of 8 with no more than 1/sex/litter from PNDs 21-31 after which, they were separated by sex. Total numbers were 39 males and 45 females, but how animals were assigned to treatment groups was unclear. Each group consisted of 13-15 mice which were injected subcutaneously daily on PNDs 23-30 with 50 µg/kg BPA, 10 µg/kg estradiol (used as a positive control), or sesame oil. At PNDs 60-70, behavioral assessments began and included open field activity, elevated plus maze activity, novel cage test activity, and social behaviors with a same- and opposite-sex mouse. BPA-treated females were reported to be hypoactive in the open field and the elevated plus maze test. Further, they spent less time in the central area of the open field and the open arms of the elevated plus maze (indicating anxiogenic effects). BPA-treated females engaged in longer durations of affiliative behaviors with a same-sex mouse but shorter durations with an opposite-sex mouse. There were fewer effects in males, but these included increased time in the open field central area. This study was of interest because of the novel period of treatment (adolescence) and the use of a positive control. Given the continued brain maturation that occurs during adolescence and the potential increased sensitivity of the adolescent brain to perturbation, studies investigating the effects of BPA treatment during this time are important. However, **this study cannot be used for HI or RA** due to significant unknowns such as dietary and/or environmental estrogen exposure via feed, caging, or water bottles and unclear assignment of siblings to treatment groups (potential litter confounding).

<u>Abnormal synaptic plasticity in basolateral amygdala may account for hyperactivity and attention-deficit in male rat exposed perinatally to low-dose bisphenol-A (Zhou et al., 2011).</u>

The objective of this study was to determine the effects of prenatal and lactational BPA treatment on basolateral amygdala (BLA) alterations and open field activity in male SD rats. BPA (2 µg/kg/day) or vehicle (olive oil) treatment began on GD 10 and continued daily until lactational day 7 via subcutaneous injections. Male offspring were assessed using a number of electrophysiological measures at PND 28 as well as two behavioral tests at PND 21; however, only those BPA-treated offspring which exhibited hyperactivity in an open field were studied further (males that were not hyperactive were not assessed using any other measure). Overall, it appears there were 6-28 rats/treatment group. While the electrophysiological results of this study suggest some alterations in the BLA of BPA-treated male rats, the BPA effects themselves cannot be easily determined because the BPA male offspring that were assessed were only those exhibiting hyperactivity. In addition, there is a lack of control for potential environmental estrogen exposure via feed, caging, and water bottle type. Statistical analyses were conducted separately for each treatment group when both should have been included in an overall ANOVA. **This study cannot be used for HI or RA**.

<u>Both Oral and SC Exposure</u>

Bisphenol A interferes with synaptic remodeling (Hajszan and Leranth, 2010).

In this review paper, preliminary results are described for a study of subcutaneous and oral BPA treatment on asymmetric spine synapse counts in layers II/III of the medial prefrontal cortex and various hippocampal regions in adult male Sprague Dawley rats treated with 300 μg/kg for three days (although the figure is labeled as 300 mg/kg) either by subcutaneous injection or gavage using sesame oil as the vehicle. An additional group received BPA for one day only by either subcutaneous injection or gavage. Significant decreases in spine synapses in the prefrontal cortex and CA1 hippocampal region were reported for both oral and sc treatment in the multiple and single exposure groups. Total BPA blood serum concentrations after SC treatment were significantly higher than those obtained after oral administration. Despite increased serum levels in the SC treatment group, spine synapse levels in the medial prefrontal cortex and hippocampal CA1 region were virtually identical in both the SC and oral groups. This study was performed to correlate previous subcutaneous injection data from this lab with those obtained after exposure via the route most likely to be relevant for most human exposures (oral). **This study cannot be used for RA or HI** due to a lack of control for potential environmental or dietary estrogen exposure, and an unknown sample size. In addition, the lack of any synaptic differences associated with different exposure routes and, thus, internal doses, without a potential explanation lowers confidence in the findings.

Reproductive and Developmental Studies

Summary

Thirteen studies were reviewed in this area: seven of which used rat models [SD (1), Long-Evans (1), Wistar (3), and Holtzman (2)], five of which used mice [CD-1 (1), C57BL/6 (3), and Balb/c (1)], and one of which used ovariectomized prepubertal lambs. Four of the rat studies and three of the mouse studies utilized the subcutaneous route of administration, while the remaining rodent studies used oral routes of administration. The lamb study utilized intravenous and intramuscular exposures. Eight studies focused on females, three on males, and two evaluated both sexes.

In general, the studies reviewed here were exploratory and focused on specific endpoints or mechanisms. Few studies utilized enough different doses of BPA to provide useful dose-response information. Studies that reported BPA effects used SC or oral routes of exposures. The internal BPA aglycone exposure levels at which the reported effects occurred after either route of administration, and their relevance to human internal exposure levels, are questions that require resolution. Several of the authors state that they observed effects at doses that are relevant to human exposure levels, but the data supporting this claim are questionable.

While the overall conclusion of the review group was that none of the studies as presented were useful for risk assessment to modify current levels of concern, there were data that suggested endpoints for more detailed study that may inform HI. In particular, a number of the studies reviewed here, together with previously published data, suggest an

effect of BPA on the ovary (see the review below of (Signorile et al., 2010), the HPG axis, and/or the estrous cycle (Adewale et al., 2009;Mendoza-Rodriguez et al., 2011;Fernandez et al., 2009;Collet et al., 2010). The three studies that focused on males indicated effects on sperm motility (Minamiyama et al., 2010;Salian et al., 2009b;Salian et al., 2009a), but it was felt that these studies also had serious limitations.

Individual Study Reviews

Oral Exposure

Endocrine disrupter bisphenol A increases *in situ* estrogen production in mouse urogenital sinus (Arase et al., 2011).

This study investigated the effects of BPA on the urogenital sinus (UGS) and other fetal organs following an oral dose (corn oil vehicle) of 20 µg/kg bw/day to the dam on embryonic days 13 to 16 (E13-E16) in the C57BL/6 (Japan SLC) mouse. DES at 0.2 µg/kg bw/day was used as a reference estrogen. There were 12 dams per dose group, and fetuses or pups from 3 dams in each group were taken at E17, E18, PND0, and PND1. UGSs from 15-18 fetuses or pups of both sexes were dissected and 5-6 were pooled for the analyses (so n=3) for each time and dose. It was not clear if tissues from littermates were pooled. The authors report that BPA, but not DES, induced elevated estradiol and aromatase activity in the UGS of PND 1 animals of both sexes. Likewise, expression of Cyp19a1, Cyp11a1, and Nr5a1 was induced in both sexes by BPA, but not DES, at PND 0 and 1. Using cultured mesenchyme, both aromatase and ERRγ were reported to be elevated by BPA, but not DES. Both DES and BPA induced ESR1 in females and AR in both sexes. Cerebellum, heart, kidney, ovary, and testes were evaluated although, except for sex specific tissues, the sex evaluated was not indicated. The authors suggest that the induction of steroidogenic enzymes occurs only in tissues expressing ERRγ, a proposed target of BPA. The consequences of the molecular changes are not clear, i.e. the changes and magnitude of the changes in molecular endpoints reported are not clearly tied to an adverse effect in the mouse or human. Similar elevations in steroid hormone receptor levels have been reported by others, so this replication of those effects is of interest. The data in this report are in themselves **not useful for HI or RA** based on the small sample size and lack of clarity on the pooling of the pups for analysis.

Dietary exposure to low levels of bisphenol A: effects on reproduction and development in two generations of C57BL/6J mice (Kobayashi et al., 2010).

This is a dietary exposure study in C57BL/6J mice (Charles River, Japan) using doses of 0, 0.333, 3.3, and 33 ppm BPA, which translate to approximately 0.05, 0.5, and 5 mg/kg body weight per day. The F_0 dams (14-16 per group) were placed on diet at GD6, and the F_0 animals and their offspring were maintained on the same diets until termination of the F_2 generation pups at 15 weeks of age. To produce the F_2 generation, 6 F_1 males were mated with 12 F_1 females. F_1 and F_2 litters were culled to 8 on PND 1, and litters with less than 8 pups were not culled. No details were provided on caging, water, or water delivery system, nor were diet mixing procedures, homogeneity, dose certification, or

phytoestrogen levels described. Endpoints evaluated included body weight and food consumption, F_0 gestation length, body length, tail length, anogenital distance (AGD), ratio of AGD to cube root of body weight, organ weights, sperm count, sperm motility and sperm progressivity. Histological evaluation was limited to testes and epididymides. Twelve males and 12 females were randomly selected for F_1 and F_2 necropsy, and it is stated that the litter was used as the experimental unit. The authors conclude that there were no treatment-related effects observed, although there were a few statistically significant effects on body weight (increased), weights of epididymides (increased), and sperm motility (decreased) observed and dismissed by the authors as relatively small in magnitude and not consistent with age or across generations. This **study meets the criteria for providing data of utility for HI**, but not RA due to concerns in the reporting of the study, including lack of confirmation of the dietary dose levels and a lack of description of possible environmental confounders.

Administration of bisphenol A to dams during perinatal period modifies molecular and morphological reproductive parameters of the offspring (Mendoza-Rodriguez et al., 2011).

This study investigated the effects of perinatal exposure to BPA on several reproductive parameters in female offspring. Pregnant Wistar rats (n= 5) were dosed with BPA (~1.2 mg/kg bw/day) from GD 6 to PND 21 via drinking water via glass bottles. After weaning on PND 21, the female pups (n = 15~25) received untreated water. Estrous cyclicity (n=15) was examined at 3 months of age for 4 consecutive weeks. Rats were then sacrificed at 13:00 on the day of estrus. Uterine and ovarian structures, apoptosis and ERα expression in uterine tissue, serum levels of progesterone (P4) and estradiol (E2) were evaluated. Reportedly, BPA caused abnormal estrous cycles in 79% of female offspring and significant increases in the thickness of uterine luminal and glandular epithelia and stroma. Significantly reduced ERα expression and apoptosis in uterine tissues were also reported. The authors suggest that perinatal BPA exposure affects the reproductive cycle of the female offspring by the deregulation of ERα. Despite the findings in the report, this study in itself **did not meet the criteria for HI or RA** due to the lack of description of environmental contamination control and offspring treatment (culling/cross-fostering), use of an inappropriate test unit (pups instead of litter), small sample size, and the use of only a single dose level.

Gene Expression in the Fetal Mouse Ovary is Altered by Exposure to Low Doses of Bisphenol A (Lawson et al., 2011).

This study investigated the effect of oral dosing with BPA at the single dose level of 20 μg/kg bw/day to pregnant C57BL/6 dams on gene expression in the fetal ovary. This dose had previously been reported by the authors to affect meiosis in this model, however the referenced study employed a longer administration period and utilized SC dosing. Dosing began on GD 11 and fetal ovaries were examined on GDs 12, 12.5, 13.5, and 14.5. Background BPA or phytoestrogen levels were not reported. Total RNA was isolated from three replicates of 6 sets of ovaries per day. The ovaries were pooled across litters although the dam was dosed and was the experimental unit. Gene

expression was analyzed using Affymetrix Mouse Gene 1.0 GeneChips and one-way ANOVA was used to assess significance and fold-change in gene expression. Using a low stringency analysis, the authors identified numerous changes in gene expression following BPA exposure. However, most of these gene expression changes were less than 2-fold and there was little overlap in those changes from day to day. The authors noted that some meiosis-specific genes were up-regulated while mitotic cell-cycle genes were down-regulated. Using more appropriate analyses to correct for multiple comparisons resulted in dramatically reduced gene lists. There was no direct linkage of the subtle gene expression changes to a biological response. Changes in meiosis, germ cell expansion, the development of aneuploid eggs, or reproductive senescence were not examined. While potentially providing mechanistic data, **the study was not considered to be useful for HI or RA** given the statistical issue of pooling ovaries across litters and the lack of a clear understanding of the biological relevance of the reported gene changes.

Generation of reactive oxygen species in sperm of rats as an earlier marker for evaluating the toxicity of endocrine-disrupting chemicals (Minamiyama et al., 2010).

The objective of this study was to look for BPA-induced increases in reactive oxygen species (ROS) in rat sperm as a potential mechanism for adverse effects on sperm. Groups of 10 8-week old male Wistar rats were administered 1 or 10 mg/L BPA (about 0.3 or 3 mg/kg, respectively) or 0.1 or 0.3 mg/L DES via drinking water. Action water consumption was not reported. One group receiving 1 mg/L BPA and one 0.1 mg/L DES group also received dietary N-acetyl cysteine (NAC) 2 days before exposure to the BPA or DES. Dosing continued for 1 or 8 weeks. Background BPA or phytoestrogen levels were not reported. Epididymal sperm counts and motility were reported. ROS generation in sperm solutions was assessed using luminol L-2 and a chemiluminescence reader to measure superoxide release from spermatozoa with and without superoxide dismutase (SOD). A mitochondrial fluorescent probe was also used to evaluate the sperm mitochondrial potential utilizing a 20-fold dilution of sperm. Protein adducts and HNE-modified proteins as biomarkers for lipid peroxidation were also assessed. There were no functional effects measured. No effect on sperm concentration was seen but sperm motility was reduced in BPA treated animals. However, there was no difference in effect between the 1 and 10 mg/L BPA groups. No morphologic changes in structure of sperm were seen for BPA, but were seen for DES. ROS products increased for DES and BPA to a lesser extent, but there was no difference between the 1 and 10 mg/L BPA groups. The effect was inhibited by NAC. While these results suggest possible mechanisms of action, they **were not considered appropriate for either HI or RA**. The lack of a dose-response is also problematic for interpretation of the results.

Perinatal Exposure of Rats to Bisphenol A Affects the Fertility of Male Offspring (Salian et al., 2009b).

The authors examined the effects of perinatal exposure of male rats to BPA on fertility parameters in the F_1, F_2, and F_3 generations. Pregnant in-house bred Holtzman rats (n = 8 per group) were dosed orally by gavage with BPA at 1.2 and 2.4 µg/kg bw from GD 12 until PND 21. DES was included as a positive control for several endpoints. Animals

were fed an in-house prepared soy-free diet. BPA dose certifications or background measurements were not provided. F_1 males (PND 75) were randomly selected (n=24) from each dose and housed with normal cycling adult females. One set of pregnant females (n=24) was killed on GD 20 while a second set (n=24) was kept until delivery. In the experimental design only the F_1 generation was dosed with BPA during gestation and lactation. That the F_0 dam was dosed and, thus, was the experimental unit was not taken into account in the statistical analysis. The authors report a significant increase in post-implantation loss, a decrease in litter size, as well as a decrease in male sperm count and motility in F_1, F_2 and F_3 males. Levels of LH, T, E, and FSH relative to control were significantly reduced in adult male rats exposed perinatally to BPA at the single time point at which measurements were made. A positive control was not included for these measures and the relevance of these single time point hormone measurements remains to be determined. The authors conclude that perinatal exposure to BPA affects the male germ line leading to impairments in the fertility of F_1 male offspring and their subsequent F_2 and F_3 generations. This study was **not considered to be of utility for HI or RA** given the lack of understanding that the F_0 dam was the experimental unit. The use of an in-house bred strain of rat together with an in-house prepared diet also limits its usefulness because replication outside of this laboratory may be problematic.

Subcutaneous Exposure

Neonatal bisphenol-A exposure alters rat reproductive development and ovarian morphology without impairing activation of gonadotropin-releasing hormone neurons (Adewale et al., 2009). [Note: This study is also reviewed under *Neurobiology studies*. This summary addresses only the reported reproductive effects.]

This study addresses the effect of neonatal exposure to BPA on female rat reproductive development, ovarian morphology, and the hypothalamus. Two doses of BPA were tested, 50 µg/kg bw/day and 50 mg/kg bw/day. Estradiol benzoate (EB) and an ESR1-specific agonist (PPT) were also used as controls. Long Evans rats (Charles River) pups were cross-fostered at birth and dosed SC from the day of birth (PND 0) through PND 3 using a 10% ethanol/90% sesame oil vehicle. No statements of BPA (Sigma) purity or steps taken to evaluate BPA background are mentioned. AIN-93G, a defined phytoestrogen-free diet, was used. Both EB and PPT accelerated vaginal opening as did the low dose, but not the high dose, of BPA. EB and PPT affected the proportion of animals cycling, and BPA was reported to decrease cycling relative to control. The cycle data were not statistically evaluated. The numbers of corpora lutea were significantly reduced in ovaries from the high BPA dose group. Qualitative evaluation indicated that folliculogenesis was abnormal in the high dose BPA animals and that the ovaries would not likely be able to support ovulation. With respect to the reproductive effects, this study is of **low utility for HI and not useful for RA**. While the high dose of BPA had clear effects on the ovary, low dose data are difficult to interpret due to insufficient control of potential litter effects and the limited evaluation of the data (no statistical evaluation of estrous cycle data, qualitative histological evaluation of low dose ovaries).

Perinatal exposure to environmentally relevant levels of bisphenol-A decreases fertility and fecundity in CD-1 mice (Cabaton et al., 2011).

This study evaluated the effects of exposure to low doses of BPA on fertility in the CD-1 mouse (Charles River) using a forced breeding protocol. Doses were administered from embryonic day (E) 8 through LD 16 via osmotic pump implanted in the F_0 dam using 50% DMSO as solvent. Doses, based on the dam weight at E6, were 0, 25, 250, and 25,000 ng/kg body weight per day. DES was used as a reference estrogen control at 10 ng/kg bw/day. The F_1 litters were culled to 8 on the day after birth, weaned at PND 21, and one female per litter (n=18-21) was mated with untreated proven male breeders starting at 8 weeks of age. F_1 females were continuously housed with proven breeder males for 32 weeks starting at 2 months of age. Resulting litters were removed on the day of delivery. Cumulative pup number, time to first litter, number of deliveries per day, number of pups per litter, sex of pups, and number of dams having litters were recorded. BPA purity was assessed and extracts of all study materials were assessed for estrogenicity using E-SCREEN (MCF-7 proliferation assay). There were no effects on dam body weights, sex ratio, percentage of dead pups, or time to first delivery. The cumulative number of pups was significantly decreased relative to control in the 25 and 25,000 ng/kg/day dose groups and the DES group, but not in the 250 ng BPA/kg bw/day dose group. The high dose BPA group also had fewer pregnancies than controls and a more rapid decline in the number of pups delivered over time. This study is not useful **for HI** given the inappropriate solvent used for dose delivery and the unusual dose-response. Forced breeding, which alters maternal hormones, negates the use of the data for risk assessment since it may increase sensitivity to BPA through mechanisms that cannot readily be extrapolated to humans.

Neonatal exposure to bisphenol A reduces the pool of primordial follicles in the rat ovary (Rodriguez et al., 2010).

This study evaluated the effect of neonatal BPA exposure on follicle development in neonatal Wistar rats. After parturition, the pups were culled and cross-fostered to minimize the litter effect. The females ($n \geq 8$) were dosed by SC injection on PNDs 1, 3, 5, and 7 with BPA (0, 0.05 or 20 mg/kg bw in corn oil) or DES (0.2 or 20 µg/kg bw). Follicular dynamics were evaluated morphologically on PND 8 as was protein expression of ERα, ERβ, PR, AR, Ki67 and p27. BPA purity and phytoestrogen content in the diet were not reported. BPA (20 mg/kg bw) caused a reduction in primordial follicle population but an increase in growing follicles along with increased p27 expression in granulosal cells and oocytes in both primordial and growing follicles. Increased ERβ expression and proliferation in recruited follicles, and increases in ERα positive follicles were also reported for the high dose BPA group. BPA did not have an effect on oocyte survival rate, incidence of multioocyte follicles, ERβ positive follicles, AR expression in granulosal cells or PR expression in ovarian/follicular cells. The authors suggest that follicle activation is a potential effect of BPA caused by altering follicular dynamics. However, the biological significance and/or functions of the molecules assessed in this study to follicle development and physiology have not been clearly demonstrated. Considering the lack of information on BPA purity, potential phytoestrogen exposure

from the diet, and dosing regime (two dose levels and 48 hrs dosing interval), the data in this study are **not useful for HI or RA**.

Prenatal exposure of mice to bisphenol A elicits an endometreosis-like phenotype in female offspring (Signorile et al., 2010).

Groups of 6 pregnant female Balb/c mice from Regina Elena Cancer Institute of Rome were administered 100 or 1000 ug BPA/kg bw/day by SC injection in 2% ETOH in saline, from GD 1 until PND 7 day. All pups in a group were pooled, separated by sex, and then fostered to moms of the same treatment group. Five males and 5 females were retained per litter. Pups were weaned at PND 21 and held without further treatment until 3 months of age: There were 6 litters and 20 pups per treatment group were examined. The mouse chow tested negative for estrogenic activity by the E-screen assay; tap water was provides from glass bottles. Cages and bedding tested negative for estrogenic activity (not mentioned how tested). H and E histology of pelvic organs was conducted, as was immunohistochemistry. Liver samples from dams were assessed for free BPA, although the potential for hydrolysis of conjugated BPA during tissue processing was not examined. No macroscopic defects were found in the reproductive tract, but cystic ovaries were present (10% in controls vs 45% in BPA 100 and 50% in BPA 1000) and these differences from controls were statistically significant. An increased incidence of endometriosis in adipose tissue surrounding pelvic organs in BPA-treated animals was also statistically significant. There was no dose-response for the presence of free BPA in liver. **The study did not meet the criteria for HI or RA** based on the statistical analysis, which did not adequately address litter effects. Use of an isolated mouse colony and unusual diet are also possible concerns. It was notable, however, that cystic ovaries, which were reported at both tested doses of BPA, have been observed in other BPA studies utilizing subcutaneous administration in neonatal SD rats (Fernandez et al., 2010); Long-Evans rats (Adewale et al., 2009); and CD-1 mice (Newbold et al., 2009;Newbold et al., 2007).

Neonatal exposure to bisphenol A and reproductive and endocrine alterations resembling the polycystic ovarian syndrome in adult rats (Fernandez et al., 2010).

The purpose of this study was to evaluate the effects of BPA on GnRH from hypothalamic explants, sex hormone levels in serum, ovarian morphology, ovulation, and fertility. Female Sprague Dawley rats from an in-house colony gave birth and litters were culled to 8 on PND1. On PNDs 1-10, female pups were dosed subcutaneously daily with BPA in castor oil at doses of 5 μg /50 μL (0.25-0.62 mg/kg); 50 μg/50 μL (2.5-6.2 mg/kg); 500 μg/50 μL (25-62.5 mg/kg). Each litter included one female pup in each exposure group (housed in the same cage). Feed was a commercial lab chow (Gepsa Feeds, phytoestrogen levels not tested) and cages were steel, with wood shavings bedding (phytoestrogen levels not tested). When the offspring were 4-5 months old, studies were performed on animals in estrus. Findings included: 50 μg and 500 μg/d BPA groups had "higher" levels of testosterone and estradiol. All BPA groups had lower levels of progesterone than controls and the 50 μg and 500 μg/d dose groups exhibited increased interpulse intervals for GnRH. Ovarian weight, but not body weight, was lower at 50

µg/d and 500 µg/d. At 500 µg/d there were a large number of ovarian cysts, a lower number of corpora lutea, and a higher number of atretic follicles. The 500 µg/d animals did not ovulate and had no pups; 50 µg/d animals had fewer pups, and 5 µg/d animals showed no effect. For ovarian cysts and ovulation, 0.25-0.62 mg/kg seems to be a no-effect level for neonatal exposure in these SD female when examined as adults at 4 months of age and 2.5-6.2 mg/kg appears to be an effect level for having fewer pups. Effects on the HPG axis seem consistent in this study. While experimental design issues, including the use of an in-house SD rat, housing pups in different dose groups in the same cage, and approximate dosing (i.e. the animals were not weighed before dosing) are of concern, the data do show a dose-response. **The data were thus considered to be of utility for HI based on the dose-response**.

Neonatal Exposure of Male Rats to Bisphenol A Impairs Fertility and expression of Sertoli Cell Junctional Proteins in the testis (Salian et al., 2009a).

The authors examined the effect of neonatal exposure to BPA (99.8% purity) on adult male rat sperm parameters, fertility, and Sertoli cell junctional proteins. The latter studies, while of possible mechanistic interest, are not clearly related to an adverse effect and are not discussed here. An in-house bred strain of Holtzman rats was dosed SC with BPA on postnatal days 1 through 5 (n=32). The doses of BPA were 0.6, 1.2, 2.4, 5, and 10 µg/ rat. Exact dosing was not determined but estimated by the authors to be approximately 100, 200, 400, 800 and 1600 µg/kg bw. Male fertility was assessed during adulthood (PND 75). Females mated with male rats exposed to doses of 2.4 ug and higher showed significant loss in post-/pre-implantation survivability and a decrease in litter size. Sperm count and motility were reduced in male rats exposed to doses of 1.2 ug BPA/animal and higher. Serum hormone levels (LH, FSH, T, and E) and testis histopathology were examined in the control, 2.4, and 10 µg/ rat groups. The pattern of serum hormone changes were not consistent in the dosed groups, with decreased LH at both doses, increased T at the mid dose and decreased E at the high dose, with no change in FSH. Sloughing of germ cells in Stage I-V tubules was reported at both doses examined. The use of an in-house strain of rats, together with the 'approximate dosing' approach limit the usefulness of this study for risk assessment. It is not clear whether the neonates were cross-fostered at the time of culling to limit the litter effect and the fact that 1 treated male was mated to two untreated females was not taken into account in the statistical analysis. A nested ANOVA (within the treated male) would have been appropriate. Because of these statistical issues, this study was considered **not useful for HI or RA**. However, the reported effects of BPA on sperm numbers and motility and fertility may warrant further investigation.

IV and IM Exposure

Estrogenicity of Bisphenol A: a concentration-effect relationship on luteinizing hormone secretion in a sensitive model of prepubertal lamb (Collet et al., 2010).

This study investigated the effects of BPA on luteinizing hormone (LH) pulsatile secretion and the correlation of internal concentrations of BPA and E_2 and LH pulsatile

secretion in prepubertal ovariectomized (OVX) Lacaune lambs in response to acute (intravenous) and long-term (intramuscular) exposures. The lambs were either OVX at 2.5 to 3 months of age (about 2 to 4 weeks before the treatment) or 36 and 134 days of age (about 107 to 9 days before the treatment). For the acute exposure, the lambs (n=2-3) received a single dose of E_2 or BPA by IV infusion for 54 hrs corresponding to total doses of 0.03, 0.14, 0.72, 3.6, or 18 µg E_2/kg bw/day or 0.5, 1.0, 2.5, 5, 10, 20, 40, and 80 mg BPA/kg bw/day, during two different periods using two different sequences: E_2 first then BPA and BPA first then E_2. The vehicle for the IV studies was ethanol-propylene glycol. LH pulsatile secretion, serum levels of E_2 or BPA, steady-state concentrations of LH, terminal half-life and overall mean clearance of E_2 and BPA were measured. The results showed a dose-dependent relationship between the fitted steady-state plasma concentrations and pulsatile LH secretion with E_2, but not with BPA. High dose BPA (≥ 40 mg/kg bw) abolished the LH pulsatile secretion and decreased the basal secretion levels of LH, but did not show a dose-dependent pattern as was observed with E_2 treatment. For the long term exposure, lambs (n=4-5) received vehicle, BPA (3.5 mg/kg) or DES (0.175 mg/kg) twice a week for a total of 8 weeks by IM injection (dissolution vehicle was ethanol-corn oil). The results showed an absence of BPA accumulation in the treated lambs. DES inhibited LH pulsatile secretion, but the data were inconclusive for BPA. A discrepancy in the effective BPA internal dosage for inhibition of pulsatile LH secretion reported in the main text and that shown in the supplemental results was noted. Due to the data presentation and analysis, lack of information on environmental contamination control and purity of BPA, and sample size, this study in itself is **not useful for HI or RA**.

Carcinogenesis Studies

Summary

Several papers were reviewed that focused on the potential carcinogenic properties of BPA. Of the studies reviewed, one utilized IP administration (Doherty et al., 2010), two utilized SC administration (Prins et al., 2011;Jones et al., 2010) (minipump)), and three utilized oral (gavage) administration (Prins et al., 2011;Betancourt et al., 2010a;Betancourt et al., 2010b). These studies attempted to examine a wide variety of changes important in the carcinogenesis process (e.g., loss of Brac 1 function). However, all had design limitations that severely limit their ability to influence the HI or RA for BPA. Of the studies reviewed, the work of Prins et al. (Prins et al., 2011) suggests the need for additional investigation. This study reports that SC injection or oral administration of 10 µg BPA/kg bw/day on PNDs 1,3, and 5 increased the incidence and severity of prostatic intraepithelial neoplasia (PIN) in SD rats given BPA followed by a testosterone/estrogen implant. This is a follow-up study to a previously reviewed study by the same group (Ho et al., 2006) which also reported PIN lesions. The addition of exogenous hormones used to drive the development of PIN lesions is a confounding factor. The method of utilizing constant, sustained levels of testosterone-estrogen introduced by pellet eliminates the natural diurnal pattern of hormone levels, and the relevance of the hormonal treatment regimen to the aging human male is not clear. The

effect noted is not an effect of BPA alone but of BPA followed by a testosterone-estrogen implant. There was a 64% incidence of PIN in the lateral prostate (LP) in the corn oil vehicle controls that confounds the interpretation since testosterone and estrogen have been implicated in the development of PIN lesions in rats. A control group that only received hormone pellets would have been helpful to determine if the effect was due to BPA or to the hormones. PIN lesions, despite the name, are considered by many to be a type of hyperplasia, part of the carcinogenesis continuum. Progression from hyperplasia is necessary to result in actual neoplasms. Some, but not all, hyperplasia progresses to neoplasms over time. Thus the study would have benefited by increasing its length to allow for a better characterization of PIN and its potential as a marker for prostate cancer. Additionally, the SD rat strain is not commonly used as rodent model of prostate cancer due to the lack of spontaneous development of prostate cancer comparable to humans. The Lobund-Wistar, ACI/seg, and Nobel rat strains are more commonly used as rodent prostate cancer models that recapitulate certain aspects of human prostate cancer. However, the effects on the hormone-induced PIN lesions induced by a low oral dose of BPA at a time critical to prostate development in the rat are of interest. The use of only a single BPA dose and the use of the hormone implant model limit the utility of the data for RA. The prostate had previously been identified in the HI process, and this study does not alter the conclusions of the previous CFSAN/FDA review.

Individual Study Reviews

Oral Exposure

In Utero Exposure to Bisphenol A Shifts the Window of Susceptibility in Mammary Carcinogenesis in the Rat (Betancourt et al., 2010a).

This study investigated whether in utero exposure to BPA would predispose the adult rat mammary gland to carcinogenesis after carcinogenic insult and if the associated protein signature could provide an insight into the possible targets of BPA in the mammary gland. Pregnant SD rats were gavaged with 25 or 250 ug BPA/kg bw or an equivalent volume of sesame oil (controls) from GDs 10- 21. Animals were housed individually in BPA-free cages with glass water bottles and fed phyto-estrogen-free (AIN-93G) diet. On PND 0 litters were culled to 10 females per lactating dam. Animals were weaned on PND 21 and continued on the AIN-93 diet. On PND 50 and PND 100, two sets (n = 8) were killed while in estrus. For tumorigenesis experiments, one female offspring from each litter (BPA exposed and control) received a single oral treatment (gavage) of 7, 12-dimethylbenz(a)anthracene (DMBA) at 30 mg/kg bw on PND 50 or 100. Animals were palpated twice weekly to monitor tumor development and necropsied at 12 months of age or when tumor burden exceeded 10%. The protein levels of the steroid receptor coregulators SRC-1, -2, and -3 were measured in control and treated animals. Body weights, time to vaginal opening, circulating levels of estradiol or progesterone, or estrous cyclicity of female offspring exposed to BPA prenatally were not significantly different from those in control animals. On PND 50, SRC-3 was significantly increased. On PND 100 all three SRC proteins were significantly increased. At PND 100, BPA

significantly increased expression of EGFR. Ki-67 expression in epithelial cells of PND 100 rats treated with 250 µg/kg/bw BPA was increased as compared to controls. For the tumorigenesis experiments, no significant difference between BPA-exposed PND 50 DMBA treated animals and control animals was found for average time to first tumor or in multiplicity of tumors at necropsy. There was a significant increase in tumor incidence, significantly decreased tumor latency and increased number of grade II mammary tumors between controls and animals exposed to 250 µg/kg bw BPA prenatally and then treated with DMBA at PND 100. Though the authors concluded that prenatal exposure to 25 or 250 µg/kg BPA in sesame oil increases mammary cancer susceptibility in offspring and shifts the window of susceptibility for DMBA-induced tumorigenesis in SD rat mammary glands from PND 50 to PND 100, the relevance of the DMBA carcinogenesis model to humans is not apparent and the control incidence of mammary tumors in this study was 54%. It is noted that SD rats are quite susceptible to mammary neoplasms, compared with other rat strains. Accordingly, the use of the SD DMBA model **precludes this study from being useful for HI or RA.**

Proteomic analysis in mammary glands of rat offspring exposed in utero to bisphenol A (Betancourt et al., 2010b).

The purpose of this study was to determine the effects of prenatal exposure to BPA on the rat mammary gland proteome in postnatal rats as a first step toward the investigation of translational markers of susceptibility in the human population. Pregnant rats (n=29-33) were treated orally by gavage with 0, 25, or 250 ug BPA/kg/bw from GDs 10-21. Sesame oil was used as the control. Females were housed in polypropylene (BPA-free) cages and fed a phyto-estrogen free diet (AIN-93G). Female offspring were sacrificed at PND 21 and PND 50 and mammary glands were collected for proteomic analysis using either 2-DE/mass spectrometry (8-10 from different litters) or western blot analysis (6-8 from different litters). Prenatal exposure to BPA did not alter body weight, uterine weight, time to vaginal opening or circulating levels of estradiol or progesterone in female offspring as compared to controls. A short list of proteins (25 proteins on day 21, 21 proteins on day 50) were identified by 2-DE that were differentially expressed between control and BPA-exposed rats, including vimentin, SPARC, and 14-3-3. Using the Panther classification system, 21 proteins were grouped into 12 major subcategories according to "biological processes." The majority of the altered proteins were found in the cytoplasm. Western blot analysis of key downstream signaling proteins demonstrated increased phosphor-AKT, cRaf, phosphor-ERKs-1 and 2 and decreased TGF-B in mammary glands of the PND 50 rats exposed to BPA compared to controls. The authors concluded that prenatal exposure to BPA altered the mammary gland proteome in PND 21 and PND 50 rats but that this does not appear to be related to any overt toxicity associated with BPA exposure. However, the report does not clearly describe whether there were differences in the proteome data for low and high dose BPA exposures at the two time points. Though these data are interesting few animals were used in the study and replication is needed to confirm the findings. **While the data are not of utility for HI (findings do not demonstrate a hazard), they may be useful in the future for helping to delineate a mechanism of action for BPA in rodent mammary carcinogenesis if**

confirmed by other laboratories. The relationship of the findings to a mechanism of action for BPA in humans is not known.

Subcutaneous Exposure

Loss of BRCA1 leads to an increased sensitivity to Bisphenol A (Jones et al., 2010).

The objective of this study was to determine the effect of BPA exposure on the proliferation of (1) mammary epithelial cells in which BRCA1 is no longer present and (2) brca1-/- mammary epithelial tissue. Groups of 13 3-month-old female postpubertal Brca1 conditional knockout mice (generated with two floxed Brca1 alleles and bacteriophage P1 cre recombinase under control of the mouse mammary tumor virus long terminal repeat promoter) on a C57Bl/6 background or non transgenic C57Bl/6 mice were implanted with Alzet osmotic pumps in the intrascapular region. The pump delivered vehicle and only one dose level (250 ng BPA/kg in 50% DMSO at a flow rate of 0.22 µL/h for 4 weeks). The authors reported the following: more dense mammary epithelial growth at the ends of the terminal ducts in the Brca1-deficient mice and slightly increased alveolar buds in wild-type mice compared to the respective DMSO controls, which had no abnormalities; increased ductal hyperplasia in the Brca1-deficient mice (69% vs 28% in DMSO mice) and only a few foci of mild ductal hyperplasia in wild type mice, with no DMSO-related structural effects in wild-type mice. No naïve controls were included to evaluate whether DMSO itself had an effect. This model with continuous pump exposure to BPA in 50% DMSO cannot be readily extrapolated to humans. Although the conditional knockout model may be useful for mechanistic studies with the Brca1 gene, its utility for toxicological assessments has not been adequately demonstrated. **The animal model, the vehicle, and the mode of administration, all preclude using this study for HI or RA. Only one dose was used, so no dose-response can be evaluated.**

IP Exposure

In Utero Exposure to Diethylstilbestrol (DES) or Bisphenol-A (BPA) Increases EZH2 Expression in the Mammary Gland: An Epigenetic Mechanism Linking Endocrine Disruptors to Breast Cancer (Doherty et al., 2010).

In this study, the authors hypothesized that BPA-induced epigenetic effects might occur after in utero exposure that could predispose animals and humans to breast cancer. 15 pregnant CD-1 mice (Charles River Wilmington) were dosed IP with 5 mg/kg BPA from Sigma (purity not stated) in sesame oil on GDs 9-16 (per a previous paper, although the current paper says 9-26 in two places). The authors also conducted a PK study for this ip BPA dose and stated that the plasma levels of free BPA obtained are consistent with those that have been reported in the plasma of pregnant women. The feed was Purina Chow but water, cages, and bedding were not described. The female offspring were then killed during postnatal week 6. Sesame oil was the vehicle and there was no sham control. Comparisons were made with animals similarly dosed with 10µg/kg DES. The authors also conducted in vitro studies with BPA and DES in the MCF-7 breast cancer

cell line. The authors reported: a) no effect of BPA on EZH2 expression but an effect of DES; b) BPA increased EZH2 protein levels as did DES; and c) BPA and DES increased trimethylated histone H3, but not total levels of Histone H3. This study addressed a possible mode of action but did not demonstrate a hazard; as **such this study has no utility for HI or RA**. Additional concerns include that the days of dosing during gestation are given as 9-26, which is unusual since it extends beyond the usual gestation period of the mouse; IP injection may increase exposure of fetuses to BPA and cause effects to the fetus not seen after administration by other routes of exposure; and the possible confounder of litter effects are unknown as the methods did not describe how possible litter effects were accounted for. Lastly, the choice of diet and the lack of information on other sources of environmental estrogens is another limitation of the study design and reporting.

Oral and Subcutaneous

Serum bisphenol A pharmacokinetics and prostate neoplastic responses following oral and subcutaneous exposures in neonatal Sprague-Dawley rats (Prins et al., 2011).

This study follows earlier work that reported subcutaneous injection of 10 µg BPA/kg bw/day on PNDs 1, 3, and 5 increased the incidence and severity of prostatic intraepithelial neoplasia (PIN) in SD rats given testosterone (T)/estradiol (E) implants as adults (Ho et al., 2006). In the present study, SD rats were from a different source (Harlan) and the dose was administered both orally (gentle feeding via a pipette) and SC. The vehicle was only administered orally. Polysulfone cages, glass water bottles, double distilled water, and a low phytoestrogen diet were used. Tocopherol-stripped corn oil was the vehicle and BPA characterized by the NTP was used. Pups were from 20 litters, 15-25 per treatment group, and pups within each litter were randomly assigned to treatment groups to minimize litter effects. At PND 90, all rats received silastic T/E implants to produce normal T levels for aging male rats and 3-fold higher E levels. Implants were replaced after 8 weeks for a 16-week total exposure to T+E. Rats were sacrificed at 28 weeks of age and the prostates examined histologically. Multiple sections from each prostate were read blind by a pathologist who scored PIN lesions by criteria described in the paper. Atypical and epithelial hyperplasia and inflammation were also evaluated and tabulated. Incidence data were analyzed using a Chi-square test, while PIN scores were analyzed by ANOVA and Fisher's exact test. Ventral and lateral lobes from BPA-treated animals showed an increase in PIN scores, and the incidence of PIN was increased in the lateral prostate. Epithelial hyperplasia was increased in BPA-treated VP, but not in BPA-treated LP or DP, and inflammatory cell infiltration was increased in the LP, but not in BP-treated VP or DP. Importantly, BPA effects were observed after treatment from both routes of exposure. There were no significant differences in the dorsal prostate, which differs from the results reported in the earlier study from this group that used SD rats from a different source. The strengths of this study include the attention paid to background exposures to BPA and phytoestrogens, the evaluation of internal doses, adequate sample sizes, and the blind reading of the slides. The authors addressed earlier criticism of the use of SC dosing only and report significant effects for both oral and SC exposures. A study weakness is the use of only one dose of

BPA and lack of a sc vehicle control. Differences between this and the earlier study from the same authors in terms of lack of a significant response in the DP may, as the authors mention, be due to substrain differences. The dorsal and lateral lobes of the rat prostate are of most interest as having most homology to the peripheral zone of the human prostate where carcinomas develop. A question as to the relevance of the hormonal treatment to the situation in the aging male human exists. **The prostate had previously been identified in the HI process and this study does not alter the previous conclusions of the CFSAN/FDA review.** The effect is not an effect of BPA alone but of BPA followed by a testosterone-estrogen implant. The fact that the BPA effects on the hormone-induced PIN lesions were induced by a low oral dose of BPA at a time critical to prostate development in the rat is of interest. The use of only a single BPA dose and the use of the hormone implant model limit its utility for HI or RA. Furthermore, the incidence of PIN in the LP in the corn oil vehicle controls, 64%, is a confounder. Although the authors state that the lesions are relevant to pre-neoplastic lesions in the development of human prostate cancer, the model used (hormone implants in adults) is controversial with regard to human relevance. In addition, it is unclear that the PIN lesions observed following treatment with BPA will in fact lead to tumors. As such, increasing the length of this study type to allow for a better characterization of PIN and its potential as a marker for prostate cancer is necessary to fully interpret the finding.

Other Endpoint Studies

Summary

Papers in this group evaluated endpoints that span a wide range of effects, including inflammation, molecular and cellular-level changes in the reproductive tract, and glucose and lipid homeostasis. Three of the papers described physiological or gene-level endpoints that are not thought to be "adverse" and were therefore classified as supporting or mode of action studies. One paper did not include a statistical evaluation of the data and was therefore rejected from further consideration and is only highlighted in the Appendix table as opposed to this summary (Holladay et al., 2010),. Among the remaining studies, three papers, Midoro-Horiuti et al. (2010), Ryan et al. (2010) and Somm et al. (2009), failed to account for litter effects. As a result, these studies were rejected since they did not meet an important criterion for appropriateness as either a HI or RA paper. Two of the papers, Braniste et al. (2010) and Alonso-Magdalena et al. (2010) included studies on adult female animals and offspring. Since neither of these studies properly accounted for litter effects, the component of the study that used offspring from BPA-treated dams was not considered for HI or RA; however, the aspects of the studies conducted on adult female animals have utility as supporting information (Braniste et al., 2010) or for HI (Alonso-Magdalena et al., 2010).

Individual Study Reviews

Oral Exposure

Perinatal exposure to bisphenol-a and the development of metabolic syndrome in CD-1 mice (Ryan et al., 2010).

Questions have been raised about the role that BPA may have in the development of obesity and diabetes in humans. The objective of this study was to determine if exposure of male and female CD-1 mice to an "ecologically relevant" dose of BPA during gestation and lactation will result in obesity in adulthood and whether this exposure exacerbates the effect of a high fat diet on weight gain and glucose metabolism. In this study, vaginal plug-positive female CD-1 mice were fed either an unmodified AIN93G control diet, feed containing 1 ppb BPA (maternal dose, 0.25 μg/kg/day), or feed containing 4 ppb DES as a positive control. Dams remained on the diets until the pups were weaned at PND 21. Although some effects were seen in offspring from BPA-treated dams (e.g., increased body weight in both male and female PND21 pups), litter effects were not accounted for in this study; as a result, it **cannot be used for either HI or RA.** Since some studies do show an effect on glucose homeostasis in BPA-treated rodents or their offspring (Alonso-Magdalena et al. 2010, below), it is notable that BPA exposure had no effect on this parameter in this study. Rather, female pups from DES-treated (but not BPA-treated) dams demonstrated impaired glucose tolerance at 8 weeks and this effect was also seen in female (but not male) mice on the high-fat diet at 15 weeks.

Perinatal exposure to bisphenol a alters early adipogenesis in the rat (Somm et al., 2009;Ryan et al., 2010).

This study was conducted to determine if perinatal exposure to BPA alters adipose storage at weaning and body weight/fat distribution following post-weaning consumption of a high fat diet. BPA was administered to pregnant SD rats via drinking water from GD 6 to the end of lactation (PND 21). BPA was dissolved in 1% ethanol and the dose by the end of gestation was estimated to be 70 μg/kg/day. Control animals received water containing 1% ethanol. On PND21, two groups of weanling animals were formed from three different litters/group (presumably accounting for litter effects); a control group and a BPA expose group (n=10 each). These animals were used immediately for fat analysis and determination of hormone levels. A second group of control and BPA-exposed PND 21 weanling animals were fed either a standard diet or a high-fat diet for 14 weeks. As in the Ryan et al. (2010) study, there were effects seen in offspring from BPA-treated dams, notably, increased body weight of male and female pups on PND 1 and changes in the weight of peri-gonadal white adipose tissue and intrascapular fat pads was seen in female but not male offspring. Litter effects were not controlled for, therefore, the results of the study cannot be used for either HI or RA per the evaluation criteria for these endpoints.

Maternal bisphenol a exposure promotes the development of experimental asthma in mouse pups (Midoro-Horiuti et al., 2010)..

This study examined the hypothesis that BPA could enhance allergic sensitization as well as bronchial inflammation and responsiveness. In this study, female BALB/c mice

received 10 µg/mL BPA in their drinking water 1 week prior to impregnation and throughout pregnancy and lactation. Airway hyper-responsiveness was induced in pups using OVA. Although BPA-treated and OVA-sensitized pups showed increased airway responsiveness, the study did not account for litter effects; consequently, the results can **not be used for either HI or RA.**

Impact of oral bisphenol A at reference doses on intestinal barrier function and sex differences after perinatal exposure in rats (Braniste et al., 2010).

This study evaluated the impact of oral exposures to BPA and estradiol benzoate (EB) on intestinal barrier function, the response to chemically induced colitis, and on pain perception following rectal dilation. The study was conducted on both ovariectomized (OXV) rats and, in a separate experiment, in offspring of BPA or EB-treated animals. Since the second part of the study did not account for litter effects, only the results of the study on OVX rats was considered. In the first part of the study, OVX adult female Wistar rats were given an oral gavage for 15 days of either corn oil vehicle control, BPA in corn oil at a dose of 5 mg/kg/d, BPA in corn oil at 50 µg/kg/d, or EB at 0.6 mg/kg/d. Three groups receiving the BPA at 5 mg/kg/day and 2 groups treated with EB received a daily SC injection of the ER antagonist ICI 182.780 (2 mg/kg/day in olive oil) for the last 5 days of treatment. Rats were used for permeability assays (5-13/group) or induction of experimental colitis (5-13/group), or assessment of visceral pain (5-14 rats/group). A dose-response experiment in which OVX rats were treated orally with BPA (0.5 µg/kg/d to 5 mg/kg/d) for 15d (n=6-8 rats/group) was conducted for a permeability assessment. This group reported that BPA caused a dose-dependant decrease in colonic paracellular permeability (CPP) with a half-maximal inhibitory dose of 5.2 µg/kg/day and a maximal inhibitory dose of 0.5 mg/kg/day. The functional results are supported by mechanistic data showing that BPA at 5 mg/kg/day caused a statistically significant increase in the expression of occludin and JAM-A protein levels in the colon of OVX rats. Also, the effects of BPA, as well as EB, were in both cases prevented by the presence of an estrogen receptor (ER) antagonist, ICI 182.780. In the inflammation study, BPA at a dose of 5 mg/kg/day decreased colonic myeloperoxidase activity (MPO) in induced colitis and also decreased macrophage migration inhibition factor (MIF), an effect which was blocked by the ER antagonist ICI 182.780. Although this study may have utility for HI, there are several factors to consider. First, the data showing a dose-dependent effect on intestinal permeability in OVX rats is convincing, but the n may be less than 10. Also, the anti-inflammatory effect of BPA at higher doses (5 mg/kg/day) on the response to induced colitis is not necessarily adverse, in fact, it may be beneficial. Therefore, it's difficult to characterize these physiological responses (altered intestinal permeability, anti-inflammatory effect on chemically-induced colitis) as being adverse for the purposes of safety assessment; therefore, the results of this study should **not be used for either HI or RA.**

Subcutaneous/IP Exposure

Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring (Alonso-Magdalena et al., 2010).

This study was conducted to determine if exposure of pregnant mice to BPA results in altered glucose homeostasis in dams during pregnancy and in dams and offspring later in life. Molecular- and cellular-level endpoints (insulin secretion from islet cells, changes in intracellular calcium dynamics in islet cells, pancreatic ß cell area) were also assessed. In this study, pregnant OF-1 mice were injected with either corn oil vehicle (control) or BPA (10 or 100 μg/kg) on GDs 9-16. Glucose tolerance tests were conducting using a standard protocol at GDs 16-18 and 4 months after delivery in pregnant mice/dams and at 3 and 6 months of age in offspring. Plasma was analyzed for insulin, leptin, triglycerides, and glycerol using appropriate methods. Since litter effects were not accounted for, only the component of the study that used pregnant animals was considered for the safety assessment. Glucose homeostasis was altered during pregnancy (10 μg/kg dose group) and at 4 months (100 μg/kg group) after delivery in F0 mice. Plasma hormone and metabolite levels (insulin, leptin, triglycerides, and glycerol) were increased during pregnancy and 4 months after delivery in a dose-dependent fashion. The investigators attributed the lack of elevated blood glucose in F0 mice treated with 100 μg/kg/day to elevated levels of insulin in that dose group; therefore, the lack of a dose-response for this endpoint can be explained. The strengths of the study include use of a well characterized diet that was free of soy or alfalfa components, use of multiple doses along with a negative control group, and evaluation of the effects at various life stages of exposed animals. Although the results in F1 animals were not considered due to the lack of control for litter effects, these results are notable as supporting data for the effects seen in dams. Specifically, at 6- but not 3-months of age, BPA exposure resulted in altered glucose tolerance and insulin sensitivity in male (but not female) offspring of dams treated with 10 μg/kg (but not 100 μg/kg) BPA. These changes were associated with differences in plasma insulin and glycerol, compared to controls, in 6-month-old male offspring. In addition, the effect of BPA on cellular- and molecular-level endpoints (e.g., insulin release from isolated pancreatic islets) provide support for the functional endpoints (e.g., hyperglycemia) seen in the study. It is notable that the effects on glucose homeostasis seen in BPA-treated mice are directly clinically relevant for patients. Consequently, the results of this study are **useful for HI.** Despite these strengths, use of the study for risk assessment is limited by uncertainties associated with the potential effect of the corn oil vehicle on glucose homeostasis and reproduction (as noted by (Sato et al., 2000)), the use of only female animals in the study, and a limited number of dose groups.

Effects of neonatal exposure to bisphenol A on steroid regulation of vascular endothelial growth factor expression and endothelial cell proliferation in the adult rat uterus (Bosquiazzo et al., 2010).

The goal of this study was to determine if neonatal exposure to BPA can alter expression of vascular endothelial growth factor (VEGF) and proliferation of endothelial cells in female rats on Day 80 after the animals had been ovariectomized (OVX). In this study, newborn female Wistar rats were injected s.c, with either vehicle (corn oil), BPA (either

0.05 or 20 mg/kg), or DES (0.2 μg/kg) on PNDs 1, 3, 5, and 7). On Day 80, rats were ovariectomized and treated with progesterone alone or progesterone + estrogen. The results of the study showed that decreased VEGF expression and endothelial cell production were observed in uterine tissue after hormonal treatment in OVX rats exposed to the low dose of BPA. A non-monotonic dose-response was observed, with no effects seen at the higher BPA dose (20 mg/kg). The endpoints evaluated in the study, VEGF expression and endothelial cell proliferation, may be important for an understanding of the mechanism by which BPA produces adverse reproductive effects, but by themselves do not constitute an "adverse" effect from a toxicological perspective. As a result, this report should **not be used for either HI or RA, but as a supporting study.**

Bisphenol-A exposure in utero leads to epigenetic alterations in the developmental programming of uterine estrogen response (Bromer et al., 2010).

This study evaluated whether the permanent epigenetic effects seen with *in utero* exposure to BPA following ip administration to dams are mediated through aberrant methylation of the *Hoxa 10* gene. Since this study dealt with a mechanistic endpoint, it should **not be used for either HI or RA, but may be useful as a supporting study.**

Epidemiology Studies

Summary

Nineteen epidemiologic reports in the BPA literature were reviewed. Ten studies used a cross-sectional design, six studies used a case-control design (three used data from the same investigation conducted in China), and three studies used a cohort design. Approximately half of the studies assessed the relationship between BPA levels and specific diseases or adverse outcomes, such as cardiovascular disease, sexual dysfunction, lower semen quality, premature birth, and others. As described in the detailed reviews that follow, the three occupational studies by Li et al. (2010), which assessed sexual dysfunction and semen quality in workers with and without occupational BPA exposure are of interest, but of limited impact due to potential confounders in this population. The study by Melzer et al. (2010), which was a follow-up of a study by Lang et al. (2008) on cardiovascular disease and other endpoints and urinary BPA concentrations from NHANES, may subsequently have utility for HI. Some studies assessed the relationship between BPA and reproductive hormone levels or other physiologic measures and did not provide clear evidence of links to specific potential hazards. While such studies do not have current utility for HI or RA, information from these studies may be of future utility for HI in the context of similar findings from future studies.

Of particular concern with regard to the utility of the findings of many of the epidemiology studies is whether or not the methods used for BPA exposure measurement accurately characterized the true exposure of study subjects over time. For example, many studies used spot urine samples to assess BPA exposure. However, urine BPA concentrations are highly variable within individuals and over time, and the within-day

variability of the spot test is greater than between-day/within-person variability; both are greater than the between-person variability (Ye, 2011). Because of the variability in urinary BPA excretion, exposure measurements based on a single urine sample may lead to bias in the exposure data and incorrect conclusions from analyses of these data. A related concern has to do with the timing of the exposure measurements. Most of the studies assessed exposure at the same time that health outcomes were assessed. Even if such measurements provided accurate estimates of current BPA exposure, such data may not be relevant with regard to the particular health outcome assessed.

Individual Study Reviews

Bisphenol a exposure in Mexico City and risk of prematurity: a pilot nested case control study (Cantonwine et al., 2010).

This pilot study was a nested case-control study (30 cases and 30 controls) nested within a Mexican birth cohort study (n=670), the *Early Life Exposure in Mexico to Environmental Toxicants* (ELEMENT) study in Mexico City, Mexico, in which women were recruited during prenatal visits at one of four clinics of the Mexican Institute of Social Security in Mexico City between 2001 and 2003. Cases were a random sample of women who had delivered before 38 weeks of gestation, which included 12 women who delivered prior to 37 weeks. Controls were a sample of the total number of women who had completed 38 or more weeks of gestation at the time of delivery. Gestational length was estimated by date of maternally-recalled last menstrual period. A spot (second morning void) urine sample was collected from each woman during a third trimester visit (earliest urine collection occurred during the 30th week of gestation). The dates of urine collection were not noted, although these dates were used in the analysis in a similar study which found phthalates to be related to prematurity in the same 60 women. All urinary BPA concentrations (corrected and uncorrected for creatinine or specific gravity) were log transformed in order to stabilize variance. The LOD for BPA in a 0.1-mL urine sample was 0.4 μg/L. For samples with concentrations below the LOD [N = 12 (20.0%)], an imputed value equal to one-half the LOD (0.2 μg/L) was used. BPA concentrations were corrected for urine dilution by specific gravity (SG) using the following formula: Pc = P[(1.014 - 1)/SG - 1)], where Pc is the SG-adjusted BPA concentration (ng/ml), 1.014 is the median SG value among the present study population, P is the measured BPA concentration, and SG is the specific gravity of the urine sample.

Cases had a geometric mean urine BPA concentration of 1.84 ± 1.86 μg/L (1.71 ± 1.57 μg/L SG adjusted) compared to controls 0.97 ± 0.92 μg/L [1.20 ± 1.02 μg/L SG adjusted (t-tests; p-value = 0.01 and 0.11, respectively). No significant differences were reported by maternal age, maternal education, pre-pregnancy body mass index, parity, marital status, or sex of infant when comparing term and preterm infants. In a logistic regression model adjusting for maternal age, maternal education, parity, and infant's sex, the adjusted odds ratio in relation to specific gravity-adjusted third trimester urinary BPA was 1.91 (95%CI 0.93, 3.91, p-value = 0.08). The authors state that inclusion of third trimester urinary phthalate metabolites didn't appreciably change the odds ratios in the regression (results not presented in article).

This study hypothesizes that BPA exposure may impact placental tissue development and thyroid function in humans. However, no measurements were presented to assess these intermediate outcomes. There is high potential for both exposure and outcome misclassification in this study, as well as confounding. In addition, with only 30 cases and controls, inclusion of even 4 covariates in the regression model to adjust for confounding may lead to 'sparse-data' biases. A spot (second morning void) urine sample was collected from each woman during a third trimester visit. This single assessment is assumed to be representative of the mother's exposure to BPA throughout the term of her pregnancy yet exposure is likely to be variable over time. In another study using the same 60 women and outcomes, with phthalate metabolites measured in the spot urine samples as the markers of exposure (Meeker et al., 2009), the authors mention that the cases and controls differed on creatinine and specific gravity levels and that urine collection date was a confounder in one of their phthalate models. This is a further concern for the method of exposure measurement and adjustment. Secondly as stated by the authors, gestational length was estimated by date of maternally-recalled last menstrual period which may be an unreliable measure, varying as much as ± 7-21 days, depending on a host of factors including nutrition, physical activity, alcohol consumption, stress, and inter-pregnancy interval. One possible uncontrolled confounder is low socioeconomic status. While the authors examined age as a confounder, they did so using it as a continuous variable. However this is a non-linear relationship. It is the extremes of maternal age (<17 y or >35 y) which are at higher risk for preterm birth. This distinction is completely lost in the current analysis. In the phthalate analysis of these women, the effects on gestational length of some metabolites were stronger than those in this study of BPA. The influence of this co-exposure should be assessed beyond stating that addition of phthalates to the model did not have an appreciable effect. Because of these limitations, **this study was not useful for HI, dose-response determination, or RA.**

<u>The impact of bisphenol A and triclosan on immune parameters in the U.S. population, NHANES 2003-2006 (Clayton et al., 2011).</u>

This study used NHANES (2003-2006) cross-sectional study data to examine whether urinary levels of BPA and triclosan were associated with reported allergies or measured serum antibody titers to a common pathogen of the herpes virus family, cytomegalovirus (CMV). Serum CMV/CMV antibody levels are considered as markers of altered cell-mediated immune function. CMV-specific IgG was measured in the 2003-04 wave NHANES for respondents aged 6-49 using an ELISA. Sera samples near the ELISA cutoff were confirmed with a second ELISA assay. When these 2 tests disagreed, an immunofluorescent assay (IFA) was performed and the result of the IFA was used as the final result. CMV-specific IgG optical density was reported (measured in Arbitrary Units / ml) and used as a continuous outcome variable. CMV seropositivity was defined by NHANES based on this optical density measure. This study also utilized self-reported diagnoses of allergies or hayfever by NHANES questionnaire data: "Has a doctor or other health professional ever told you that you have allergies/hayfever?" Spot urine samples were collected at a single time point and analyzed for BPA levels using an online

solid-phase extraction procedure coupled with HPLC-isotope dilution and tandem-MS. This cross-sectional study sample consisted of 3,728 US adults and children age ≥ 6 years with both BPA and triclosan measurements, with 1/3 random subsample of those 6 years and older (N=5,250); [the study started with 5,065 had both BPA and triclosan measurements, and the following were further excluded: 541 with missing data on 1 or more covariates; 796 with missing continuous CMV data or allergy diagnosis]. Multivariate analyses included age, sex, race, BMI, creatinine levels, family income, and educational attainment. Multivariate ordinary least squares linear regressions were used to model continuous CMV. Logistic regressions were used to model allergies/hay fever. Interaction by age was explored using stratified analyses of <18 years and ≥18 years age group due to the potential difference in length of BPA exposure. All analyses included sampling weights and were adjusted for NHANES complex survey design. BPA < LOD were excluded.

The lower LOD for BPA was 0.36 ng/mL in 2003-04 and 0.4 ng/mL in 2005-06. Of the study sample, 46.7% were seropositive for CMV. Increasing urinary BPA levels were associated with increasing CMV antibody titers among participants ≥18 years old ($p<0.001$). For each unit increase in CMV antibody levels, there was a mean increase of 0.158 (SE=0.035) increase in log-transformed BPA (ng/mL) ($p<0.001$). Conversely, in the <18-year-old age group, lower levels of BPA were associated with higher CMV antibody titers ($p<0.05$). For each unit increase in CMV antibody levels, there was a mean increase of 0.113 (SE=0.047) decrease in log-transformed BPA (ng/mL) ($p<0.05$). There was no association of BPA with allergy/hayfever diagnosis [adjusted OR (SE) for ln-transformed BPA/ng/mL <18 years: 0.93(0.12) and for ≥18 years: 0.90 (0.09)]. Data from this large, population-based cross-sectional study suggested that BPA exposure is positively associated with CMV antibodies in adults (≥18 years) but in contrast, is negatively associated with CMV antibodies in children (<18 years). This interaction is unclear and there isn't any strong biological plausibility for this finding.

This was a population-based study with a nationally representative sample and a relatively large sample size for a cross-sectional study. In terms of BPA and CMV seropositivity, the study used a relatively accurate measurement for one of the endpoints, CMV seropositivity. However, in terms of BPA and immune function, CMV seropositivity is a less sensitive biomarker for immune function because it affects only the typeII helper (Th2) balance of the immune system and does not reflect the Th1 arm. Several limitations were noted in the study design and reporting. For examples, BPA was measured in a spot urine sample that may not reflect the actual BPA exposure. 24-hour urine samples are preferable for less measurement error of BPA exposure. In addition, BPA urine levels may not reflect the concentration of free (unconjugated) BPA in the circulating system (bloodstream) that has more direct access to organs. Additionally, the cross-sectional study design with prevalent cases of immune-related diseases precludes conclusive inference on the direction of causality of BPA and immune system and the measurement of self-reported allergies and hayfever may not capture the lower range of allergies. Results from this large, cross-sectional study selected from the US national survey are generalizable to the American public. The observations of a positive association of BPA with CMV antibody levels in adults that is contrasted with negative

association in children is intriguing. Given that there is currently no replication of this observation in either independent cross-sectional studies or prospective studies, these initial findings warrant further investigations. **The current findings alone have no direct utility for HI.**

Measurement of bisphenol A and bisphenol B levels in human blood sera from healthy and endometriotic women (Cobellis et al., 2009).

This study was conducted to validate a new technique for measuring and monitoring BPA and bisphenol B (BPB) levels simultaneously using mass spectrometric analyses, and to assess a possible association between levels of each of the two bisphenol compounds and endometriosis. The authors claim that by use of RP-HPLC with fluorescence detection, employing a monolithic column as the stationary phase to simplify extraction, the new technique minimizes effects from residual biological components in sera, while increasing simultaneous BPA and BPB recovery. The participants in the study included cases and controls; enrolled participants consisted of a convenience sample of fertile women (n=69) referred to the Second University of Naples' Department of Gynecology, Obstetrics and Reproductive Medicine for ovarian cysts, pelvic pain, or dysmenorrhea. After diagnostic or operative laparoscopy, the women were divided into endometriotic cases(n=58; mean age: 32.8±6.7y; range: 21-42 y) and non-endometriotic controls (n=11; mean age: 34.5±4.1y; range: 18-44y). Endometriosis diagnoses were ascertained by histological examination and lesion classification according to the revised American Fertility Society system. Age-matched controls were ascertained as "healthy" if they did not show macroscopic evidence of disease; however, like the cases, these women were referred for investigation of ovarian cysts, pelvic pain, or dysmenorrhea, potentially conditions with abnormalities not evident macroscopically. Clean glass syringes, tubes, and vials were used for blood samples to avoid possible contamination, stored at -20ºC, and assayed within a week of collection. RP-HPLC validation used spiked sera to verify no interference from other biological compounds, based on elution within 4 min in chromatograms and absence of signals interfering with those of BPA and BPB. Mass spectrometric analyses and triplicate RP-HPLC measurements of standards, reagent blanks, and samples, using appropriate calibrations and calculations of LOD and LOQ, were performed and compared. LODs were 0.18 ng/mL for BPA and 0.20 ng/ mL for BPB.

Although study participants included cases and controls, the study lacked elements of case-control design. Case and control definitions were incomplete, exclusion and inclusion criteria were not fully detailed, refusals and nonparticipant characteristics were not addressed, and the study period was not given. As such, this investigation was not a properly designed case-control study. Lack of BPA detection in any of the control sera suggests the possibility of control selection bias, measurement error, or misclassification bias. Both BPA and BPB were detected in sera from 30 (51.7%) and 16 (27.6%) cases, respectively. Only 15 of the BPA (range: 0.79-7.12 ng/mL; mean: 2.91±1.74 ng/mL) and 10 of the BPB serum samples were quantitated because of LOQ. This study started out with a sample size too small to be representative and its analytical sample size became even smaller. BPA and BPB detection by the novel RP-HPLC method may have been

adequately compared to mass spectrometric results to address the study's first aim. However, this study was unable to adequately address its second aim, which was to assess the association between endometriosis and BPA or BPB levels, given its weaknesses, which included study design flaws, especially with regard to the choice of controls, possible bias and measurement error, and limited data analyses failing to take into account potential confounders and risk factors. Because none of the controls had BPA or BPB levels, no comparisons between control and case mean levels could be made or examined for statistically significant association with risk of endometriosis; therefore, there was no identified association to adjust for potential confounders or risk factors. Due to the stated limitations and the small, unique population examined, **this study is not useful for HI, dose-response determination, or RA.**

Serum unconjugated bisphenol A concentrations in women may adversely influence oocyte quality during in vitro fertilization (Fujimoto et al., 2011).

In this cross-sectional study, the authors investigated the relationship between serum BPA and oocyte maturation and fertility outcomes in women undergoing IVF. The study subjects were derived from a cohort of 58 infertile female patients and 37 male partners undergoing a first IVF cycle. Female patients underwent gonadotropin-induced ovarian stimulation "…per clinic protocols." A fasting blood specimen was obtained from women at the time of oocyte retrieval. Specimens were collected in serum separator Vacutainer tubes and later aliquoted into polypropylene cryovials and frozen; serum was assayed for concentrations of unconjugated BPA by HPLC. Oocytes were fertilized by conventional insemination or intracytoplasmic sperm injection (ICSI). Approximately 16-18 hours after insemination, zygotes were identified by the appearance of two pronuclei. For each ICSI patient, the proportion of mature oocytes collected was defined as the total number of oocytes in metaphase II (MII) arrest divided by the total number of oocytes collected; the authors do not mention what was done in this regard for conventional insemination patients. In ICSI patients, the proportion of oocytes fertilized was determined from the number of zygotes formed divided by the number oocytes injected; in conventional insemination patients, the number of zygotes formed was divided by the number oocytes with a visible polar body. The authors state that "To maximize statistical power the denominators were collapsed and a single value generated," but they do not describe how that single value was calculated. Log binomial regression models were used to estimate adjusted associations between log-transformed serum BPA concentrations and oocyte maturity for 31 women undergoing ICSI and 26 IVF couples; only participants with complete covariate data (age, race/ethnicity, cigarette smoking) were included.

The median BPA concentrations were 2.53 ng/ml (range 0.0 – 67.4, 86.4% > LOD) for women and 0.34 ng/ml (range 0.0 – 22.7, 51.6% > LOD) for men. In the multivariate analysis, there was no association between BPA and oocyte maturation when all cases were considered; an association was seen for the nine Asian women in the ICSI group. There was an association between BPA and fertility among the 26 cases of ICSI or conventional insemination; a doubling of the female serum BPA concentration was associated with a 55% decrease in the probability of fertilization [adjusted relative risk (aRR): 0.45; 95% confidence interval (CI): 0.31, 0.66; $p<0.001$)].

Several limitations were noted with regard to the conduct and reporting of this study. These included: the fact that the cross-sectional design precludes assessment of temporality because the exposure and outcome data collection occurred in the same time period; the small number of study subjects precluded adjustment for additional covariates; however, the authors noted that the source study for the subjects in this study (Bloom et al. 2010) is a hypothesis-generating investigation which collected data on lead, cadmium, and mercury exposures; examining the effects of these other exposures on the observed associations in this study may have been informative; measurement of BPA in serum may not be as reliable as measurement of BPA in urine; (Bloom et al., 2010;Calafat and Needham, 2008) the study findings may not be generalizable to the population of women who are not undergoing evaluation for subfertility; the authors did not address how the timing for the blood specimens--which were taken after fasting among the women but not after fasting among the men--may have impacted the measurements or study findings; furthermore, although gonadotropin was used to induce ovarian stimulation and human chorionic gonadotropin was administered 36 hours before oocyte retrieval, which was also when the blood specimens were obtained from the women, there was no discussion of the possible impact of either on BPA metabolism or serum measurement; and serum BPA levels were substantially higher in women compared to men in this study (the investigators did not discuss any possible explanations, or indicate if they considered and explored the possibility that another confounding exposure associated with higher BPA might be related to decreased fertility in this study). Based on these concerns, the findings of this study **do not have current utility for HI and RA but may be of future utility for HI in the context of similar findings from additional studies.**

<u>Daily Bisphenol A excretion and associations with sex hormone concentrations: results from the InCHIANTI adult population study (Galloway et al., 2010).</u>

This study was conducted by the same research group that reported correlations between BPA levels and disease outcomes based on analyses of NHANES data (Melzer et al., 2010;Lang et al., 2008b). The current study was based on cross-sectional samples (urine and serum) and questionnaire data for 720 individuals collected as the base-line measures in 1998-2000 from two sites for the Italian InCHIANTI cohort study (Ferrucci et al., 2000). The total InCHIANTI cohort included 1268 persons selected through a multistage sampling plan in Tuscany, Italy who provided blood samples and had physical examinations. The method for sub-sampling from the 1268 to get the 720 was not described. BPA was measured in a 24 hour urine sample using the same methods as in the NHANES study (solid-phase extraction with HPLC; LOD 0.50 μg/L). Sex hormone concentrations were measured using a commercial RA for total testosterone (LOD 0.08 ng/mL), sex hormone binding globulin (SHBG; sensitivity 3 nmol/L) and estradiol (sensitivity 2.2 pg/mL). Materials used for blood and urine collection, storage, and lab assays and their possible impacts on measurement were not discussed. Although participants were reported to have consumed a diet free of meat and fish for 3 days before blood and urine collection, no limits on intake of dietary phytoestrogens or other-than-animal sources of fat were mentioned. Geometric means and distribution percentiles were

presented by sex and age. An upper age cutoff of 75 years was used to minimize the problem of comorbidity without discussing the reasoning for that choice or addressing comorbidities among those in the included age-groups. Geometric means, adjusted for age, sex and site were presented by education level, BMI, smoking history, waist circumference, weight and urinary creatinine. Other plausibly important confounders and risk factors were not taken into account such as prescription drug use and alcohol intake. Twenty separate multivariable linear regression models were developed to evaluate the association between BPA and estradiol, testosterone or SHBG for men, premenopausal and postmenopausal women, adjusted first for age and site, and then for age, site, smoking, BMI, weight, waist and urinary creatinine concentration. The only significant associations were, for men, higher BPA excretion was associated with higher testosterone levels and higher BPA excretion was associated with higher SHBG in premenopausal women. Post-hoc evaluations of estradiol:testosterone ratios did not detect associations.

This study provides estimates of BPA levels in 24 hour urine collections for a general population sample in Tuscany, Italy. The high proportion of the population exposed and the estimated levels were similar to findings from other studies across the globe. In a recent study, variability in urine BPA levels within the same person between days was higher than between persons, but averages across a population may give reasonable summary estimates (Ye et al., 2011b). However, this type of variability would make it more difficult to detect true relationships between BPA exposure and health outcomes. In other studies, the association between BPA and testosterone has varied from no association to both negative and positive associations. This cross-sectional study, with BPA and hormonal levels measured at a single point in time, does not help clarify the relationship. In addition, as the authors note, there are potential biological explanations for reverse causation. Residual confounding within age class may have contributed to the association, since testosterone declines with older age and BPA showed a small decline with age in this study. The authors did not discuss the equally strong association of SHBG with BPA in premenopausal women. Although SHBG appears to have been measured predominantly to allow determination of free testosterone, a number of studies have explored the relationship of SHBG with insulin resistance and diseases (Pasquali et al., 1997;Akin et al., 2009). Additionally, SHBG has been reported to increase in response to estrogenic stimulation, although it would be difficult to explain the differential effects seen between groups in this study. SHBG has also been demonstrated to regulate the estradiol – testosterone balance and to vary between individuals, but the authors did not address its potential as an effect modifier (Maggio et al., 2011;Xita and Tsatsoulis, 2010). Previous studies have reported varying patterns of association or lack thereof between BPA and testosterone. This study does not clarify this relationship nor does it provide evidence of new risk information. As such**, this study is not considered useful for HI or RA but may be of future utility for HI in the context of similar findings from other studies.**

<u>Endocrine disruptors and polycystic ovary syndrome (PCOS): elevated serum levels of bisphenol A in women with PCOS (Kandaraki et al., 2011).</u>

The objectives of this study were to measure serum BPA levels in women with PCOS and to explore a possible link between BPA and PCOS. Although described as cross-sectional, the design is case-control, with 71 PCOS cases that were referred to an Athens, Greece university hospital endocrine clinic for menstrual irregularities. Those who met NIH PCOS criteria were included, and those with androgen excess disorders other than PCOS were excluded. Enrolled cases were described as “in good health” without “chronic or acute diseases.” Age- and BMI-matched controls consisted of 100 “healthy” women with regular periods and no hyperandrogenemia, hirsutism, or acne, further described as euthyroid, normoandrogenemic, and normoprolactinemic, with 17OHP not exceeding 1.0 ng/mL; however, the source and selection of controls are not indicated. Current smokers, those > 40 y old, and those with known cardiovascular disease, neoplasms, diabetes mellitus, renal impairment (serum creatinine >120 umol/L), or hypertension (bp >140/85 mm Hg) were excluded, but other than as specified, methods of ascertainment for “good” health, lack of chronic disease, and “healthy” were not addressed. Oral contraceptives or other (unspecified) drugs affecting carbohydrate metabolism were noted to have ceased ≥3 mo pre-study, but no verification was indicated. Refusals to participate, response rates of those recruited and non-respondent characteristics were not addressed. From each blood sample taken after a 3-d high carbohydrate (300 g/d) diet, overnight fasting, and a standard 2-h oral glucose tolerance test, BPA level was directly measured in duplicate using an ELISA kit (with a somewhat high intra-assay coefficient of variation range: 5.5% - 14.0%). Directly measured PCOS components included insulin, total testosterone, SHGB, androstenedione, LH, and FSH. Alexandraki et al. (Alexandraki et al., 2006) was cited for 17OHP and dehydroepiandrosterone sulfate measurement methods, which were not found described in that paper. Other PCOS components were indirectly measured. Statistical methods were appropriate but incompletely presented.

Mean BPA levels (ng/mL) were found to be significantly higher in PCOS cases compared to BMI-matched controls [PCOS-Lean (BMI <25 kg/m2) 1.13±0.63 vs. Control-Lean: 0.70±0.36, $p<0.001$; PCOS-Overweight (BMI ≥25 kg/m2): 0.96± 0.46 vs. Control-Overweight: 0.72±0.39, $p<0.05$)]. BPA levels were significantly associated with (1) testosterone ($r=0.192$, $p<0.05$) and (2) androstenedione ($r=0.257$, $p<0.05$). Multiple linear regression showed significant correlation of BPA with PCOS ($r=0.497$, $p<0.05$), and BPA was also significantly correlated with insulin resistance (indirectly measured by the Matsuda index) among PCOS cases ($r=0.273$, $p<0.05$).

Strengths of this study included direct measures of several PCOS variables and serum BPA. However, the suitability of the ELISA method for measuring BPA in human sera is questionable because of low BPA levels in blood and non-specific binding of anti-BPA antibody, which may compromise results. In addition, previous evidence indicates that serum BPA levels may be unreliable because of (1) possible contamination during sampling, storage, and processing, (2) variable analytical methods, and (3) variable human factors, including age, diet, route/frequency/magnitude of exposure from all sources, potential interaction of BPA products with other blood chemicals and compounds (such as lipoprotein), and genetics (Calafat and Needham, 2008). Temporality was also a limitation, with analyses based on BPA levels from blood

samples taken after one assay at one point in time; therefore, evidence of a causal association between serum BPA levels and PCOS risk based on temporality cannot be assessed from this study, and a prospective investigation would be needed to determine if increased levels of BPA preceded the onset of PCOS. Furthermore, blood was drawn from ovulatory participants during the early follicular phase (d 2-4 from the 1st day of spontaneous bleeding episode) but "at any time" for the anovulatory women with progesterone levels <5 ng/mL, meaning that unaddressed confounding exposures could be involved. Other limitations included the lack of clarity/description with regard to the defined study period, the case and control ascertainment, the source and selection of controls, the impact of lab plastics on measurement, and the partial presentation of regression results, with unspecified use of the Bonferroni correction affecting evaluation of significance. Because of these limitations as well as this study's small, unique population, **it is not of utility for HI, dose-response determination, or RA.**

Occupational exposure to bisphenol-A (BPA) and the risk of self-reported male sexual dysfunction (Li et al., 2010a).

In this case-control study conducted from 2004-2008 by investigators from the Kaiser Foundation Research Institute and the Department of Environmental Health at the University of Washington in collaboration with two Chinese academic and research institutions, study participants were recruited from among current workers at a BPA manufacturer and three epoxy resin manufacturers in China (all BPA-exposed workers were eligible to participate). Unexposed workers were recruited from among several other companies in the same city where the BPA-exposed workers were recruited; the controls had no known exposures to BPA or known reproductive toxins. Unexposed workers were matched to exposed workers on age (5 year interval), gender, educational level, and length of employment. Of 373 eligible BPA-exposed male workers, 230 (62%) agreed to participate. Of 515 eligible unexposed male controls, 284 (55%) agreed to participate. The male control group was expanded to 404 by the addition of 120 male spouses whose wives had been selected as unexposed controls for the BPA-exposed female workers. The investigators stated that the study was presented to all participating factories (exposed and unexposed) as a study of health effects of general occupational hazards and that, therefore, all participants were blinded as to the specific hypothesis regarding the potential effect of BPA. At the four BPA-exposed factories, exposure was assessed with air sampling ("spot" air samples and personal air samples) and work history information obtained through worker interviews. Workplaces in each factory were categorized into subgroups with similar BPA exposure levels. A cumulative exposure index for participants was calculated based on the time spent in a particular workplace and the exposure level in that workplace. For current workers, the exposure levels obtained from personal air sampling were used. For positions held in the past, historical air sampling data was used. In-person interviews were conducted to obtain information on demographics, work history, medical history, personal behaviors and sexual activities. The investigators used two questionnaires reported in the urology medical literature to ascertain sexual functioning among male study participants. Four areas of sexual function were assessed: sexual desire, erectile function, orgasmic function, and overall satisfaction with sex life. Study participants were asked about changes in sexual function over time

(one year or less after employment, 2-5 years, and greater than 5 years). Logistic regression was used to estimate odds ratios and calculate 95% confidence intervals to measure the association between BPA-exposure and the risk of male sexual dysfunction.

BPA-exposed workers and controls were similar with regard to age distribution and marital status (approximately 88% of workers in each group were married). Smoking and alcohol intake were similar in each group, as was history of chronic disease which might affect sexual function (urogenital diseases, autoimmune diseases, endocrine disorders, hypertension and other cardiovascular diseases, kidney diseases, and injury to genital organs). More BPA-exposed workers than non-BPA-exposed workers (59% vs. 13%) reported exposure to other chemicals or heavy metals (includes organic solvents, pesticides / herbicides, and heavy metals). After adjustment for potential confounders including age, education, marital status, current smoking status, a history of chronic diseases and exposure to other chemicals, and employment history, the BPA-exposed workers had a significantly higher risk of sexual dysfunction among all indices measuring sexual function in four domains (sexual desire, erectile function, orgasmic function, and overall satisfaction with sex life). Adjusted prevalence odds ratios ranged from 3.5 (95% CI 1.8–8.6) to 4.5 (95% CI 2.1–9.8), except for "no difficulty" vs. "some difficulty" ejaculating, for which the AOR was 7.1; all confidence intervals excluded "1". These same associations remained when analyses were restricted to workers with less than one year of work in the factory or without history of exposure to other chemicals. When BPA-exposed workers' cumulative exposure index measures were grouped into tertiles, increasing BPA exposure was associated with greater risk of sexual dysfunction in three of four domains (erectile function, orgasmic function, and overall satisfaction with sex life).

This study had several strengths, including: (1) It utilized a population with likely relatively high exposure to BPA in which to assess measures of sexual dysfunction and then compared these measures to those of populations that are likely far less exposed to BPA; (2) it employed a mechanism for assessing dose-response relationships; and (3) it attempted to blind the study participants to the study hypothesis. However, it also had several limitations including: (1) the measures of exposure to BPA that are used to classify workers' exposure status are based on air sampling data and work history and not on biological samples - the investigators do include data in the article on urine BPA levels in BPA-exposed workers and controls; the 50th percentile level from 123 pre-shift urine samples in exposed workers was 57.9 μg/g creatinine, compared to 1.2 μg/g creatinine in 254 urine samples from non-exposed workers; the investigators do not report the results of any analyses of the association of urine BPA levels and sexual function indices, if they conducted such analyses; (2) the investigators do not provide any information on whether or not interviewers were blinded as to the study hypothesis - given the subjective nature of the sexual history assessment, there is the possibility that bias could have occurred during the interviews if the interviewers were aware of the investigational hypothesis regarding BPA and sexual dysfunction and also were aware of workers' exposure status; (3) exposures were measured at the same time as outcomes, a design that does not assess temporality; (4) other aspects of work might explain self-reported sexual dysfunction among BPA-exposed workers compared to those without

occupational BPA exposure such as BPA-exposed workers might have worked more hours or performed more physical labor on average than non-BPA-exposed workers; no information was provided on the nature of the production processes and co-exposures to other chemicals for BPA-exposed workers. Despite the limitations noted, the findings of this study may have **some utility for HI; however, repeatability of the finding in another population or by another research group is needed.**

Relationship between urine bisphenol-A level and declining male sexual function (Li et al., 2010b).

This study is similar to the other studies by Li et al., conducted from 2004 to 2008, assessing the hypothesis that there is an association between increased urinary BPA and increased risk of male sexual dysfunction among workers in China with and without occupational BPA exposure. All male BPA-exposed workers in one BPA factory and three epoxy resin factories (n=373) were eligible for the study, and 230 (62%) agreed to participate. In the same areas, controls were recruited from other factories without known occupational BPA exposure. The exposed were matched to unexposed on 5-y age intervals, sex, education, and employment time. Of 515 eligible unexposed male controls, 284 (55%) agreed to participate. Excluding 58 without urine and 29 without function data, the analytical sample included 427 participants. Occupational BPA exposure was measured directly using spot air and personal air sampling with glass fiber filters for collection and spot urine from BPA-exposed and -unexposed workers. For BPA-exposed factories, spot air exposure was calculated by averaging time-weighted averages of individuals in each factory subgroup with similar BPA exposure. Urinary BPA was measured by both volume-based and creatinine-corrected concentrations using 2 spot urine samples collected pre- and post-shift from BPA-exposed workers, but using one spot urine sample collected from BPA-unexposed workers at unspecified times. In-person interviews based on instruments validated in previous reports, including Chinese population studies, indirectly measured the sexual function outcome during the past 6 mo, using discrete scale measures. In-person interviews collected data on potential confounders and risk factors. Statistical methods were appropriate for some but not all of the analyses. Because of the skewed distribution of urine BPA data and reported sexual function scales, these variables were included in the regression model after log transformation, except for the 2 sexual function outcomes with a scale of 1-4. The lower limit of BPA detection was 0.31 μg/L.

Significant dose-response associations were observed between increasing urinary BPA and declining male sexual function across the male sexual function categories (p-value for sexual desire, level of sex drive: <0.001; erectile function, ability to have an erection: 0.05, difficulty level of having an erection: <0.001; orgasmic function, difficulty level of ejaculating: 0.02, level of ejaculation strength: <0.001; overall satisfaction with sex life, level of satisfaction: 0.003) in linear regression models that adjusted for age, marital status, presence of chronic disease, education, employment history, previous exposure to other chemicals or heavy metals, smoking, alcohol consumption, and study site.

Limitations in the study included the fact that some of the sexual function variables were not appropriately analyzed; ordinal categorical variables were treated as continuous and frequently log transformed, further eliminating the ranked nature of the data and violating assumptions of these self-report categorical metrics. Also, the analysis presented beta coefficients as they refer to changes in one log unit of BPA without explaining how this relates to the overall distribution on the normal scale. Because participants were blinded to the study hypothesis, the likelihood of differential misreporting of sexual function between the two groups was considered low. The authors acknowledged and explored the possibility of participation bias, by comparing participation patterns in the BPA-exposed and -unexposed groups; nonparticipants in both groups were slightly older and had slightly longer employment times than participants, but did not show evidence of participation bias that explained the observed associations. However, the issue of interviewer bias (with regard to questionnaire data collected on the sexual function outcome, as well as potential confounders and risk factors) and its possible impact on the study's findings, were not addressed. Strengths of the study included direct exposure measurement, but adjustment for diurnal variation in urinary BPA and its possible impact on the findings were not addressed (Mahalingaiah et al., 2008); also, timing of spot urine specimens taken from non-BPA-exposed workers was not specified, and averages of pre- and post-shift urinary BPA for the BPA-exposed were used to make the measurement more stable without checking for any consistent differences in the pre- and post-shift measurements. Possible impacts on measurement from (1) environmental exposure and contamination of samples; (2) plastics used for collecting, aliquoting, processing, and storing specimens; and (3) variable human factors, including diet, route/frequency/ magnitude of exposure from all sources, hormonal status, and genetics (Calafat and Needham, 2008) were not discussed. Therefore, some uncertainty persists regarding this study's urinary BPA measurement. The authors claimed a cohort design but did not describe exposure specimens or outcome data collected or analyzed from other time-points. Given the cross-sectional nature of the data, with exposure (urinary BPA) measured during the same time period as outcome (sexual function parameters), evidence of a causal association between urinary BPA levels and declining sexual function risk based on temporality cannot be assessed from this study. BPA exposures and sexual function outcome were well-defined and appropriately measured, and although some important confounding exposures, such as stress, dietary intake, and workplace or home exposures that could contribute to accounting for the effects observed, were not addressed, other potentially important confounding factors were identified and taken into account. Despite the limitations noted, the findings of this study may have **some utility for HI; however, repeatability of the finding in another population or by another research group is needed.**

<u>Urine bisphenol-A (BPA) level in relation to semen quality (Li et al., 2011)</u>.

This is another study by the Li et al. group examining men in China with and without occupational BPA exposure and conducted from 2004 to 2008. The goal of this study was to test the hypothesis that increasing urinary BPA concentration is associated with decreasing semen quality [as determined by sperm concentration ($x10^6$ sperm/mL), count ($x10^6$), vitality (% alive), motility (% moving forward), volume (mL), and morphology

(% normal)]. All male workers at the participating factories in China with and without workplace BPA exposure were eligible. The factories and workers were blinded to the study hypothesis. Of 888 eligible men, 514 (57%) agreed to participate. After exclusions of those whose semen specimens did not meet WHO guidelines (n=278) and those who did not provide urine samples (n=18), the final analytical sample consisted of 218 men, from whom additional demographic, medical and occupational, and other data were collected by in-person interviews. Urinary BPA was measured by both volume-based and creatinine-corrected concentrations (μg/g Cr) using 2 spot urine samples collected pre- and post-shift from BPA-exposed workers, and 1 spot urine sample collected at unspecified times from BPA-unexposed workers. For BPA values below the LOD of 0.31 μg/mL, a value of LOD/√2 was used. Only results for μg/g Cr were reported. Each participant provided 2 semen specimens, with 7-to-21-day intervals between samples. WHO semen standards were followed, including 2-to-7-day abstinence before collection, and analyses within 1 hour of ejaculation. The same technician conducted all semen parameter assessments on all parameters by manual examination and by Computer Assisted Sperm Analysis (CASA) on all except morphology for which CASA has not been considered reliable. Statistical methods were appropriate.

Linear regression modeling showed statistically significant associations between increasing urinary BPA (expressed as μg/g Cr, log10-transformed) and declining semen quality, including lower sperm concentration (βadj=-15.6), lower sperm count (βadj=-42.1), lower sperm vitality (βadj=-4.6), and lower sperm motility (βadj=-3.1), but not volume (βadj=0.1) or morphology (βadj=0.05). Results from logistic regression modeling for the associations between urinary BPA and reproductive outcomes below the group median showed a similar pattern: sperm concentration (AOR: 3.4, 95% CI: 1.4-7.9); count (AOR: 4.1, 95% CI: 1.7-9.9); vitality (AOR: 3.3, 95% CI: 1.4-7.5); and motility (AOR: 2.3, 95% CI: 1.0-5.1); but not volume (AOR: 1.2, 95% CI: 0.5-2.6) or morphology (AOR: 0.7, 95% CI: 0.3-1.6). The linear modeling approach was repeated for the 88 men whose only BPA exposure was 'environmental' (below 17.9 μg/g Cr, median 1.4). Linear associations between increasing urinary BPA and declining semen quality remained statistically significant for decreasing sperm concentration (βadj=-22.3) and count (βadj=-79.0), but not for the other parameters. Modeling adjusted for age, education, chronic disease history, previous exposure to other chemicals and heavy metals (as a single yes/no variable), employment history, marital status, age at first intercourse, smoking, alcohol consumption, and study site.

One weakness of this study was in the urinary BPA measurement. There was a lack of adjustment for diurnal variation in urinary BPA (Mahalingaiah et al., 2008) and lack of discussion of its possible impact on the findings; unspecified timing of spot urine specimens from non-BPA-exposed workers (with possible impact on detectability and levels); and possible measurement error or bias from use of pre- and post-shift urinary BPA averages for the BPA-exposed without checking for consistent differences; lack of explanation for the temporal appropriateness of pre- and post-shift measures; no information on number of men below the limit of detection; and use of only μg/g Cr, log10-transformed values. Other weaknesses involved (1) uncertainty regarding the semen quality determination given the lack of explanation regarding how two semen

specimens were analyzed to generate single measurements for each parameter; (2) unaddressed possible impacts of the issue of interviewer bias (with regard to questionnaire data collected on potential confounders and risk factors) on the study's findings; (3) undiscussed possible impacts of environmental contamination, lab plastics, and variable human factors, including diet, route/frequency/magnitude of exposure from all sources, hormonal status, and genetics on measurements; and (4) limited consideration of potential confounders and risk factors without taking into account others, such as stress, dietary intake, or environmental or residential exposures that may have accounted in part for observed effects. Since both BPA exposed and unexposed workers were drawn from manufacturing plants in China, confounding or synergistic effects of exposure to other chemicals would be of particular concern and this was not discussed. Given the cross-sectional nature of the data, with exposure (urinary BPA) measured during the same time period as outcome (semen quality), evidence of a causal association between urinary BPA levels and risk of declining semen quality based on temporality cannot be assessed from this study. Strengths included direct measurement of urinary BPA and semen quality, with use of WHO semen standards; appropriate steps to minimize, acknowledge, and address possibilities of participation bias and differential misreporting of self-reported data; and appropriate statistical methods. Despite the limitations noted, the findings of this study may have **some utility for HI; however, repeatability of the finding in another population or by another research group is needed.**

Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic (Meeker et al., 2010b).

This cross-sectional study assessed the relationship between urinary BPA concentrations and (1) semen quality and (2) sperm DNA damage among men recruited from subfertile couples seeking treatment at Massachusetts General Hospital's Vincent Andrology Lab between 2000 and 2004. Of ~290 eligible non-post-vasectomy 18-to-55-year-old men, 190 (~65%) consented to participate. The authors note that most (n, % not given) who refused cited lack of time as the reason, but other differences between participants and nonparticipants were not addressed. Direct measurements of BPA from urine samples, as well as semen quality and DNA damage from semen samples, were performed appropriately. A single-spot urine sample was collected from each participant using a sterile polypropylene cup on the day of the clinic visit; second and third samples were collected from a subset of men at follow-up clinic visits between 1 week and 2 months after the first sample. Semen quality was directly measured from a single specimen collected from each participant in sterile plastic cups (type of plastic not described) onsite after a recommended 48-hour abstinence period. A portion of each specimen was measured on 7 semen quality parameters according to WHO guidelines by trained staff using a validated computer-aided semen analyzer (CASA) method. Because some parameters were found highly correlated, only three measures of sperm motion [straight line velocity (VSL), curvilinear velocity (VCL), and linearity (LIN=VSL/VCL*100)] were included in the analysis. Morphological assessment was performed on a minimum of 200 sperm cells counted from two thin-smear slides of each fresh semen specimen, using validated Kruger scoring criteria to determine the normal and subnormal. The

remaining unprocessed portion of each specimen was used in the 'comet' assay, a validated method for assessing sperm DNA damage.

The statistical analysis used four modeling approaches, and significant associations (but with p-values near 0.05) were observed in only one model between increasing urinary BPA interquartiles and (1) declining sperm concentration, (2) morphology [β=0.77 (95% CI 0.6, 1.0), p=0.047, and β= -0.9 (−1.79, −0.004), p=0.049, respectively], and (3) sperm DNA damage as Tail% measure in the comet assay [β=3.88 (0.01, 7.74), p=0.048] for men from whom urine and semen samples were collected on the same day.

Strengths of this study included direct measurements of urinary BPA exposure with and without specific gravity correction, direct measurement of semen quality and sperm DNA damage outcomes, and use of WHO semen guidelines, as well as thorough and appropriate statistical methods. Weaknesses of the study included (1) a comparison group consisting of only 76 men with semen quality parameters above WHO reference levels but undescribed sperm DNA damage levels, (2) use of plastic lab materials in semen collection, processing, and analysis without addressing impact, (3) inability to assess for differential versus nondifferential urinary BPA measurement error given that only a subset of participants had multiple urinary BPA measures, (4) regression models adjusted for limited potential confounders without explanation of how the data on those were collected or why others were not considered instead or in addition (age, body mass index, abstinence time, race, and smoking status were taken into account, but not, for example, alcohol or drug use, other chemical exposure history, cholesterol levels, blood pressure, or chronic disease history), (5) not addressing possible effect modification in the regression modeling or otherwise, (6) possible selection bias, given that the study sample consisted of male partners from couples seeking treatment for infertility, and (7) the possibility of reverse causation, given that unaddressed factors involved in infertility mechanisms on the part of some of the men could also be involved in BPA metabolism. Because of this study's limitations and its small, unique population, the findings were determined **not to be of utility to HI, dose-response determination, or RA.**

<u>Urinary bisphenol A concentrations in relation to serum thyroid and reproductive hormone levels in men from an infertility clinic (Meeker et al., 2010a).</u>

This is a cross-sectional study of the relationship between urinary BPA concentrations and serum thyroid and reproductive hormone levels in adult men. Participating men were partners in subfertile couples seeking treatment at Massachusetts General Hospital (MGH). Men between 18 and 55 years of age without post-vasectomy status were eligible to participate. A single spot urine sample was collected in a sterile polypropylene cup from all participants (n=167) on the day of their clinic visit. In a subset of men, a second and third urine sample (n=75 and n=4, respectively) was collected between one week and two months following the original sample collection. After measurement of urine specific gravity (SG), each urine sample was divided into aliquots and then frozen and shipped on dry ice to CDC for BPA analysis. A single non-fasting blood sample was drawn between 9 a.m. and 4 p.m. on the same day and at the same time that the first urine sample was obtained. Concentrations of testosterone, estradiol, SHBG, inhibin B, FSH, LH, prolactin,

free T4, total T3, and TSH were measured. For BPA concentrations or hormone levels below the LOD, an imputed value equal to one-half the LOD was used. Multivariable linear regression models were adjusted for age, BMI, smoking status, and season and time of day blood/urine samples were collected. Four models were constructed: (1) using only urinary BPA concentrations from a single urine sample collected on the same day as the serum sample (n=167); (2) using the geometric mean urinary BPA concentration for each participant, where between one and three values were used to calculate each individual's geometric mean (n=167); (3) using the geometric mean BPA concentration among only participants that contributed BPA data from at least two urine samples (n=75); and (4) using only the single urinary BPA measure collected on the same day as the serum sample among men with BPA data from at least two urine samples (n=75).

The model for (1) showed a positive association between urinary BPA and serum FSH ($p = 0.0005$) and a suggestive inverse association between BPA and inhibin B ($p = 0.053$). Urinary BPA was also positively associated with the FSH:inhibin B ratio ($p = 0.005$) and inversely associated with the estradiol:testosterone ratio ($p = 0.03$). The findings of significant associations varied across the four models, with the only outcome to remain consistent across all four modeling approaches being the inverse association between urinary BPA concentrations and the estradiol:testosterone ratio.

Limitations noted in the study included: (1) the findings of this study may not be generalizable to the population of men who are not undergoing evaluation for subfertility; (2) due to the cross-sectional nature of the study, temporality cannot be assessed – the authors point out that "reverse causation" (hormonal status affecting BPA metabolism) cannot be ruled out; (3) some of the associations seen with some modeling approaches but not others may be chance findings as a result of the multiple comparisons made; (4) the inconsistency of some the associations across the four modeling approaches may be due to the inclusion of urinary BPA data from urine samples collected weeks or months after serum was collected for hormone levels (i.e., data from urinary BPA samples collected at an appropriate point in time before serum collection would have been more relevant for these analyses); (5) the authors state that urine was collected in polypropylene containers but do not indicate if the devices used for dividing the sample into aliquots and the containers for those aliquots were also BPA-free. Based on these concerns, the findings of this study **do not have current utility for HI and RA** but may be of future utility for HI in the context of similar findings from other studies.

<u>Association of urinary bisphenol a concentration with heart disease: evidence from NHANES 2003/06 (Melzer et al., 2010).</u>

In a follow up study to the report of Lang et al. (2008), Melzer et al. estimated the associations between urinary BPA concentrations and the diagnoses of cardiovascular diseases (CVD), diabetes and serum liver enzyme levels using NHANES data (a cross-sectional analysis) from 2003/04 (n = 1455) and 2005/06 (n = 1493) of adults aged 18–74 years. A one-third random subset of subjects was generated from NHANES 2003/04 and 2005/06 responders. The selected subjects supplied urine samples and were asked questions relating to medical conditions before the physical examination in the

participant's home. Self-reported information relating to race, education, income and behaviors were abstracted from the NHANES survey. The levels of BPA (free and conjugated) were determined by the CDC using online solid-phase extraction coupled to HPLC–isotope dilution tandem MS with peak focusing. The LLOD for BPA concentrations was 0.36 ng/ml in 2003/04 and 0.4 ng/ml in 2005/06. BPA results below the LLOD (7.97% in 2003/04 and 8.44% in 2005/06 for individuals aged 18 to 74 years of age with measured BPA and urinary creatinine levels) were replaced with a value equal to the LLOD divided by the square root of two in order to distinguish between a non-detectable laboratory test result from a measured laboratory test result. Respondents aged 20 years and over were asked ''Has a doctor or other health professional ever told you that you have…'' angina, coronary heart disease, heart attack, stroke, asthma, emphysema, chronic bronchitis; arthritis, thyroid problems, any kind of liver condition, or cancers. CVD was defined as any reported diagnosis of angina, heart attack or coronary heart disease; diabetes was defined as diabetes or sugar diabetes, self-reported diagnosed and borderline diabetes; and the three original liver enzyme markers associated with BPA in 2003/04 (Lang et al., 2008b) were measured (γ-glutamyl transferase, lactate dehydrogenase,; alkaline phosphatase). Self reported race/ethnicity (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, and other race); education [less than high school, high school diploma (including GED), more than high school, and unknown education]; annual household income (less than $20,000;$20,000 to $35,000; $35,000 to $65,000; over $65,000, and unknown); smoking (never smoked, former smoker, smoking some days, smoking every day, and unknown), BMI (underweight (18.5), recommended weight (18.5 to 24.9), overweight (25.0 to 29.9), obese I (30.0 to 34.9), obese II (35.0 or above), and unknown BMI; waist circumference (in quartiles, with a missing value group); and, urinary creatinine concentration in mg/dl. Per standard deviation increases of BPA concentration Prevalence Odds Ratios (PORs) and linear regression β coefficients were calculated adjusting for the covariates.

Urinary BPA levels were lower in the 2005/06 cohort than in the 2003/04 cohort: unadjusted geometric means: 1.79 ng/ml (95% CI: 1.64 to 1.96) vs 2.49 ng/ml (CI: 2.20 to 2.83); unadjusted arithmetic means: 3.30 ng/ml (CI: 2.88 to 3.72) vs 4.59 ng/ml (CI: 3.95 to 5.24). In regression analyses of logged BPA concentration adjusting for age, gender, ethnicity and urinary creatinine, the difference in BPA levels between NHANES waves was significant. In the prevalent CVD analysis, higher BPA concentrations were associated with coronary heart disease in 2005/06 (POR per z-score increase in BPA = 1.33, 95%CI: 1.01 to 1.75) and in pooled data (POR = 1.42, CI: 1.17 to 1.72) in the fully adjusted model. When comparing 2003/04 to 2005/06 fully adjusted associations with MI (POR: 1.3 vs 1.3, respectively) and angina (POR: 1.3 versus 1.2 respectively), 2005/06 estimates were in the same direction, but did not reach statistical significance. In the prevalent diabetes analysis, associations with diabetes in 2005/06 were around the null 1.07 (0.85 to 1.34), but pooled estimates remained significant (POR = 1.24, CI: 1.10 to 1.40). The association between logged liver enzymes and per standard deviation increase of BPA concentration previously examined was not statistically significant in any of the adjusted models in 2005/06. No overall association with γ-glutamyl transferase concentrations, but pooled associations with alkaline phosphatase (β =0.03) and lactate dehydrogenase remained (β =0.01) significant in adjusted models.

The strength of this study is the use of NHANES data, which are a good representative of the general population. Limitations included the fact that the concentrations of BPA are only measured at one point in time with the assumption that this is representative of long term exposure. Orally administered BPA may be rapidly and completely excreted, however a recent NHANES article refutes this. Urine concentrations would only represent the most recent exposure to BPA, thus, using those values would mean assuming that participants are always exposed to the same amount of BPA. The authors report on a study which demonstrates that a single sample of urine can only provide moderate sensitivity for estimating the long-term tertile of BPA exposure. Secondly, there is imprecision with which prevalent disease status was assessed. Each disease was self-reported with no validation of these self-reported outcomes. This source of study error should receive as much consideration as the potential for BPA exposure misclassification, yet this important methodological consideration is unaddressed. The NHANES dataset contains other data, such as medications used by the participant, which could assist in better specification of prevalent conditions. Thirdly, this is an exploratory analysis of the association of BPA with a great number of health metrics. This increases the potential for Type I error where a statistically significant effect is observed when there is actually no real effect. Fourthly, it is questionable whether or not the data from 2003/2004 cohort and 2005/2006 cohort should be pooled. The population distribution of BPA concentrations varies substantially between the two cohorts and no explanation for this variation in the exposure was presented. Finally, some covariates included in the analysis are highly correlated such as BMI and waist circumference. This may result in analysis bias. Despite the methodological issues of this study, it provides **some useful information on HI and exposure assessment.** The relationship between increased urinary BPA concentrations and prevalent coronary heart disease persisted within the 2005/06 sample. However, the uncertainty associated with these data described above limits the utility of the data; confidence in the interpretation of the findings would be strengthened if replicated by additional studies.

<u>Are Environmental Levels of Bisphenol A Associated with Reproductive Function in Fertile Men? (Mendiola et al., 2010).</u>

This is a cross-sectional study of male participants in the Study for Future Families (SFF), a multicenter study of pregnant women and their partners conducted at prenatal clinics affiliated with university hospitals in four U.S. cities (Harbor-UCLA and Cedars-Sinai Medical Center in Los Angeles, CA; University of Minnesota Medical Center in Minneapolis, MN; University Physicians in Columbia, MO; and University of Iowa, Iowa City, IA) between 1999 and 2005. The aim of the present study was to examine the associations between urinary BPA concentrations and reproductive function (semen quality and hormone levels) in a population of fertile men. Couples were recruited at the prenatal clinic, and only those whose pregnancy was conceived without medical assistance were eligible. The men completed a questionnaire, received a physical examination, and gave blood, semen, and urine samples on the same day during the prenatal visit. The urinary concentrations of BPA in 375 men were determined at the CDC. Urine was not collected until the second year of the study. The total urinary

concentration of BPA (free plus conjugated species) was assessed. Most BPA concentrations were > LOD (89.7%); those < LOD were assigned a value of LOD divided by the square root of 2. Serum samples were analyzed for reproductive hormones, including FSH, LH, testosterone, inhibin B, estradiol, and SHBG, as well as the free androgen index (FAI). Questions included demographics, recent fever, and history of sexually transmitted diseases, as well as lifestyle factors (smoking, and alcohol and caffeine consumption) and diet. Approximately 85% of men provided a semen sample. Yet, this analysis is based on the 360 (39%) men for whom data on serum hormones and urinary BPA concentrations were available (n = 302 for complete covariates) and the 375 men with data on semen parameters and urinary BPA concentrations (n = 317 for complete covariates).

The frequency distribution of serum hormones (except E2) and urinary BPA concentrations showed skewed (non-normal) distributions and were transformed using the natural log (ln) before analysis. Almost 90% (n = 235) of the urinary samples had concentrations of BPA > LOD (0.4 μg/L). The geometric mean of BPA (μg/L) was 1.5 with an interquartile range of 0.80- 3.0. The mean (± SD) urinary creatinine concentration was 144 ± 79.8 mg/dL (median, 138 mg/dL). There was a week inverse correlation between creatinine-adjusted urinary BPA concentrations and FAI level (-0.11), FAI/LH ratio(-0.13), T/LH ratio (-0.11), and FT/LH ratio(-0.13). However, only FAI and FAI/LH ratio correlations remained statistically significant after adjustment for age, age squared, BMI, smoking status, ethnicity, study center, urinary creatinine concentration, and time of sample collection. Neither T nor SHBG were correlated with urinary BPA. No significant associations between semen parameters and BPA exposures were observed.

As with other studies of this nature the use of one urine sample as an assessment of BPA exposure is a limitation, as is the cross-sectional design. Measures derived from single semen sample measures are also questionable. One concern not addressed by the authors is the possibility of measurement error and misclassification bias as a result of assigning these values to >10% of the participants without knowing whether the undetected actually contained BPA below or above LOD. Concern for environmental contamination due the impacts of lab plastics on possible measurement error, etc., was also not addressed. The distribution of BPA and correlations described within the study may have low generalizability given that only one third of the already highly selected SFF population elected to participate in this study. In addition, the multivariate analyses adjusted for only a very limited set of potential confounders and risk factors but not others with possibly important effects, such as dietary intake, prescription drug or alcohol use, chronic disease history, and genetics. As stated by the authors, BPA levels in this fertile population of men is not associated with sperm quality but is weakly associated with markers of androgenic action. Finally, associations between BPA exposure and measures of reproductive function in fertile men were small and of uncertain clinical significance. The findings of this study **do not have current utility for HI or RA** but may be of future utility for HI in the context of similar findings from additional studies.

<u>Endocrine disruptors and childhood social impairment (Miodovnik et al., 2011).</u>

This study examined the association between BPA levels measured in a maternal spot urine sample collected between 25 and 40 weeks of gestation and the child's T-score on the Social Responsiveness Scale (SRS) completed by the mother when the child was between 7 and 9 years of age. Demographic information was collected from the mothers using questionnaires at the time of urine collection and SRS evaluation. The mother-child pairs were part of the Mount Sinai Children's Environmental Health Study. Women were recruited from East Harlem or a private practice on the Upper East Side of Manhattan and all delivered at Mount Sinai Hospital between May 1998 and July 2002 (Berkowitz et al., 2003). This article also applied the same analysis to evaluate associations between urine phthalate levels and SRS. BPA was measured by CDC using solid-phase extraction with HPLC (Ye et al., 2005). From the 404 mothers who started the study, 134 participated in the follow-up for SRS data collection and were included in this study. The women who returned were more likely to be single or divorced; more educated; and had a slightly lower BPA level (median of 1.2 μg/L compared to 1.3 μg/L).

BPA was detectable in over 90% of subjects; those below the LOD were assigned the value of LOD divided by the square root of 2. On the SRS assessment, there were 103 children in the normal range (SRS T-score < 60); 25 children in the mild social impairment range (T-score 60 - 74) and 6 children in the severe range (T-score > 75). In the unadjusted analysis, BPA level was positively correlated with high SRS T-score (Spearman rho 0.25, p=0.004), however the difference was not significant when adjusted for child race, sex, caretaker marital status and urinary creatinine [1.18 (95% CI -0.75 – 3.11) increase in the natural log transformed BPA concentration for each SRS T-scores]. The adjusted difference (1.73, 95% CI 0.02-3.45) became significant when 6 outlier values were deleted from the analysis. Phthalate concentrations for monoethyl phthalate and combined low molecular weight phthalates were significantly associated with increased SRS T-score.

A strength of this study was the use of a cohort approach to study BPA exposure with a subsequent assessment of behavior in children. However, a single, third trimester, maternal, spot urine test was used to quantify exposure of the fetus and child to BPA. Urine BPA concentrations are highly variable within individuals and over time, and the within-day variability of the spot test is greater than between-day/within-person variability; both are greater than the between-person variability (Ye et al., 2011a). Additionally, there were a large number of samples that were near or below the limit of BPA detection. Those below this limit were given a value of LOD/$\sqrt{2}$, distorting the relationship at this lower level. Misclassification is very likely to result from using a single measure to characterize the longer term exposure in the fetal environment (Braun et al., 2011). Neurogenesis is complete by week 25, so the measured BPA value may not have corresponded well to this important exposure window. Children may also be exposed in the perinatal period through breast milk, bottles and through food over a longer period, when brain development is still on-going. The single measure would be appropriate only if exposure remained constant over time or exposures in other time periods were not relevant for the outcome. Additionally, only 33% (134/404) of mothers returned for follow-up with notable differences between them and the entire base cohort.

It is unclear if study attrition is associated with poorer SRS scores and BPA exposure. This study did find a positive association of the outcomes of interest with the phthalates, another type of endocrine disrupting compound. They did not examine the correlation between phthalate and BPA levels; other studies have found phthalate exposure to be positively associated with BPA exposure in pregnant women (Braun et al., 2011). The results of the SRS analyses were based on a relatively small number of children with values above the 'normal' range. SRS T-scores have been reported to have a genetic component (Coon et al., 2010); therefore, information on the parents' T-scores should be considered in adjusted models. Additionally, socioeconomic status was not included in the model nor was psychiatric history of the mothers, both of which are potentially important confounders of the SRS T-scores and which might be associated with lifestyle differences in BPA exposure. The study does highlight the complication of interpreting single analyte studies given that that study subjects are likely to have simultaneous exposure to a range of potential endocrine disrupting compounds. The results do not provide evidence of an association between BPA exposure and early brain development, as measured by social responsiveness. The findings were not significant and there were limitations in the analysis of both exposure and outcomes variables. This study was determined **not to be of utility for HI or RA.**

Urinary bisphenol A concentrations and ovarian response among women undergoing IVF (Mok-Lin et al., 2010).

This study is described as a prospective cohort study. However, based on the timing of collection of the urinary BPA samples in relation to the outcome measures (described below), the study is cross-sectional in nature. Study subjects were recruited from among women seeking infertility evaluation at the Massachusetts General Hospital (MGH) from November 2004 through August 2008. To be eligible for the study, a woman had to be between 18 and 45 years-old and had to use her own oocytes for IVF. Questionnaires were used to obtain information on demographics, reproductive and other medical history, lifestyle factors such as smoking, and diet. FSH was measured on the third day of the menstrual cycle to assess ovarian reserve. Serum samples to measure estradiol were collected throughout the monitoring phase of the subject's IVF treatment cycle. Serum estradiol was used as a marker of ovarian stimulation and follicular development. The peak estradiol concentration was defined as the highest level of estradiol prior to oocyte retrieval. Oocyte retrieval was performed when follicle size reached 16-18 mm and the peak estradiol reached at least 500 pg/ml. Retrieved oocytes were cultured; trained embryologists at MGH identified the total number of oocytes retrieved per IVF cycle. Women provided two urine samples per IVF treatment cycle: one at the beginning of the cycle on day 3 or 4 of the gonadotropin phase and the second on the day of oocyte retrieval. Urine was collected in clean polypropylene containers. Urine SG was measured; the urine was then frozen and shipped to CDC for BPA analysis. The LOD for BPA was 0.4 μg/L; BPA concentrations less than the LOD were assigned a value equal to one-half the LOD prior to adjustment by SG. SG was used instead of creatinine to adjust for urine volume because creatinine concentrations may be confounded by muscle mass, physical activity, urine flow, time of day, diet and disease states. The geometric mean of the two SG-adjusted urinary BPA concentrations was used as the cycle-specific BPA

concentration to reduce within-cycle variability. Mixed effect models were used to evaluate the association of the log SG-adjusted cycle-specific urinary BPA concentration with peak serum estradiol concentration. Poisson regression models using a GEE approach were used to evaluate the association of the urinary BPA concentration with the total number of oocytes retrieved. Both models used autoregressive 1 correlation structure to account for correlation between repeated IVF cycles in the same woman, and adjusted for potential confounders.

Urinary BPA concentrations were measured on 84 women who underwent IVF. Sixty-one women contributed one IVF cycle, 18 women contributed two cycles, and five women contributed three cycles, for a total of 112 IVF cycles during the study period. Peak estradiol concentrations ranged from 551 to 4455 pg/ml, with a mean +/- SD of 2004 +/- 838. The total number of oocytes retrieved per IVF cycle ranged from 1 to 27 with a mean +/- SD of 10.4 +/- 5.3. Peak serum estradiol levels showed a strong positive correlation with the total number of oocytes retrieved per cycle ($r = 0.65$, $p < 0.001$). Urinary BPA concentrations were measured in 203 urine samples collected during 112 IVF cycles. Fifteen samples had BPA concentrations below the LOD. SG-adjusted BPA concentrations ranged from <LOD to 65.3 μg/L. The authors report that the distribution was similar to previous studies by Calafat et al. and Mahalingaiah et al. in 2008. There was no association between SG-adjusted cycle-specific urinary BPA concentration and BMI ($r = -0.06$, $p = 0.61$). There were statistically significant univariate associations of day 3 FSH concentrations with peak estradiol concentration and the number of oocytes retrieved, measures of ovarian response to hyperstimulation. For each unit increase in day 3 FSH (IU/L), there was an average decrease of 9% (95% CI: 5,14%; $p = 0.00001$) in the number of oocytes retrieved and an average decrease in peak estradiol of 116 pg/ml (95% CI: -187,-45; $p = 0.002$). Age was not associated with decreased ovarian response. Using a Poisson regression model with a GEE approach and controlling for BMI, age, and day 3 FSH, there was an average decrease of 12% (95% CI: 4,23%; $p = 0.007$) in the number of oocytes retrieved per cycle for each log unit increase in the cycle-specific SG-BPA. In a mixed effect regression model accounting for multiple IVF cycles, and adjusting for age, BMI, and day 3 FSH level, there was an average decrease of 213 pg/ml (95% CI: -407,-20; $p = 0.03$) in peak serum estradiol levels for each log unit increase in SG-BPA.

This study had several strengths, including: (1) the study collected objective data on BPA dose and on outcomes of interest; (2) as designed, the study protocol allowed for standardization of exposure and response measurements (i.e., FSH levels and urinary BPA measured at the same times relative to the menstrual cycle and IVF treatment, respectively, for all subjects in the study); (3) age, BMI, and day 3 FSH (factors known to be associated with decreased ovarian response during IVF) were adjusted for in the statistical analyses; (4) urinary BPA concentrations were comparable to those measured during the NHANES study. Potential limitations of the study included: (1) The findings of this study may not be generalizable to the population of women who are not undergoing evaluation and treatment for infertility; (2) the small sample sized limited the power necessary for stratum-specific analyses by specific type of infertility (the authors indicated that these study results should be considered preliminary as the study is ongoing); (3) the study methods do not indicate if the diurnal variation that is known to

occur with urinary BPA excretion was considered with regard to timing of collection of urine samples (collecting samples at different times of the day in different subjects may have biased the data); (4) the authors state that urine was collected in polypropylene containers but do not indicate if the devices used for dividing sample into aliquots and the containers for those aliquots were also BPA-free. Urine samples for BPA measurements were collected at the beginning of the IVF treatment cycle and on the day of oocyte retrieval. It may be that one of these time points is more relevant than the other with regard to ovarian response to controlled ovarian hyperstimulation. The authors did not indicate if they conducted analyses using the individual BPA measurements in addition to the analysis using the geometric mean of the two measurements from each subject that they reported in the article. Such analyses should be considered as this research continues. Based on the considerations summarized, the findings of this **study do not have current utility for HI or RA** but may be of future utility for HI in the context of similar findings from future studies.

Investigation of relationships between urinary biomarkers of phytoestrogens, phthalates, and phenols and pubertal stages in girls (Wolff et al., 2010).

This study tested hypotheses that phenols (including BPA) are associated with earlier onset puberty and that obesity modifies that association, as well as hypotheses relating to other chemical exposures. ("Earlier puberty" was not defined as happening at a specific younger versus older age; however, it was measured by breast development and pubic hair changes.) The sample consisted of 1151 eligible 6-to-8-year-old girls (enrolled between 2004 and 2007 and followed through puberty) from one of three sites in the Breast Cancer and Environment Research Centers (BCERC) multiethnic longitudinal study: (1) Mount Sinai School of Medicine (MSSM), (East Harlem, New York City), (2) Cincinnati Children's Hospital (Cincinnati and the Breast Cancer Registry of Greater Cincinnati), and (3) Kaiser Permanente Northern California (KPNC) (San Francisco Bay area KPNC Health Plan members). Eligibility criteria included age, female sex, absence of underlying medical conditions (how that was determined is not described), and black or Hispanic race/ethnicity at MSSM. Comparisons between participants and non-participants (refusals) were not addressed. The main BPA exposure was determined directly using Visit 1 urine spot samples from MSSM and KPNC but early-morning samples from Cincinnati. The CDC performed the urinary BPA analysis with appropriate HPLC-isotope dilution tandem MS quantification and quality control procedures. When results were below the LOD participants were assigned a value of the LOD/$\sqrt{2}$. Trained and tested examiners assessed breast and pubic hair stages at study Visits 1 (defined for MSSM and KPNC as the baseline visit but for Cincinnati as the 6-months-post-baseline visit when urine was also collected) and 2 (noted to have occurred ~1 year later) to determine earlier puberty, the main outcome evaluated for possible association with urinary BPA. Obesity was determined by age- (in months) and sex-specific BMI percentile calculations based on CDC growth charts, with weight and height measurements using calibrated scales and stadiometers at each visit. Participants' parents or guardians completed questionnaires providing other data. Statistical methods were appropriate.

Ln-urinary creatinine adjusted geometric mean BPA levels were highest in summer (2.4 µg/L compared to spring 1.8 µg/L, fall 2.0 µg/L) and winter (1.6 µg/L). The corrected geometric BPA means were similar across BMI groups and age groups (6.0-6.9; 7.0-7.9; >= 8.0). Asians had a lower adjusted geometric mean BPA (1.5 µg/L) compared to Whites 2.0 µg/L, Blacks 2.2 µg/L, and Hispanics 2.1 µg/L. This study did not detect a statistically significant association between urinary BPA (by creatinine-corrected quintile) and earlier puberty (as determined by breast development and pubic hair changes): [quintiles of creatinine-corrected biomarker concentrations; adjusted prevalence ratio (APR): (2)1.03, 95% CI: 0.96-1.09; (3) APR: 1.06, 95% CI: 0.99-1.13; (4) APR: 0.99, 95% CI: 0.92-1.05; (5) APR: 1.00 (0.94-1.07)], and it also did not detect a significant trend (adjusted p-trend: 0.57) for the association. Given that the association observed was not significant, further assessment regarding its modification by obesity was not meaningful.

Weaknesses in the study design centered around the measurement of urinary BPA and included: (1) uncertainty given that time of day, diurnal, and seasonal variations were not taken into account; (2) unaddressed impact of inconsistent specimen collection, with spot urine from MSSM and KPNC participants but early morning urine from Cincinnati; (3) possible misclassification given that no specific gravity correction was used and creatinine correction may not have been sufficient; (4) possible measurement error bias leading to an estimate toward the null given the use of LOD/√2 for results designated below the LOD without explanation regarding how those with BPA below LOD were distinguished from those with BPA above LOD but whose levels went undetected because of assay failure or other reasons; and (5) unaddressed measurement impacts of plastics in sample collection cups and lab materials, as well as environmental exposures of the specimen during collection and processing. Although trained and tested examiners followed a written, illustrated protocol and showed somewhat reasonable concurrence (87%), as well as inter-rater agreement by the kappa statistic (0.67), variability in pubertal stage assessment still may have contributed to outcome misclassification. Multiple comparisons also could have been an issue, where chance may have accounted for some of the findings because regression modeling involved >100 comparisons. Residual potential confounding by prenatal and perinatal exposures, as well as genetic and other factors, may have also affected the results. The strengths of the study included its truly prospective design, with urine sample collections a year before puberty measures; its large sample size; multicenter participants from three US geographic areas; and thorough statistical methods. The authors acknowledged that other markers may have better measured "earlier puberty" and that the peripubertal period may not be the only critical window of exposure for puberty. Given that the authors indicated that as the study cohort matured, they would conduct longitudinal analyses, future follow-up to see if any results of such analyses are available would be of interest. The findings of this study **do not have current utility for HI or RA** but may be of future utility for HI in the context of similar findings from additional studies.

<u>Bisphenol A exposure is associated with oxidative stress and inflammation in postmenopausal women (Yang et al., 2009b).</u>

In this cross-sectional study of a Korean urban population, researchers investigated the relationship between BPA levels with either oxidative stress or inflammation with respect to gender and menopausal status as a result of differences in the expression of the ER and/or the ER occupancy. The study sample consisted of 485 adults: 259 men, 92 premenopausal women, and 134 postmenopausal women living in large Korean cities. Oxidative stress was examined using malondialdehyde (MDA) as a biomarker for lipid peroxidation and 8-hydroxydeoxyguanosine (8-OHdG) as a biomarker for DNA oxidation. Systemic inflammation was measured using white blood cell (WBC) count C-reactive protein (CRP). BPA level was assessed for a spot urine sample using HPLC-MS (500-ul urine sample) in a single time-point measurement. The analyses used log10 cotinine levels to adjust for tobacco exposure and also adjusted for age, BMI, alcohol, and exercise. Creatinine adjustment was used to correct for urine dilution. The data were log-transformed. Linear regression modeling was used to estimate β and generate p-values to evaluate significance of observed associations. SEs for each β estimate were not provided. The ANOVA was used to test the difference among the 3 groups. No adjustment for multiple comparisons was performed. BPA measurements less than the LOD were excluded.

Urinary BPA levels were detected in 76% of subjects. Geometric means of urinary BPA were similar among men, premenopausal women, and postmenopausal women, i.e., 0.53, 0.61, 0.58 μg/g cr respectively. Geometric means of oxidative stress and inflammation biomarkers were similar among men, premenopausal women, and postmenopausal women, except for WBC count, which was higher in men than pre- or post-menopausal women. In postmenopausal women, urinary BPA was associated with MDA, 8-OHdG and CRP (adjusted β and p-value, 0.066, p=0.007; 0.103, p=0.008; 0.113, p=0.029; 0.113, p=0.029). SEs for each β were not provided. No associations were observed in men or pre-menopausal women. No association was observed between urinary BPA and WBC count in post-menopausal women.

The authors concluded that postmenopausal women may be more susceptible to BPA-induced detrimental health effects based on the significant p-value of the β estimate. The interpretation of β is the expected change in BPA for a one-unit change in the inflammatory markers when the other covariates are held constant (or adjustment for confounders). It appears that the authors based their conclusions on the p-value of the β estimates. However, the p-values were not adjusted for multiple comparisons. For CRP, the magnitude of the β between pre- and post- menopausal women is not materially different. The significant p-value of the β in post-menopausal women could be an artifact due to the larger sample size. This is supported by the observation that mean geometric BPA levels between pre- and post- menopausal women were similar. The authors also speculated that their results may be influenced by estrogen levels and receptor occupancy (data not presented). The measurement for the confounder, tobacco exposure, uses a relatively accurate measure, cotinine, as compared to derived metrics from self-reported smoking behavior. The study uses the less sensitive measurement of spot urine BPA as compared to a 24-h urine collection. BPA urine levels may not reflect free concentration of BPA in the blood, which would be a more robust biomarker of exposure. The study lacked generalizability to a specific population of interest. Noteworthy, the one-time

BPA levels measurement reported by the authors is lower than the US population estimates in the NHANES and lower than in a limited sample (N=48) of Japanese women. It is unclear if the lower BPA measurements reflect the type of BPA testing or actual lower BPA exposure. The study provides no evidence of an association between BPA exposure and certain biomarkers of effect in postmenopausal women, premenopausal women, or men. The author's conclusion that postmenopausal women may be more susceptible to health effects from BPA acting through the ER is not supported by their data. Thus, this study of BPA exposure in a convenience sample of Koreans near urban cities is **not useful for HI or RA.**

Effects of bisphenol A on breast cancer and its risk factors (Yang et al., 2009a).

This case-control study evaluated associations between serum BPA levels and breast cancer risk among Korean women (n=167). Cases were patients who had visited the Seoul National University Hospital clinic between 1994 and 1997 and were diagnosed with breast cancer for the first time. Controls were patients who had visited the same clinic during the same period and "had worried about" but had not been diagnosed with breast cancer. After matching on age, the sample consisted of 70 cases and 82 controls, with no case relatives among controls. No further definition, inclusion, or exclusion criteria were mentioned for cases or controls; refusals to participate were not addressed. Used as the exposure biomarker, conjugated BPA was directly measured by reverse-phase HPLC-FD via liquid-liquid extraction from 1 mL of each patient's 5-mL blood specimen (collected before breakfast, aliquoted into 1.5-mL Eppendorf tubes, and stored in a -80ºC freezer). The HPLC-FD method was erroneously cited(Yang et al., 2003), which referred to urinary BPA only. The possible impact of lab plastics used for blood specimen collection, aliquoting, processing, and storage on BPA measurement was not discussed. Total blood BPA analysis added sodium acetate and beta-glucuronidase to 0.5 mL of each blood sample in 15-mL glass tubes and used liquid chromatography-tandem MS to identify the HPLC/FD BPA fraction. Other data were collected by questionnaire (administration and validation unspecified). Statistical methods described were appropriate but incompletely performed and reported.

The authors reported significantly less breast feeding, greater menstrual irregularity, earlier menarche, later menopause, and more frequent alcohol drinking among cases than controls, as well as higher breast feeding frequency, later age at menarche and menopause, earlier age at first birth, and lower alcohol drinking frequency among older compared to younger participants; however, they did not stratify further analyses by age. BPA was detected in only 50.8% of specimens [LOD of .012 μg/L; LOQ of 0.04 μg/L]; for the statistical analyses, those with undetected BPA levels were assigned a value one-half the minimum detected level (0.06 μg/L), or 0.03 μg/L.

Evidence of a causal association between serum BPA levels and breast cancer risk based on temporality cannot be assessed from this study because exposure and outcome data collection occurred in the same time period. Furthermore, this study was insufficiently powered, and its weaknesses outweighed its limited strengths. The assignment procedure for undetected BPA levels could be a source of exposure measurement error and

misclassification bias, given that it is unknown if each specimen without detected BPA actually contained no BPA, or BPA was present but was not detected, and therefore not quantified. In addition, previous evidence indicates that serum BPA levels may be unreliable because of (1) possible contamination during sampling, storage, and processing, (2) variable analytical methods, and (3) variable human factors, including age, diet, route/frequency/magnitude of exposure from all sources, potential interaction of BPA products with other blood chemicals and compounds (such as lipoprotein), and genetics (Calafat and Needham, 2008). Moreover, the blood specimens this study used for direct measurement of BPA exposure were described as "well stored under -80ºC" for over 10 years subsequent to collection between 1994 and 1997. The authors do not address possible thawing that may have occurred if, for example, the power supply failed or the specimens were removed from the freezer for analyses conducted by other researchers (frequency, duration, and conditions). They also do not describe any procedures used to verify that specimen integrity was not compromised during the prolonged storage period. A higher "median value" of BPA was noted among cases (0.61 μg/L) compared to controls (0.03 μg/L), and the overall mean was given (1.69±SD 2.57 μg/L), but the group means were not reported; however, the authors indicated that the BPA levels among cases and controls did not differ significantly (p=0.42). No statistically significant association was observed between BPA level and breast cancer risk or BPA and the other breast cancer risk factors examined, with one exception: a borderline negative association (p=0.07) was noted between BPA levels and age at first birth. The authors describe conducting regression modeling, but they report no regression modeling results: no adjusted odds ratios, 95% confidence intervals, or p-values. Because of its serious limitations, this study is **without utility for either HI or RA**.

<u>Renal function, bisphenol A, and alkylphenols: results from the National Health and Nutrition Examination Survey (NHANES 2003-2006) (You et al., 2011).</u>

This cross section study using NHANES (2003-2006) data examined the hypothesis that urinary excretion of BPA and APs may be reduced among people with insufficient renal function, as measured by estimated glomerular filtration rate (eGFR). The study sample consisted of 2573 adults (20 years and older), with a 1/3 random subsample of those 6 years and older (N=5250) where the following were excluded: 185 with missing BPA and AP, 953 with missing creatinine measurements, 61 with renal diseases (self-reported weak/failing kidneys or dialysis in past 12 months), 1302 participants < 20 years old because they lacked renal disease history data, 176 pregnant or lactating females. Endpoints examined included insufficient renal function (eGFR) and serum creatinine. Serum creatinine-based GFR was estimated using the modified 4-variable Modification of Diet in Renal Disease Study (MDRD) equation (Levey et al., 2006) and the Chronic Kidney Disease Epidemiology Collaboration equation /CKD-EPI (Levey et al., 2009). The outcome of insufficient renal function was categorized in the analyses as (1) normal renal: eGFR≥90 mL/min/m2; (2) mildly decreased renal function: 90>eGFR≥60 mL/min/m2; (3) chronic kidney disease (CKD): eGFR<60 mL/min/m2. Spot urine, measured from single time-point sample by online solid-phase extraction coupled with HPLC-isotope dilution and tandem-MS was used to measure BPA. The analyses adjusted for age, sex, education level, annual household income, BMI, waist

circumference, dietary intake of energy, cigarette smoking status; alcohol drinking status, and daily activities. Creatinine adjustment was used to correct for urine dilution. Weights for sampling procedure were included in the analyses (Proc Survey, SAS 9.2). Values for BPA were log-transformed and the association between renal function and geometric means of BPA was assessed via a trend analyses in regression models and adjusted for covariates.

Of the study sample, 41.7% had normal renal function, 47.8% had mildly decreased renal function, and 10% had undiagnosed CKD. There was marginal significance for a dose response where adjusted geometric means for urinary BPA excretion decreased with decreasing levels of eGFR using MDRD equation that tends to underestimate measured GFR at higher values (P for trend, 0.04). However, the trend was not supported when using the CKD-EPI equation that is reported to be a more accurate measure of GFR at higher values than MDRD (P for trend, 0.4). There was no evidence of effect modification of BPA on renal diseases by age or gender. The authors concluded that "… possibly, urinary BPA decreased with decreasing renal function." However, while the trend test using the MDRD measurement of GFR was marginally significant, indicating a linear dose-response of BPA and renal function, this was not supported by another measure of GFR that is reported to have less measurement error at higher ranges. Although the authors concluded that "The associations appeared primarily in females (P for trend, 0.03)", the formal test of interaction for association of BPA and renal diseases by gender was not significant.

Strengths of this study included the fact that it was population-based that was nationally representative (generalizable to the American public) and had a relatively large sample size for a cross-sectional study. Limitations included the use of a single spot urine sample to measure BPA which may not reflect the actual BPA exposure; 24-hour urine samples are preferable for less measurement error of BPA exposure. In addition, BPA urine levels may not reflect the concentration of BPA circulating in the bloodstream, which has more direct access to organs. Adjustment for diurnal and seasonal variations in urinary BPA and their possible impact on the findings were not addressed (Mahalingaiah et al., 2008); also, timing of spot urine specimens was not specified, and variable human factors affecting urinary BPA measurement, including individual variation in hormonal status, genetics, and other factors (Calafat and Needham, 2008) were not discussed. The measurement of the endpoint/outcome was not consistent between two published methods. A more consistent measurement of renal diseases is recommended. The analysis adjusted for some important potential confounders and risk factors but did not consider others that could plausibly have had an effect, such as comorbidities, drug intake, and environmental exposures. The cross-sectional study design with prevalent cases of renal diseases precludes conclusive inference on the direction of causality of BPA and renal diseases. Specifically, subjects with prevalent renal diseases may experience dietary changes and, thus, exposure to dietary-related BPA. A dose-response relationship was estimated with a trend test. However, the authors did not present any associations for adjusted geometric mean concentrations of BPA and the three categories of renal function. Examples of analyses for those associations is either multinomial logistic regression or ordinal regression (ascending trend of renal

function), with weights for sampling. BPA measurements at less than the LOD values were presented as equal to the LOD divided by the square root of 2; however, assigning these values could be a source of exposure measurement error and misclassification bias, given that it is unknown if each specimen without detected BPA actually contained no BPA or BPA was present but was not detected, and therefore not quantified.. The detection limit of the assay imposes an artificial left-truncation on the distribution of the measured BPA levels that may not reflect the actual BPA levels that are related to health outcomes. While assuming a value of one-half of the LOD may generate a less biased estimate of the analytes as compared to omitting undetectable samples from analyses (Helsel, 2006) errors in estimates may still exist. Data from this study do not seem to support an association between BPA exposure and renal diseases. However, the possibility of an association cannot be excluded due to uncertainty with regard to measurement of (1) the exposure (urinary BPA) based on one spot urine sample, and (2) the endpoint/outcome (insufficient renal function). Based on the limitations, this study was concluded **not to be useful for HI or RA.**

Conclusions

FDA has updated the review of bisphenol A considering new low dose animal studies, as well as pharmacokinetic and epidemiology studies. Given that hundreds of BPA studies have been conducted, FDA used a tiered approach in its evaluation of the data. The tiered steps were as follows: 1) search criteria identified updated literature (including in vivo and low dose studies employing direct BPA exposure; epidemiology; or pharmacokinetics); 2) identified studies were evaluated to determine if they were adequate (met criteria) for use in hazard identification (using the NTP CERHR hazard identification report to qualify studies as 'adequate'); 3) criteria commonly used in guideline studies were used for determining which studies were useful for human health risk assessment purposes (see above). The charge to this group was to determine:

1) what hazards should be added or removed from FDA's continuing review/research evaluation;
2) what dose/response level for a specific effect/endpoint should be changed and to what level; and
3) how new exposure data or improved assessments should be incorporated into risk assessment.

The function of this group was strictly limited to performing a review of the most recent literature for the purposes of informing the risk assessment for BPA.

Based on the literature reviewed, this WG concludes the following in response to the charge questions:

1) With regard to hazard identification, data currently available maintain the previously identified endpoints. In addition, the updated review expands these to include:

a. Cardiovascular disease-related factors based on human epidemiology studies (Melzer et al., 2010), which continued the analysis of Lang et al. (2008) of NHANES data.
b. Perturbations in glucose homeostasis based on limited supporting evidence from a subcutaneous study in mice (Alonso-Magdalena et al., 2010). Data supporting this conclusion are based on exposure to adult females during pregnancy.

Sperm parameters based on human epidemiology data (Li et al., 2011) and some very limited, recent supporting data from rodent studies utilizing multiple routes of exposure (Minamiyama et al., 2010;Salian et al., 2009b) were also considered for their impact on hazard identification. However, findings in large, multigenerational rodent studies have not demonstrated decreased reproductive function or effects on sperm parameters at *low doses* (Tyl et al., 2002;Tyl et al., 2008;Ema et al., 2001;Tinwell et al., 2002) and other reviewed epidemiology studies also do not provide clarity on this issue (Meeker et al., 2010a;Meeker et al., 2010b;Mendiola et al., 2010). Given the observations in humans and the mixed results in animals, these findings in human occupational exposure studies based on high exposure levels suggest that further evaluation in non-occupational (low exposure) populations is needed.

2) No new information was identified to inform the issue of dose-effect level. As such the existing NOAEL identified in the previous review (5 mg/kg bw/day) is not altered.

3) No new studies were identified as useful for informing the BPA risk assessment. However, the hazard identification endpoints identified in this and previous reviews are currently being investigated by NIEHS and FDA/NCTR, and the results of those studies should directly address the risk assessment issue.

Common problematic procedural issues occurred in many of the articles in this updated review of the BPA literature. The most common issues were inappropriate study design and/or inappropriate statistical analyses, primarily with regard to control for the litter effect. The vast majority of studies did not adequately account for litter, resulting in the inappropriate use of individual pups as the statistical units. Proper use of the litter as the statistical unit would have resulted in a very low number of subjects/treatment group (sometimes as low as 2). While cross-fostering pups at birth can minimize the litter confound, it does not eliminate it. In addition, very few studies utilized an estrogenic reference as an additional type of control treatment and many studies investigated only a single dose of BPA. These issues were not considered to be as serious as those previously mentioned. Additional concerns included the possible effect of diet and vehicle on the endpoints examined. For example, oils used routinely as vehicles in the reviewed studies can potentially be additional sources of estrogenic activity (Ashby and Lefevre, 1997) or may otherwise confound interpretation of some of the endpoints measured (Sato et al., 2000). From the updated PK information it is also clear that studies utilizing only lactational exposure would effectively limit pup exposures to BPA levels

hundreds of fold lower than those given the dams, complicating interpretation of results. Thus, studies reporting a lack of BPA effect using lactational only exposure protocols should be suspect due to minimal pup exposure to unconjugated BPA.

The epidemiology studies reviewed varied widely in the completeness of the information provided to describe the conduct of the study. Thus, information which would have been very valuable for gauging the quality of the study was missing. For example, for some case-control studies the information provided on how controls were chosen was insufficient to provide assurance that the controls were appropriate for the cases. Studies also varied with regard to the level of detail provided as to whether contamination of biological samples from BPA in specimen containers, instruments, and laboratory equipment used in analyses had been avoided or even considered. Another critical shortcoming pertinent to most of the reviewed studies was the lack of data or information concerning other chemicals/compounds—in addition to BPA--to which subjects may have been exposed. This is particularly important for populations exposed to BPA in occupational settings in which a variety of chemicals are used.

As detailed in the *Methods* section, studies reviewed in this update--as in FDA's previous review--were evaluated with regard to whether the study design met specific criteria. Adherence to the specified criteria can have a strong impact on confidence in the reported results, especially as they relate to use of the information in a regulatory context. Incorporation of the study design criteria utilized for regulatory consideration will greatly improve the usefulness of scientific research into the effects of BPA for hazard identification and risk assessment purposes.

On Behalf of the BPA Joint Emerging Science Working Group:

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Appendix

Common Table Abbreviations: PND (Postnatal day), GD (Gestation day), PC (Post Coitum) ↑, increase; ↓, decrease; ↔, no change
^^Criteria Limitations Key: A = Administration; N = Sample Size or Statistical Analysis; M = Endpoint Measure (Validity); D = Dose Response; S = Sex; R = Repeatability; E = Environmental Contamination; HI = Hazard Identification; RA = Risk Assessment; [b] Effective Dose (E) and Non-effective Dose (NE), as applicable; see text for additional definitions

Table 1: Assessment of Pharmacokinetic Studies (*Detailed description provided in ***Introduction*****)**

Study Description - Author	**Study Description - Animal model/Cell model**	**PBPK Utility Statement**	**Measurement of Aglycone and Total BPA**	**Use of native or labeled BPA**	**Methodology**	**Internal Standard**	**Quality Controlled**
Balakrishnan et al. 2010	Human placental transfer	Some utility	Both	Native	LC/MS/MS	d_{16}-BPA	Yes
Doerge et al., 2010a	SD rat serum PK	High Utility	Both	D6	LC/MS/MS	$^{13}C_{12}$-BPA	Yes
Doerge et al., 2010b	Monkey serum PK	High Utility	Both	D6	LC/MS/MS	$^{13}C_{12}$-BPA	Yes
Doerge et al., 2010c	SD rat lactational transfer	High Utility	Both	D6	LC/MS/MS	$^{13}C_{12}$-BPA	Yes
Jiménez-Díaz et al., 2010	Human placental tissue biomonitoring	Some utility	Aglycone only	Native	LC/MS/MS	d_{16}-BPA	Yes
Kurebayashi et al., 2010	cryopreserved hepatocytes from rat, monkey and human	Some utility	Aglycone only	Native	LC-UV	no	No
Mazur et al., 2010	hepatic and intestinal microsomes from rat and human	Some utility	Aglycone only	Native	LC-UV	no	Minimal
Mørck et al., 2010	BEWo cell line, human placental transfer	Some utility	Total only	^{14}C	LSC (liquid scintillation counting)	no	No
Nishikawa et al. 2010	SD rat ex vivo placenta perfusion	Some utility	Both	Native	LC-TOF/MS	no	Yes

Study Description - Author	**Study Description - Animal model/Cell model**	**PBPK Utility Statement**	**Measurement of Aglycone and Total BPA**	**Use of native or labeled BPA**	**Methodology**	**Internal Standard**	**Quality Controlled**
Okabayashi and Watanabe, 2010	SD Rat, lactational transfer	Some utility	Aglycone only	Native	LC/MS	d_{16}-BPA	No
Prins et al. , 2010	SD rat limited serum PK	Some utility	Both	Native	HPLC, ESI-MS/MS	d_{16}-BPA	Yes
Tanaka et al. 2010	Pregnant and fetal mouse, tissue distribution of BPA	Some utility	Total only	^{14}C	Autoradiography	no	No
Taylor et al., 2010	Rhesus Monkeys and CD-1 Mouse serum PK	Some utility	Both	D6	LC/MS	$^{13}C_{12}$-BPA	No
Twaddle et al. 2010	SD rat serum, tissue, and excreta method validation	High utility	Both	D6	LC/MS/MS	$^{13}C_{12}$-BPA	Yes
Verner et al., 2010	Hepatocytes and hepatic microsomes from rats	Some utility	Aglycone only	Native	LC-Fluorescence	no	Minimal
Völkel at al.2011	Infant urine biomonitoring	Some utility	Both	Native	LC/MS/MS	d_{16}-BPA	Yes
Wan et al. 2010	Pregnant female and fetal cord blood biomonitoring	Some utility	Aglycone only	Native	LC/MS/MS	d_{16}-BPA	No
Zhang et al., 2010.	Human placental and fetal liver biomonitoring	Some utility	Both	Native	GC/MS	d_{16}-BPA	Yes

Table 2: Neurobiology Data Summary.

Reference	Species/Strain/ Source	Sex	Route	N	Exposure Period	BPA Doses	E & NEb	Effects	Criteria Limitations^^	Study Utility
Adewale et al,, 2009	Long Evans rats (Charles River Laboratories)	F	SC	7-14	PNDs 0-3	50 µg/kg, 50 mg/kg	E=50 µg/kg for day of vaginal opening	No change in co-localization of GnRH and FOS in the OVLT, normal lordosis quotient, advanced vaginal opening (50 µg/kg)	N, E, M	
Adewale et al, 2011	Long Evans rats (Charles River Laboratories)	F	SC	4-10	PNDs 0-3	50 µg/kg, 50 mg/kg	E = 50 µg/kg for # of OT-ir cells in PVN	↑ adult body weight (high-dose only); ↑ #, but not activity, of oxytocin (OT) producing neurons in the rostral PVN (high and low dose)	N, E	
Cardoso et al, 2010	Wistar rats (colony at Dept of Physiology, School of Medicine, Univ of Buenos Aires)	M	Oral – in drinking water (and direct to pups?)	4	GD 0 to lactationald ay 21	estimated at ~2.5 mg/kg bw/day	~2.5 mg/kg/day (dam)	GnRH and GLU concentrations ↓ significantly in BPA treated & serum concentrations of LH, FSH and testosterone also ↓	N, E, S	
Goncalves et al, 2010	Wistar rats (from University breeding colony)	M, F	Oral (dam)	12-29	GD 0-21 or lactational day 0-21 or both	40 µg/kg/day	40 µg/kg/day	Short- & long-term memory impaired in both sexes with prenatal & lactational BPA (inhibitory avoidance & object recognition) and in males with lactational only (inhibitory avoidance). Males with prenatal & lactational or lactational only had ↓ activity. Sporadic effects on water maze performance.	N, possible E	
Hajszan & Leranth 2010	Sprague Dawley rats	M	SC or oral	4 and 1	Adult – 3 days and 0-8 hours	300 mg/kg bw/day or 300 µg/kg bw/day (conflicting labeling)	300 µg/kg bw/day	↓ spine synapses in prefrontal cortex and CA1 region of hippocampus	N, M, S, R, E	
Jones et al, 2011	Long-Evans rats (Charles River)	M, F	Oral (dam)	7-12	GD 7 – lactational day 14	5, 50, 500, 5000 µg/kg/day	50 µg/kg/day	5000 µg/kg/day males show better sexual performance; 50 µg/kg/day males show worse performance. No effects in females.	N, E, M	

Reference	Species/Strain/ Source	Sex	Route	N	Exposure Period	BPA Doses	E & NEb	Effects	Criteria Limitations^^	Study Utility
Kunz et al, 2011	SD Rats (Taconic, Netherlands)	M, F	Oral dam drinking water (and pups?)	7-8 4-6 (control)	GD 6 lactational day 20	70 µg/kg/day	70 µg/kg/day	↓ NeuN-positive neuron density in hippo. ↑ GFAP density in astrocytes in cingulum, ↑ in glutamate associated with trend towards ↓ in aspartate measured by 1H MRS	N, M	
Mahoney & Padmanabhan 2010	Sheep	F	SC	3-7	GDs 30-90 of approx. 147 day gestation period	5 mg/kg/day	5 mg/kg/day	↓ in hypothalamic GnRH & mPOA ESR2; ↑ (by effect size) ESR1 in mPOA in adult female offspring	N, M, E	
Martini et al, 2010	CD-1 mice (outbred colony at Univ of Parma, originally from Charles River Italia)	M, F	Oral (dam)	5-6	GD 11 to lactational day 8	10, 20, 40 µg/kg/day	E = 20, 40; NE = 10 (and 40)	F @ 20 µg⁄kg ↑ number of MPOM nNOS cells; M @ 40 µg⁄kg ↓ MPOM nNOS cells compared to 20 µg⁄kg only; M bed nucleus of the stria terminalis, ventromedial subdivision ↓ staining @ 20 µg⁄kg compared to 10 µg⁄kg	N, M, E	
Monje et al, 2010	Wistar-derived rats (Dept of Human Physiology, Argentina)	F	Subcutaneous injection	5-30/group	4 injections, 1 every 48 hours on PNDs 1-7	50 or 20,000 µg/kg	50 µg/kg	50 µg/kg ↓ % time in proestrous or estrous; mature version of LHRH was ↓ by 20,000 µg/kg but ↑ by 50 µg/kg. Expression of unprocessed version ↓ at both doses. ERα levels ↑ in AVPV but ↓ in arcuate nuc at both doses. PR levels ↓ in AVPV by 50 µg/kg. SRC-1 levels ↓ by 50 µg/kg in AVPV.	N, E	

Reference	Species/Strain/ Source	Sex	Route	N	Exposure Period	BPA Doses	E & NEb	Effects	Criteria Limitations^^	Study Utility
Nakamura et al, 2010	ICR/Jcl mice (CLEA Japan Inc., Kyoto)	M, F	Subcutaneous injection to dam	7-8	GD 0 to lactational day 21	20 µg/kg/day	20 µg/kg/day for some endpoints	Somatosensory cortex: ↓ 5-HIAA/5-HT @ 3 weeks of age (M&F); CP: ↑ 5-HIAA at 3 & 12 weeks of age (M&F); ↑ DOPAC & ↑ DA at 12 weeks of age (M&F); LH/POA: ↑ 5-HIAA @ 3 weeks of age (M&F); Thalamus: ↑ 5-HT, ↓ 5-HIAA/5-HT at 3 weeks of age (M&F); DRN: ↑ DA at 12 weeks of age (M&F), ↑ 5-HT at 12 weeks of age (M&F), ↑ 5-HIAA at 12 weeks of age (M&F); SN: ↑ 5-HT at 3 weeks of age (M&F), ↓ 5-HIAA/5-HT at 3 weeks of age (M&F).	N, M, E	
Nakamura et al, 2011	ICR/Jcl mice (CLEA Japan, Inc.)	M, F	Subcutaneous injection	15-20	GD 0-lactational day 21	20 µg/kg/day	20 µg/kg/day	PND 21 body wt ↓ but adult wt normal. Adult activity ↓, but weaning activity normal. Elevated plus maze activity ↓ at weaning, but adult activity normal. No water maze effects.	N, E	
Patisaul et al, 2009	Long Evans rats (Charles River stock)	M, F	Subcutaneous injection	5-7/grp (from 20 cross-fostered litters)	PNDs 0-3	50 µg/kg/day, 50 mg/kg/day	50 mg/kg/day 50 µg/kg/day	↓ kisspeptin levels in arcuate nucleus but only at high dose and not in males	N, E, M	
Poimenova et al, 2010	Wistar rats (Hellenic Pasteur Institute, Athens, Greece)	M, F	Dam –oral corn flake	34-36	GD 0-lactational day 22	40 µg/kg/day	40 µg/kg/day	↓ exploratory interest (possibly ↑ anxiety) in females. Loss of sexual dimorphism in memory	N, E	
Tanida et al, 2009	ICR mice (Japan SLC, Inc)	M	Oral (method?)	6-9	GDs 8-17 and direct to pups on PNDs 3-5	5 mg/kg/day	5 mg/kg/day	↓ body weight at 2 weeks (recovery by 4 weeks); ↓ brain weight at 6 weeks. ↓ numbers of TH–ir & TH intensity in midbrain areas	E, S, M, N	

Reference	Species/Strain/ Source	Sex	Route	N	Exposure Period	BPA Doses	E & NEb	Effects	Criteria Limitations^^	Study Utility
Tian et al, 2010	Mice/ICR	M,F combined	Dam - oral	3-12	GD 7-21, possible direct pup treatment?	0, 0.1, 0.5 mg/kg bw/day	0.1 or 0.5 mg/kg bw/day	↑ central locomotion in low dose; ↓ anxiety in high dose; impaired memory at both doses; ↑ D2 in caudate at low dose; ↓ DAT in caudate at both doses; ↓ NMDA in frontal cortex and 3 hippocampal regions at both doses	N, M, E	
Xu et al, 2010a	SD rats (Experimental Animal Center, Zhejian Acad of Med Sci, Jinhua, China)	M	Dam - oral	8-9 dams; # pups/group?	GD 7 to lactational day 21	0, 0.05, 0.5, 5.0, 50.0, 200.0 mg/kg/day	0.05mg/kg/day for ↓ in NMDARs; 5.0 mg/kg/day for ↓ in ERß; 50 mg/kg/day for ↑ P450-aromatase	Dose-related ↓ in NMDARs and ERß	N (stats?), S, E	
Xu et al,. 2010b	Mice/ICR	M	Dam - oral	10	GD 7 to lactational day 21	0, 0.05, 0.5, 5, or 50 mg/kg bw/day	0.05 mg/kg bw/day for body wt; 0.5 or 5 mg/kg bw/day	↓ acquisition and retention in water maze & passive avoidance	N, S, E	
Yu et al, 2010	C57BL/6J mice; (Shaanxi, China)	M, F	Subcutaneous injection	13-15/gp	PNDs 23-30	50 µg/kg/day	50 µg/kg/day	BPA ↑ 'anxiety-like' behaviors and affiliative behaviors of females with females and ↓ behaviors of females with males.	N, E	
Zhou et al, 2011	SD rats (Oriental Bio Service Inc., Nanjing, China)	M	Subcutaneous injection	6-28/group	GD 10-lactation day 7	2 µg/kg/day	2 µg/kg/day	↓ # of GAD 67 cells in basolateral amygdala (BLA); external capsule stimulation elicited multiple population spikes in BLA; amplitude of spikes was ↑; high frequency stimulation elicited LTP ; SCH23390 (D1 agonist) ↓ spike amplitude and burst frequency; AP5 (NMDA antagonist) blocked LTP; muscimol (GABAA agonist) ↓ # of spikes and blocked LTP	M, S, E, N	

Table 3: Reproductive and Developmental Toxicity Summary

Reference	Species/Strain/Source	Sex	Route	N	Exposure Period	BPA Doses	E & NEa	Effects	Criteria Limitations^^	Study Utility
Arase et al, 2010	Mouse/C57BL/6 (Japan SLC)	M & F	Oral gavage (corn oil)	3	E13-E16	20 µg/kg bw/day	20 µg/kg bw/day	↑ estradiol, aromatase, steroidogenic enzymes, ESR1, AR expression	N, M, D, E	-
Lawson et al., 2011	Mouse/C57BL/6J/Jackson Laboratory	F	Oral gavage (corn oil)	3 per timepoint	GD 12-14.5	20 ng/g bw/day	E	Gene expression alterations in fetal ovary. ↑ Meiosis specific genes ↓ mitotic cell-cycle genes.	N, D, R	-
Kobayashi et al., 2010	Mouse/C57BL/6 (Charles River, Japan)	M & F	Oral (diet)	14-16 dams, 12 at terminal evaluation	F_0 GD 6 through F_2 at 15 weeks	0.33, 3.3, 33 ppm or 0.05, 0.5, and 5 mg/kg bw/day	NE 5 mg/kg bw/day	↔ Body weights, food consumption, organ weights, sperm paramateres, testicular histology, AGD, litter parameters	A, N (F_2),E	HI
Salian et al., 2009b	Rat/Holzman/Bred in-house	M	Oral gavage to the dam (sesame oil)	8 dams per dose group	GD 12 – PND 21	1.2 and 2.4 µg/kg bw/day	E 1.2 µg/kg bw/day	postimplantation loss, a decrease in litter size, as well as a decrease in male sperm count, and sperm motility in F1, F2 and F3 males.	A, N, R	-

Reference	Species/Strain/Source	Sex	Route	N	Exposure Period	BPA Doses	E & NEa	Effects	Criteria Limitations^^	Study Utility
Mendoza-Rodiguez et al. 2010	Wistar rats (source not indicated)	F	Oral (drinking water)	N =5 (dams), N=15~25 pups)	GD 6 to PND 21	1.2 mg/kg/d	E:1.2 mg/kg/d	Irregular estrous cycle, morphology changes in uterine epithelial cells	N, D, E	-
Minamiyama et al. 2010	Wistar rats SLC Japan	M	Oral (drinking water)	Groups of 10	8-wk old for 1 or 8 wk	about 0.3 or 3 mg/kg BPA	No dose response	NE on water consumption for BPA, but ↓for DES. NE effect on sperm conc. but ↓sperm motility for BPA. No morph. changes for sperm for BPA, but ↑ for DES. ROS product ↑ for DES and BPA to lesser extent, (ROS is a marker of sperm tox.) Effect inhib. by NAC-	Functional effects not measured. No randomization by litter; Water consumption data for this dw study not given; nominal doses only a rough estimate.	-

Reference	Species/Strain/Source	Sex	Route	N	Exposure Period	BPA Doses	E & NEa	Effects	Criteria Limitations^^	Study Utility
Cabaton et al., 2010	Mouse/CD-1 (Charles River)	F	sc osmotic minipump (50% DMSO)	18-21	E8 through lactation day 16	25, 250, 25,000 ng/kg bw/day	E 25 ng/kg bw/day	↓ cumulative pup number in forced breeding protocol	A, D	-
Signorile et al. 2010	Balb/c mice from Regina Elena Cancer Institute of Rome	F	sc (2% ETOH)	Groups of 6 dams; 20 pups	D1 gest to PND 7 Pups killed at 3mo	100 or 1000 µg/kg	No dose effect	cystic ovaries (10% in controls vs 45% in md BPA and 50% in hd BPA); ↑endometriosis in adipose tissue surrounding pelvic organs	Background rate in veh. cont. not 0; sc and cross fostering; analysis should be by litter and not pup; No DR	Does not meet criteria based on data analysis, but cystic ovaries consistent with other studies in Long-Evans, S-D rats and CD-1 mice
Adewale et al., 2009	Rat/Long Evans (Charles River)	F	sc (sesame oil)	8-9	PND 0 – PND 3	50 µg/kg bw/day and 50 mg/kg bw/day	E 50 µg/kg bw/day	↓ time of vaginal opening, abnormal folliculogenesis	N, D, R, E	-
Salian et al., 2009a	Rat/Holtzman/Bred in-house	M	sc (sesame oil)	32	PND 1- 5	Approx. 100, 200, 400, 800, 1600,	E 200 µg/kg bw/day NE 100 µg/kg bw/day	impaired fertility, sperm count and motility (dose response) and perturbed Sertoli Cell junctional proteins (single dose)	A, N, R	-
Rodriguez et al. 2010	Wistar rats (source not indicated)	F	sc (corn oil)	N ≥ 8	PND 1, 3, 5 & 7	BPA:0.05, 20 mg/kg; DES:0.2, 20 µg/kg	E:20 mg/kg	↑ recruited/ERα (+) follicles, p27 in granulosa/ oocytes	A, M, D, E	-

Reference	Species/Strain/Source	Sex	Route	N	Exposure Period	BPA Doses	E & NEa	Effects	Criteria Limitations^^	Study Utility
Fernandez et al., 2010	SD rats from IByME colony from Charles River in 1985	F	sc (castor oil)	7 per group	PNDs 1-10	(0.62-0.25 mg/kg); (6.2-2.5 mg/kg); (62.5-25 mg/kg); in castor oil	md, hd all md.hd hd md ld NE	↑testosterone and estradiol, ↓progesterone ↑interpulse interval for GnRH; ovarian wt↓ but not bw ↑ovarian cysts, ↓no. of corpora lutea, ↑no. atretic follicles; did not ovulate and no pups; ↓ pups	possible cross contam. from way littermates dosed; purity of BPA?; sc route; most S-D rats↑ background levels prolactin and estrogen than humans; hard to compare with other S-D studies;dose uncertain since no pup wts	HI, Evidence of DR despite uncertainties concerning doses delivered
Collet et al. 2010	Lacaume Lambs	F	iv and im	iv: cont 1~3; total BPA: 5~15 Im: cont 4, BPA:5	Iv: 56 h; im: 8 weeks (twice/wk)	iv: eqt 0.5, 1, 2.5, 5, 10, 20, 40, 80 mg/kg; im: 3.2 mg/kg	E:Internal: 38 ng/ml (questionable) serum or 5 mg/kg	↓ LH pulsatile secretion	A, N, M, D, E, R	-

Table 4: Carcinogenesis Studies

Reference	Species/Strain/ Source	Sex	Route	N	Exposure Period	BPA Doses	E & NEb	Effects	Criteria Limitations^^	Study Utility
Jones et al, 2010	Brca1 knockout mice with two floxed Brca1 alleles carrying mouse mammary tumor virus recombinase gene on a C57Bl/6 background	F	Alzet osmotic pump in intrascapular region	13	4 wk starting at 3 mo	250 ng BPA/kg in 50% DMSO at a flow rate of 0.22µL/h	Only 1dose	more dense mammary epithelial growth, ↑alveolar buds in wild-type mice, compared to DMSO; ↑ductal hyperplasia in the transgenic mice was 69% vs 28% in DMSO mice); only a few foci of mild ductal hyperplasia in wild type, and no DMSO effects in wild-type mice.	Not way normal animals exposed nor humans. Continuous pump exposure not clearly relevant, nor is 50% DMSO; findings are not clearly relevant to humans; presence of mouse mammary tumor gene, and BRCA deficiency in mice; contrib. of cages and bedding; only 1 dose	

Reference	Species/Strain/ Source	Sex	Route	N	Exposure Period	BPA Doses	E & NEb	Effects	Criteria Limitations^^	Study Utility
Doherty et al, 2010	pregnant CD- 1 mice (Charles River Wilmington	F	IP in sesame oil	15 dams	D 9-16 (or D9-26) of gestation); Pups evaluated pnw 6	10 µg/kg	Only 1 dose	no effect by BPA on EZH2 expression but by DES; BPA and DES↑ EZH2 protein levels; BPA and DES ↑ trimethylated histone H3, but not total levels of Histone H3.	Days of dosing unclear; IP injection may ↑ exposure of fetuses to BPA and cause effects not seen by other routes; contributions of diet, water, bedding, and sesame oil to effect levels ? Litter effects ? sham control would have helped, as would more than one dose; Only one dose was used, albeit in triplicate	

Reference	Species/Strain/ Source	Sex	Route	N	Exposure Period	BPA Doses	E & NEb	Effects	Criteria Limitations^^	Study Utility
Betancourt et al, 2010a	SD CD rat/Charles River	F	Oral (gavage)	30/group pregnant dams; For histo- 2 sets (n=8) for each group DMBA-PN50-31C, 29LD, 33HD; for PN100, 30C, 28HD	Prenatal Pups allowed to live til PND 50 and 100	25 or 250 µg/kg/ BW Control was sesame oil	250µg/kgBW prenatal and DMBA treated at PND100	Significant ↑ in mammary tumor incidence, significantly ↓tumor latency and ↑number of grade II mammary tumors for 250 ug.kg/BW PN100 DMBA-treated animals as compared to controls	DMBA-Carcinogenesis model not relevant to humans	

Reference	Species/Strain/ Source	Sex	Route	N	Exposure Period	BPA Doses	E & NEb	Effects	Criteria Limitations^^	Study Utility
Betancourt et al, 2010b	SD CD rat/Charles River	F	Oral (gavage)	31 control litters, 29 LD litters, 33 HD litters For each age (PND 21 or 50), 8-10 mammary gland samples were used from each treatment group	Prenatal 10-21 days postception Pups allowed to live to PND 21 or 50	25 or 250 µg/kg/ BW Control was sesame oil	25 and 250µg/kg BPA At PND21 and PND 50	M--21 proteins identified to be different in rat mammary gland of HD and LD BPA animals compared to controls Proteins involved in cell signaling pathway may be regulated by BPA	Preliminary data not confirmed by other researchers; limited # of samples: no clear differentiated between LD and HD BPA effects	
Prins et al. 2010	Rat/SD/Harlan	M	SC and oral (vehicle control oral only)	15-25	PND 1,3, and 5	10 µg/kg bw/day	E 10 µg/kg bw/day	Increased PIN lesions in ventral (VP) and lateral (LP) prostate lobes in animals treated with T+E implants as adults; increased hyperplasia in VP, increased inflammation in LP	M, D	Endpoint previously identified; effect dependent on T+E implants; doesn't alter previous (see text discussion)

Table 5: Other Endpoint Studies

Reference	Species/Strain/Source	Sex	Route	N	Exposure Period	BPA Doses	E & NE[b]	Effects	Criteria Limitations^^	Study Utility
Alonso-Magdalena et al. 2010	Mice/OF1/Charles River (Spain)	F	Sc	6-12	GD9-16	10 or 100 µg/kg/day	E	Altered glucose homeostasis during pregnancy (10 µg/kg dose group) and 4 months after delivery (100 µg/kg group) Altered plasma hormone and metabolite levels (insulin, leptin, triglycerides, and glycerol) during pregnancy and 4 months after delivery in a dose-dependent fashion.	E, S, A Caging materials not specified) n=6-12/group Effect of corn oil control was not evaluated and no positive control was utilized.	HI (effects on dams only)
Braniste et al. 2010	Rats/Wistar	F	Oral, gavage	5-13	15 days	0.05 and 5 mg.kg/day	E	Changes in colonic permeability and visceral pain perception	Didn't account for litter effects	
Bromer et al 2010	Mice/CD/Charles River	F	i.p	7	Day 9-16 of pregnancy	5 mg/kg bw of pregnant dam	E	Decreased methylation of *Hoxa10* gene in BPA treated mice as compared to controls: altered methylation affected ER binding to the *Hoxa10* ERE and increased estrogen responsiveness of the gene	Mechanistic endpoints	

Reference	Species/Strain/Source	Sex	Route	N	Exposure Period	BPA Doses	E & NE[b]	Effects	Criteria Limitations^^	Study Utility
Busquiazzo et al. 2010	Rat/Wistar	F	Sc	5-8	PND 1,3,5,7	0.05 or 20 mg/kg	E	↓ VEGF expression and endothelial cell production	Mechanistic endpoints	
Holladay et al. 2010	Mice/C57B6/129svj		Sc	5	GD9.5-PND21	1 mg/kg/day	E	Changes in cytokines	S No statistical evaluation of data	
Midoro-Horiuti et al. 2010	Mice/Balb/c/Harlan	F	oral, drinking water	7-16	1 week prior to impregnation and throughout pregnancy and lactation.	NR	E	↑ increased airway responsiveness to methacholine eosinophils in BAL fluid	Didn't account for litter effects	
Ryan et al. 2010	Mice/CD1/	F	Oral, diet	15/group	GD1-PND21	0.25 µg/kg/day (maternal dose)	E	↓ BW in female PND21 pups from BPA-treated dams. No effect on glucose homeostasis	D, E Didn't account for litter effects	
Somm et al. 2009	Rats/CD	F	Oral, drinking water	10/group	GD6-PND21	70 µg/kg/day	E	↑ BW of M and F pups from BPA-exposed dams on PND1; ↑ BW of F pups on PND21, changes in fat depots.	Didn't account for litter effects	

Table 6: Epidemiology Data Summary

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
Cantonwine, et al, 2010	Small nested case-control study in a Mexican birth cohort ; Study hypothesis: BPA exposure may impact placental tissue development and thyroid function in humans.	Pilot study (30 cases and 30 controls) nested within a Mexican birth cohort study (n=670), the Early Life Exposure in Mexico to Environmental Toxicants study in Mexico City, Mexico. Women were recruited during prenatal visits at one of four clinics of the Mexican Institute of Social Security in Mexico City, 2001-2003. Cases: random sample of women who had delivered before 38 weeks of gestation at time of delivery, which included 12 women who delivered prior to 37 weeks. Controls: sample of the total number of women who had completed 38 or more weeks of gestation at the time of delivery. Gestational length was estimated by date of maternally-recalled last menstrual period.	A spot (second morning void) urine - third trimester visit (earliest urine collection occurred during the 30th week of gestation). The dates of urine collection were not noted, although these dates were used in the analysis in a similar study which found phthalates to be related to prematurity in the same 60 women. All urinary BPA concentrations (creatinine or specific gravity corrected and uncorrected) were log transformed in order to stabilize variance. The LOD for BPA in a 0.1-mL urine sample was 0.4 µg/L. Concentrations below the LOD [N = 12 (20.0%)], an imputed value equal to one-half the LOD (0.2 µg/L) was used. BPA concentrations were corrected for urine dilution by SG	Prematurity: women who had delivered before 38 weeks of gestation at time of delivery. Gestational length was estimated by date of maternally-recalled last menstrual period	Logistic regression models, adjusting for maternal age; maternal education; parity; infant's sex	Geometric mean BPA concentrations of 1.84 ± 1.86 µg/L (1.71 ± 1.57 µg/L SG adjusted) compared to controls 0.97 ± 0.92 µg/L [1.20 ± 1.02 µg/L SG adjusted (t-tests; p-value = 0.01 and 0.11, respectively). No significant differences were reported by maternal age, maternal education, pre-pregnancy body mass index, parity, marital status, or infant sex when comparing term and preterm infants. In a logistic regression model adjusting for maternal age; maternal education; parity; infant's sex, the adjusted odds ratio in relation to specific gravity adjusted third trimester BPA was 1.91 (95%CI 0.93, 3.91, p-value = 0.08). The authors state that inclusion of third trimester urinary phthalate metabolites didn't appreciably change the odds ratios in the regression (results not presented in article).	

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
Clayton, et al, 2011	Cross-sectional study in NHANES. The study hypothesis was Urinary levels of BPA and triclosan were associated with reported allergies or measured serum antibody titers to a common pathogen of the herpes virus family, CMV	3728 US adults and children age ≥ 6 years with both BPA and triclosan measurements. Flowchart of study population: 1/3 random subsample of those 6 years and older (N=5250) and 5065 had both BPA and triclosan measurement and the following were further excluded:541 with missing data on 1 or more covariates,796 with missing continuous CMV data or allergy diagnosis	BPA exposure: Spot urine. Measurement: online solid-phase extraction coupled with HPLC-isotope dilution and tandem-MS in 100uL urine. Duration: Single timepoint measurement	i) Serum CMV antibody levels (considered as a marker of altered cell-mediated immune function). CMV-specific IgG was measured in the 2003-04 wave for respondents aged 6-49 using ELISA. Sera samples near the ELISA cutoff were confirmed with a second ELISA assay. When these 2 tests disagreed, an IFA was performed and the result of the IFA was given as the final result. CMV-specific IgG optical density was reported (measured in Arbitrary Units / ml) and used as a continuous outcome variable. CMV seropositivity was defined by NHANES based on this optical density measure. ii) self-reported diagnosis of allergies or hayfever by NHANES questionnaire data : "Has a doctor or other health professional ever told you that you have allergies/hayfever?"	Confounder adjusted in the analyses: age, sex, race, BMI, creatinine levels, family income, and educational attainment. Multivariate ordinary least squares linear regressions were used to model continuous CMV. Logistic regressions were used to model allergies/hay fever. Interaction by age was explored with stratified analyses of <18 years and ≥18 years age group due to the potential difference on length of BPA exposure. All analyses included sampling weights and adjusted for NHANES complex survey design. BPA < LOD were excluded.	46.7% were seropositive for CMV. i. ≥18 years Increasing urinary BPA levels were associated with increasing cytomegalovirus antibody titers in ≥18 years ($p<0.001$). For each unit increase in CMV antibody levels, there is a 0.158 (SE=0.035) increase in log-transformed BPA (ng/mL) ($p<0.001$, Clayton 2010, Table 4). ii. <18 years Conversely, in the <18 age group, lower levels of BPA were associated with higher cytomegalovirus antibody titers ($p<0.05$) For each unit increase in CMV antibody levels, there is a 0.113 (SE=0.047) decrease in log-transformed BPA (ng/mL) ($p<0.05$). Allergies or hayfever There was no association of BPA with allergy/hayfever diagnosis (adjusted OR(SE) for ln-transformed BPA/ng/mL <18 years: 0.93(0.12) and for ≥18 years: 0.90 (0.09)	

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
Cobellis, et al. 2009	Reported as case-control but lacked several elements of case-control design (no case or control definitions; no study period given; exclusion and inclusion criteria not fully detailed; refusals/nonparticipants not addressed)	Fertile women (n=69) referred to Second University of Naples' Department of Gynecology, Obstetrics and Reproductive Medicine for ovarian cysts, pelvic pain, or dysmenorrhea after laparoscopy, divided into endometriotic cases (n=58) and non-endometriotic age-matched controls (n=11)	Serum BPA and serum BPB as detected by RP-HPLC with fluorescence detection compared to MS analyses	Endometriosis	Limited to calculation of mean exposure levels	BPA and BPB detected in sera from 30 (51.7%) and 16 (27.6%) cases, respectively Only 15 of the BPA (range: 0.79-7.12 ng/mL; mean: 2.91±1.74 ng/mL) and 10 of the BPB serum samples quantitated because of LOQ	
Fujimoto, et al. 2010	Cross-sectional	58 infertile female patients and 37 male partners undergoing a first IVF cycle at the University of California at San Francisco Center for Reproductive Health between 9/1/2007, and 8/31/2008. Female patients underwent gonadotropin-induced ovarian stimulation; they were also administered human chorionic gonadotropin injections subcutaneously 36 hours before oocyte retrieval	Serum BPA (HPLC)	Oocyte maturation and fertilization outcomes (visually counted, followed by calculations based on number of zygotes formed and number of oocytes with visible polar body); fertilization methods used: (1) conventional insemination or (2) ICSI	Log binomial regression models to estimate adjusted associations between log-transformed serum BPA concentrations and oocyte maturity including only participants with covariate data (age, race/ethnicity, cigarette smoking)	(1) in ICSI-only cases, decrease in probability for mature oocyte associated with doubling of female serum BPA, but only among 9 Asian women (aRR 0.91, 95% CI 0.83–1.00); (2) 55% decrease in probability for fertilization among 26 cases of ICSI or conventional insemination associated with doubling in female serum BPA (aRR 0.45, 95% CI 0.21–0.66), reduced by 2% for each year increase in female age (aRR 1.02, 95% *CI* 1.01–1.03) and further reduced by 6% for each doubling of male BPA (aRR 1.06, 95% CI 1.02–1.10); (3) doubling in male serum BPA associated with 12% reduction in probability for fertilization, but only among 5 Asian men.	Does not have current utility for HI and RA but may be of future utility for HI in the context of findings from future studies.
Galloway, et al. 2010	Cross-sectional from cohort	720 individuals from two sites for the Italian InCHIANTI cohort study (total n for that study=1268 selected through multistage sampling in Tuscany, Italy; they provided blood samples and had physical examinations)	Urinary BPA (NHANES 24-hour urine sample measured by solid phase extraction, HPLC)	Serum sex hormone concentrations [total T; SHBG, E2]	Multivariable linear regression models adjusted first for age and site, and then for age, site, smoking, BMI, weight, waist, and urinary creatinine concentration)	Higher daily BPA excretion associated with higher total T concentrations in men, in models adjusted for age and study site ($p = 0.044$), and in models additionally adjusted for smoking, obesity, and urinary creatinine concentrations ($\beta = 0.046$; 95% CI, 0.015–0.076; $p = 0.004$) No significant associations with other serum measures. No associations with primary outcomes among women overall; however, significant association observed between BPA and SHBG concentrations among premenopausal women (n=60)	Not considered useful for HI or RA but may be of future utility for HI in the context of similar findings from other studies

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
Kandaraki ,et al., 2011	Reported as cross-sectional but described as case-control	71 PCOS cases referred to Athens, Greece university hospital endocrine clinic for menstrual irregularities. Those who met NIH PCOS criteria were included; those with androgen excess disorders other than PCOS excluded. Enrolled cases described as “in good health” without “chronic or acute diseases.” Age- and BMI-matched controls consisted of 100 “healthy” women	serum BPA (directly measured in duplicate by ELISA kit)	PCOS (directly measured components: insulin, total T, SHBG, androstenedione, LH, and FSH; others indirectly measured)	multiple linear regression	BPA levels (ng/mL) significantly higher in PCOS cases compared to BMI-matched controls [PCOS-Lean (BMI <25 kg/m2) 1.13±0.63 vs. Control-Lean: 0.70±0.36, p<0.001; PCOS-Overweight (BMI ≥25 kg/m2): 0.96± 0.46 vs. Control-Overweight: 0.72±0.39, p<0.05)] BPA levels significantly associated with (1)T (r=0.192, p<0.05) and (2) androstenedione (r=0.257, p<0.05) Multiple linear regression showed significant correlation of BPA with PCOS (r=0.497, p<0.05) BPA also significantly correlated with insulin resistance (indirectly measured by the Matsuda index) among PCOS cases (r=0.273, p<0.05)	

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
Li, et al., 2010b	Case-control	Cases (n=230) BPA-exposed occupationally in China; controls (n=284) BPA-non-exposed occupationally same areas of China; matched by 5-y age intervals, education, employment time; excluding those without urine or function data, sample n=427 (years of study: 2004-2008)	BPA exposure measured directly from urine samples (creatinine-corrected concentrations / HPLC) (2 spot urine samples collected pre- and post-shift from BPA-exposed workers, and 1 spot urine sample collected at unspecified times from BPA-unexposed workers) & workplace exposure measured by averaging time-weighted averages in factory subgroups with similar BPA exposure In-person interviews with previously validated instruments in Chinese population studies, indirectly measured sexual function outcome during past 6 mo, using discrete scale measures, and also collected data on potential confounders and risk factors	Male sexual dysfunction	Linear regression modeling	Significant dose-response associations between increasing urinary BPA and declining male sexual function (p-values: sexual desire, level of sex drive: <0.001; erectile function, ability to have an erection: 0.05, difficulty level of having an erection: <0.001; orgasmic function, difficulty level of ejaculating: 0.02, level of ejaculation strength: <0.001; overall satisfaction with sex life, level of satisfaction: 0.003) in linear regression models adjusting for age, marital status, presence of chronic disease, education, employment history, previous exposure to other chemicals or heavy metals, smoking, alcohol consumption, and study site	Some utility for HI; however, repeatability of the finding in another population or by another research group is needed.

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
Li, et al. 2011	Case-control	Conducted from 2004 to 2008 among men in China with and without occupational BPA exposure; after exclusions of those whose semen specimens did not meet WHO guidelines (n=278) and those who did not provide urine samples (n=18), the final analytical sample consisted of 218 men,	BPA exposure measured directly from urine samples (creatinine-corrected concentrations / HPLC) (2 spot urine samples collected pre- and post-shift from BPA-exposed workers, and 1 spot urine sample collected at unspecified times from BPA-unexposed workers)	Decreasing semen quality [as determined by sperm concentration, count, vitality, motility, volume, and morphology; (measured by by manual examination and by CASA) on all except morphology for which CASA has not been considered reliable) additional data collected by in-person interviews	Linear regression modeling	Statistically significant associations between increasing urinary BPA (expressed as μg/g Cr, log10-transformed) and declining semen quality, including lower sperm concentration (βadj=-15.6), lower sperm count (βadj=-42.1), lower sperm vitality (βadj=-4.6), and lower sperm motility (βadj=-3.1), but not volume (βadj=0.1) or morphology (βadj=0.05) Statistically significant associations between urinary BPA and reproductive outcomes below the group median showed similar pattern: sperm concentration (AOR: 3.4, 95% CI: 1.4-7.9); count (AOR: 4.1, 95% CI: 1.7-9.9); vitality (AOR: 3.3, 95% CI: 1.4-7.5); and motility (AOR: 2.3, 95% CI: 1.0-5.1); but not volume (AOR: 1.2, 95% CI: 0.5-2.6) or morphology (AOR: 0.7, 95% CI: 0.3-1.6). The linear modeling approach was repeated for the 88 men whose only BPA exposure was 'environmental" (below 17.9 μg/g Cr, median 1.4). Linear associations between increasing urinary BPA and declining semen quality remained statistically significant for decreasing sperm concentration (βadj=-22.3) and count (βadj=-79.0), but not for the other parameters. Modeling adjusted for age, education, chronic disease history, previous exposure to other chemicals and heavy metals (as a single yes/no variable), employment history, marital status, age at first intercourse, smoking, alcohol consumption, and study site.	Some utility for HI; however, repeatability of the finding in another population or by another research group is needed.

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
Li, et al., 2010a	Case-control	Study participants: current workers at a BPA manufacturer and three epoxy resin manufacturers in China (all BPA-exposed workers were eligible to participate, 2004-2008). Controls: Unexposed workers recruited from among several other companies in the same city where the BPA-exposed workers were recruited. The controls had no known exposures to BPA or known reproductive toxins and matched to exposed workers on age (5 year interval), gender, educational level, and length of employment. The male control group was expanded to 404 by the addition of 120 male spouses whose wives had been selected as unexposed controls for the BPA-exposed female workers. Participants were blinded as to the specific hypothesis regarding the potential effect of BPA. Participation rate for exposed male worker/cases; 62% (230/373) and 55% for controls (284/ 515).	Exposure: air sampling ("spot" air samples and personal air samples) and work history information obtained through worker interviews. Current workers: personal air sampling. For positions held in the past: historical air sampling data. Workplaces in each factory were categorized into subgroups with similar BPA exposure levels. A cumulative exposure index for participants was calculated based on the time spent in a particular workplace and the exposure level in that workplace.	Demographics, work history, medical history, personal behaviors and sexual activities information: in-person interview with 2 reported questionnaires for male sexual function Measures of sexual function:sexual desire, erectile function, orgasmic function, and overall satisfaction with sex life and changes in sexual function over time (one year or less after employment, 2-5 years, and greater than 5 years).	Logistic regression	BPA-exposed workers and controls were similar with regard to age distribution and marital status (approximately 88% of workers in each group were married). Smoking and alcohol intake were similar in each group, as was history of chronic disease which might affect sexual function (urogenital diseases, autoimmune diseases, endocrine disorders, hypertension and other cardiovascular diseases, kidney diseases, and injury to genital organs). More BPA-exposed workers than non-BPA-exposed workers (59% vs. 13%) reported exposure to other chemicals or heavy metals (includes organic solvents, pesticides / herbicides, and heavy metals). After adjustment for potential confounders including age, education, marital status, current smoking status, a history of chronic diseases and exposure to other chemicals, and employment history, the BPA-exposed workers had a significantly higher risk of sexual dysfunction among all indices measuring sexual function in four domains (sexual desire, erectile function, orgasmic function, and overall satisfaction with sex life). aPR odds ratios ranged from 3.5(95% CI 1.8–8.6) to 4.5 (95% CI 2.1–9.8), except for "no difficulty" vs. "some difficulty" ejaculating, for which the aOR was 7.1; all confidence intervals excluded "1". These same associations remained when analyses were restricted to workers with less than one year of work in the factory or without history of exposure to other chemicals. When BPA cumulative exposure index measures were grouped into tertiles, increasing BPA exposure was associated with greater risk of sexual dysfunction in three of four domains (erectile function, orgasmic function, and overall satisfaction with sex life).	Some utility for HI; however, repeatability of the finding in another population or by another research group is needed.

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
Meeker, et al. 2010 b	Cross-sectional	Men recruited from subfertile couples seeking treatment at Massachusetts General Hospital's Vincent Andrology Lab between 2000 and 2004: of ~290 eligible non-post-vasectomy 18-to-55-year-old men, 190 (~65%) consented to participate	BPA directly measured from urine samples	(1) semen quality (2) sperm DNA damage directly measured from semen samples	Logistic and linear regression modeling	Associations observed between increasing urinary BPA interquartiles and declining sperm concentration and morphology [β=0.77 (95% CI 0.6, 1.0), P=0.047, and β= -0.9 (−1.79, −0.004), P=0.049, respectively], and the Tail% measure in the comet assay [β=3.88 (0.01, 7.74), P=0.048] for men from whom urine and semen samples were collected on the same day noted by another reviewer: statistically significant associations observed between (a) increasing urinary BPA interquartiles and declining sperm concentration, motility, and morphology [23% (95% CI−40%, −0.3%), 7.5% (−17%, +1.5%), and 13% (−26%, −0.1%), respectively], and (b) increasing sperm DNA damage (10% (0.03%, 19%),	

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
Meeker, et al. 2010a	Cross-sectional study. Study objective: to assess the relationship between urinary BPA concentrations and serum thyroid and reproductive hormone levels in adult men.	N=167 subjects recruited from an ongoing study on the relationship between environmental agents and reproductive health. Participating men were partners in subfertile couples seeking treatment at Massachusetts General Hospital (MGH). Eligibility: Men between 18 and 55 years of age without post-vasectomy status. Participation rate:~ 65%, with most non-participants citing lack of time on the date of their clinic visit as the reason for not participating. Exclusion: 9 men who reported use of medications that alter hormone levels (e.g., propecia, finasteride, cabergoline, clomid, GnRH, T, or prednisone taper).	A single spot urine sample collected in a sterile polypropylene cup from all participants (n=167) on the day of their clinic visit. In a subset of men, a second and third urine sample (n=75 and n=4, respectively) was collected between one week and two months following the original sample collection at a follow-up clinic visit. After measurement of urine SG, each urine sample was divided into aliquots and then frozen and shipped on dry ice to CDC for BPA analysis.	A single non-fasting blood sample was drawn between 9 a.m. and 4 p.m. on the same day and at the same time that the first urine sample was obtained. Analyses were conducted at the MGH Reproductive Endocrinology Laboratory for the following hormones: T, E2, SHBG, inhibin B, FSH, LH, prolactin, free T4, total T3, and TSH. The FAI was calculated as the molar ratio of total T to SHBG. The ratios of FSH to inhibin B and E2l to T were calculated as measures of sertoli cell function and aromatase activity, respectively.	Multivariable linear regression. BPA concentrations or hormone levels < LOD == an imputed value equal to one-half the LOD was used. SG was included as a continuous variable in all models to adjust for urinary dilution. Analyses were adjusted for age, BMI, smoking status, and season and time of day blood/urine samples were collected. Four models were constructed: "Approach 1" - using only urinary BPA concentrations from a single urine sample collected on the same day as the serum sample (n=167) "Approach 2" - using the geometric mean urinary BPA concentration for each participant, where between one and three values were used to calculate each individual's geometric mean (n=167) "Approach 3" - using the geometric mean BPA concentration among only participants that contributed BPA data from at least two urine samples (n=75) "Approach 4" -using only the single urinary BPA measure collected on the same day as the serum sample among men with BPA data from at least two urine samples (n=75).	BPA was detected in 89% of urine samples. The geometric mean and median were both 1.3 ng/mL; the highest BPA concentration was 36.4 ng/mL. Among men from whom two urine samples were collected, BPA concentrations in the two samples were weakly correlated (r = 0.17; p = 0.14). Urine samples collected in winter had a suggestively lower median BPA concentration than samples collected in spring, summer, or fall (1.3 vs. 1.8 ng/mL; p = 0.06). The multivariable linear regression model for "Approach 1" showed a positive association between urinary BPA and serum FSH (p = 0.0005) and a suggestive inverse association between BPA and inhibin B (p = 0.053), representing a 23% increase in FSH and a 11% decrease in inhibin B for an inter quartile range increase in BPA concentration compared to the median levels of these hormones. Urinary BPA was also positively associated with the FSH:inhibin B ratio (p = 0.005) and inversely associated with the E2:T ratio (p = 0.03). There was a positive dose-response trend between urinary BPA concentration quartiles and serum FSH levels (p for trend = 0.002), and an inverse trend between urinary BPA concentration quartiles and inhibin B (p for trend = 0.04); there was a positive trend between urinary BPA concentration quartiles and the FSH:inhibin B ratio (p for trend = 0.01) and a suggestive inverse trend between urinary BPA concentration quartiles and the estradiol:testosterone ratio (p for trend = 0.06). Of the above findings, the only outcome to remain consistent across all four modeling approaches was the inverse association between urinary BPA concentrations and the estradiol:testosterone ratio. The positive associations between urinary BPA and serum FSH and between urinary BPA and the FSH:inhibin B ratio seen with approach 1 were also seen with approaches 2 and 4. The suggestive inverse association between BPA and inhibin B seen in approach 1 was significant in Approach 4 (p = 0.003). A positive association between urinary BPA and SHBG was seen with approach 3 and approach 4 only. An inverse association between urinary BPA and TSH was seen with approach 2; a suggestive inverse association between these two measurements was seen with approach 3. An inverse association between urinary BPA and FAI, and a suggestive inverse association between urinary BPA and estradiol, were seen with approach 3 only.	No current utility for HI and RA but may be of future utility for HI in the context of similar findings from other studies.

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
Melzer, et al. 2010	Cross-sectional. Study objective: To estimate the associations between urinary BPA concentrations and the diagnoses of cardiovascular diseases, diabetes and serum liver enzyme levels.	Cross-sectional analysis of NHANES: subjects were n = 1455 (2003/04) and n = 1493 (2005/06) adults aged 18–74 years. A one-third random subset of subjects was generated from NHANES 2003/04 and 2005/06 responders. The selected subjects supplied urine samples, and were asked questions relating to medical conditions before the physical examination in the participants' home. Self-reported information relating to race, education, income and behaviors were abstracted from NHANES survey.	The level of BPA (free and conjugated) were analyzed by the online solid-phase extraction coupled to HPLC–isotope dilution tandem MS with peak focusing. The LLOD for BPA concentrations was 0.36 ng/ml in 2003/04 and 0.4 ng/ml in 2005/06. BPA results below the LLOD (7.97% in 2003/04 and 8.44% in 2005/06) aged 18 - 74 years with measured BPA and urinary creatinine levels) were replaced with a value equal to the LLOD divided by the square root of two in order to distinguish between a non-detectable laboratory test result from a measured laboratory test result.	Respondents aged 20 years and over were asked ''Has a doctor or other health professional ever told you that you have…'' for angina, coronary heart disease, heart attack, stroke, asthma, emphysema, chronic bronchitis; arthritis, thyroid problems, any kind of liver condition, or cancers. • CVD: any reported diagnosis of angina, heart attack or coronary heart disease. • Diabetes: diabetes or sugar diabetes, self-reported diagnosed and borderline diabetes. • Three original liver enzyme markers associated with BPA in 2003/04: GGT,; lactate dehydrogenase, alkaline phosphatase,.	Per standard deviation increases of BPA concentration PORs and linear regression β coefficients were calculated adjusting for the covariates. Covariates:Self reported race/ethnicity (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, and other race); education (less than high school, high school diploma (including GED), more than high school, and unknown education);annual household income (less than $20,000;$20,000 to $35,000; $35,000 to $65,000; over $65,000, and unknown); smoking)never smoked, former smoker, smoking some days, smoking every day, and unknown), BMI (underweight (18.5), recommended weight (18.5 to 24.9), overweight (25.0 to 29.9), obese I (30.0 to 34.9), obese II (35.0 or above), and unknown BMI; waist circumference (in quartiles, with a missing value group); and, urinary creatinine concentration in mg/dl.	Urinary BPA levels were lower in the 2005/06 cohort than in the 2003/04 cohort: unadjusted geometric means: 1.79 ng/ml (95% CI: 1.64 to 1.96) vs 2.49 ng/ml (CI: 2.20 to 2.83); unadjusted arithmetic means: 3.30 ng/ml (CI: 2.88 to 3.72) vs 4.59 ng/ml (CI: 3.95 to 5.24). In regression analyses of logged BPA concentration adjusting for age, gender, ethnicity and urinary creatinine, the difference in BPA levels between NHANES waves was significant. • Cardiovascular Disease. Higher BPA concentrations were associated with coronary heart disease in 2005/06 (POR per z-score increase in BPA = 1.33, 95%CI: 1.01 to 1.75) and in pooled data (POR = 1.42, CI: 1.17 to 1.72) in the fully adjusted model. When comparing 2003/04 to 2005/06 fully adjusted associations with MI (POR: 1.3 vs 1.3, respectively) and angina (POR: 1.3 versus 1.2 respectively), 2005/06 estimates were in the same direction, but did not reach statistical significance. Diabetes. Associations with diabetes in 2005/06 were around the null 1.07 (0.85 to 1.34), but pooled estimates remained significant (POR = 1.24, CI: 1.10 to 1.40). Liver enzyme markers. The association between logged liver enzymes and per standard deviation increase of BPA concentration previously examined was not statistically significant in any of the adjusted models in 2005/06. No overall association with GGT concentrations, but pooled associations with alkaline phosphatase (β =0.03) and lactate dehydrogenase remained (β =0.01) significant in adjusted models.	Some useful information on HI and exposure assessment

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
Mendiola, et al. 2010	Cross-sectional. Study aim: To examine the associations between urinary BPA concentrations and reproductive function (semen quality and hormone levels) in a population of fertile men.	N=375 men. Male participants in the Study for Future Families (SFF), a multicenter study of pregnant women and their partners conducted at prenatal clinics affiliated with university hospitals in four U.S. cities (Los Angeles, CA, Minneapolis, MN, Columbia, MO, Iowa City, IA),1999 - 2005. Eligibility: Of the couples recruited at the prenatal clinics,only those whose pregnancy was conceived without medical assistance were eligible. The men completed a questionnaire, received a physical examination, and gave blood, semen, and urine samples on the same day during the prenatal visit.	Total urinary concentration of BPA (free plus conjugated species) BPA were assayed at CDC. Urine was not collected until the second year of the study. Most BPA concentrations were > LOD (89.7%); those < LOD == a value of LOD divided by the square root of 2.	Serum levels of: FSH, LH, T, inhibin B, (E2), SHBG, FAI	Pearson correlations: unadjusted analyses Multiple linear regression analyses: adjusted for age, body mass index, smoking, ethnicity, urinary creatinine concentration, time of sample collection, and duration of abstinence.	Approximately 85% of men provided a semen sample. Yet, this analysis is based on the 360 (39%) men for whom data on serum hormones and urinary BPA concentrations were available (n = 302 for complete covariates) and the 375 men with data on semen parameters and urinary BPA concentrations (n = 317 for complete covariates). Almost 90% (n = 235) of the urinary samples had concentrations of BPA > LOD (0.4 μg/L). The geometric mean of BPA (μg/L) was 1.5 with an interquartile range of 0.80- 3.0. The mean (± SD) urinary creatinine concentration was 144 ± 79.8 mg/dL (median, 138 mg/dL). There was a week inverse correlation between creatinine-adjusted urinary BPA concentrations and FAI level (-0.11), FAI/LH ratio(-0.13), T/LH ratio (-0.11), and FT/LH ratio(-0.13). However, only FAI and FAI/LH ratio correlations remained statistically significant after adjustment for age, age squared, BMI, smoking status, ethnicity, study center, urinary creatinine concentration,and time of sample collection. Neither T nor SHBG were correlated with urinary BPA.	Does not have current utility for HI or RA but may be of future utility for HI in the context of similar findings from additional studies
Miodovnik, et al. 2011	Cohort	n = 134 mother-child pairs returning for follow-up out of 404 from Mount Sinai Children's Environmental Health Study (women recruited from East Harlem or private practice on Upper East Side of Manhattan; all delivered at Mount Sinai Hospital bewteen Many 1998 and July 2002)	BPA from single, 3rd trimester maternal spot urine test collected between weeks 25 and 40 used to quantify exposure of fetus and child (measured by CDC using solid-hase extraction HPLC) same study also evaluated phthalate levels	Child's T-score on Social Responsiveness Scale (SRS) completed by mother when child 7-to-9 years old (main outcome) Demographic data collected by questionnaire	Adjusted general linear modeling	No significant association between BPA exposure and SRS outcome was found (β = 1.18, 95% CI: -0.75, 3.11) .	

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
Mok-Lin, et al. 2010	The authors state the study is a prospective cohort study. However, the study may also be considered cross-sectional based on the timing of collection of the urinary BPA samples in relation to the outcome measures. The study hypothesis is that pre- and peri-conception urinary BPA concentrations correlates with lower oocyte and estradiol production among women undergoing IVF.	Women (n=84) seeking infertility evaluation at the Massachusetts General Hospital, Nov 2004 - August 2008. Eligibility criteria:18 and 45 years-old and usage of the subject's own oocytes for IVF. Information on demographics, reproductive, medical history, lifestyle factors e.g, smoking, and diet via questionnaire.	2 urine samples per IVF treatment cycle per subject: (i) at the beginning of the cycle on day 3 or 4 of the gonadotropin phase; (ii) on the day of oocyte retrieval. Urine was collected in clean polypropylene containers and urine specific gravity (SG) measured, frozen and shipped to CDC for BPA analysis. The LOD for BPA was 0.4 µg/L.BPA concentrations < the LOD == a value equal to one-half the LOD prior to adjustment by SG. SG was used instead of creatinine to adjust for urine volume as creatinine concentrations may be confounded by muscle mass, physical activity, urine flow, time of day, diet and disease states. The geometric mean of the two SG-adjusted urinary BPA concentrations was used as the cycle-specific BPA concentration to reduce within-cycle variability.	Serum E2 as a marker of ovarian stimulation and follicular development. Serum samples to measure E2 were collected throughout the monitoring phase of the subject's IVF treatment cycle. The peak E2 concentration: the highest level of E2 prior to oocyte retrieval. FSH: measured on the third day of the menstrual cycle to assess ovarian reserve. Oocyte retrieval: when follicle size reached 16-18 mm and the peak E2 reached at least 500 pg/ml. Retrieved oocytes were cultured; trained embryologists at MGH identified the total number of oocytes retrieved per IVF cycle.	Mixed effect models: log SG-adjusted cycle-specific urinary BPA concentration & peak serum E2 concentration. Poisson regression models with a GEE approach: urinary BPA concentration & the total number of oocytes etrieved. Correlation between repeated IVF cycles in the same woman accounted for with autoregressive 1 correlation structure. Potential confounders were adjusted.	There was no association between SG-adjusted cycle-specific urinary BPA concentration and BMI (r = -0.06, p = 0.61). Urinary BPA concentrations measured in 203 urine samples collected during 112 IVF cycles. SG-adjusted BPA concentrations ranged from <LOD (15 samples) to 65.3 µg/L. There were statistically significant univariate associations of day 3 FSH concentrations with peak E2 concentration and the number of oocytes retrieved, measures of ovarian response to hyperstimulation. - for each unit increase in day 3 FSH (IU/L), there was an average decrease of 9% (95% CI: 5,14%; p = 0.00001) in the number of oocytes retrieved - average decrease in peak estradiol of 116 pg/ml (95% CI: -187,-45; p = 0.002). In BMI-, age-, day 3 FSH-adjusted Poisson regression model/GEE, there was an average decrease of 12% (95% CI: 4,23%; p = 0.007) in the number of oocytes retrieved per cycle for each log unit increase in the cycle-specific SG-BPA. In BMI-, age-, day 3 FSH-adjusted mixed effect regression model and accounting for multiple IVF cycles, there was an average decrease of 213 pg/ml (95% CI: -407,-20; p = 0.03) in peak serum E2 levels for each log unit increase in SG-BPA.	Does not have current utility for HI or RA but may be of future utility for HI in the context of similar findings from future studies

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
Wolff ,et al. 2010	Prospective	n=1151 eligible 6-to-8-year-old girls (enrolled between 2004 and 2007 and followed through puberty) from one of three sites in the Breast Cancer and Environment Research Centers (BCERC) multiethnic longitudinal study: (1) Mount Sinai School of Medicine (MSSM), (East Harlem, New York City), (2) Cincinnati Children's Hospital (Cincinnati and the Breast Cancer Registry of Greater Cincinnati), and (3) Kaiser Permanente Northern California (KPNC) (San Francisco Bay area KPNC Health Plan members); eligibility criteria included age, female sex, absence of underlying medical conditions (how determined undescribed), and black or Hispanic race/ethnicity at MSSM	BPA exposure measured directly using Visit 1 urine spot samples from MSSM and KPNC but early-morning samples from Cincinnati Urinary BPA analysis HPLC-isotope dilution tandem MS quantification (CDC)	"Earlier puberty" (main outcome) determined by breast and pubic hair stages at study Visits 1 (defined for MSSM and KPNC as the baseline visit but for Cincinnati as the 6-months-post-baseline visit when urine was also collected) and 2 (noted to have occurred ~1 year later) by trained and tested examiners Obesity determined by age- (in months) and sex-specific body mass index percentile calculations based on CDC growth charts Parents or guardians completed questionnaires providing other data	Nonparametric statis-tics (Spearman or Kruskal-Wallis) and multivariate linear regression (Proc Genmod in SAS with modified Poisson regression)	Ln-urinary creatnine adjusted geometric mean BPA levels were highest in summer 2.4 µg/L compared to spring 1.8 µg/L, fall 2.0 µg/L, and winter 1.6 µg/L. The corrected geometric BPA means were similar across BMI groups and age groups (6.0-6.9; 7.0-7.9; >= 8.0). Asians had a lower adjusted geometric mean BPA (1.5 µg/L) compared to Whites 2.0 µg/L, Blacks 2.2 µg/L, and Hispanics 2.1 µg/L. Association between urinary BPA by creatinine-corrected quintile and earlier puberty (as determined by breast development and pubic hair changes) not statistically significant: (1) reference category; (2) adjusted prevalence ratio (APR): 1.03, 95% CI: 0.96-1.09; (3) APR: 1.06, 95% CI: 0.99--1.13; (4) APR: 0.99, 95% CI: 0.92-1.05; (5) APR: 1.00 (0.94-1.07), (adjusted p-trend: 0.57) for the association; given that the association observed was not significant, further assessment regarding its modification by obesity was not meaningful	Does not have current utility for HI or RA but may be of future utility for HI in the context of similar findings from additional studies.

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
Yang, et al. 2009a	case-control	n=167; after age-matching, n=152; cases: n=70 (Korean women who had visited Seoul National University Hospital clinic between 1994 and 1997 and were diagnosed with breast cancer **for first time); controls: n=82 (Korean** women who had visited same clinic, during same period, "worried about" but not diagnosed with breast cancer, no case relatives)	serum BPA (measured by reverse-phase HPLC-fluorescence detection)	breast cancer risk	regression modeling	borderline negative association between BPA levels and age at first birth (p=0.07)	

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
Yang, et al. 2009b	Cross-sectional in Korean urban population. Study hypothesis: The relationship between the BPA level with either oxidative stress or inflammation is different with respect to gender and menopausal status due to differences in the expression of the ER and/or the ER occupancy.	485 adults(259 men, 92 premenopausal women who had regular menstrual cycles at the time of the investigation, and 134 postmenopausal women who had cessation of menses >12 months) living in large Korean cities. The cross-sectional study was from the Biomarker Monitoring for Environmental Health between April and December 2005 (total of 1131 adults enrolled), of which urinary BPA was analyzed in 613 subjects. Of the 613 individuals, this study excluded subjects who reported a history of diseases possibly influencing oxidative stress levels, such as cancer, ischemic heart disease, cerebrovascular accidents, tuberculosis, acute hepatitis, chronic bronchitis, arthritis, or asthma. and further excluded 4 women who had occasional menstruation during the last 12 months, and 15 women receiving hormone replacement therapy.	BPA exposure: Spot Urine. Measurement: HPLC-MS in 500 ul urine sample. Duration:Single timepoint measurement	urinary oxidative stress biomarkers: MDA, 8- OHdG; systemic inflammation WBC; C-reactive protein (CRP)	Data was log-transformed. Linear regression. β estimate and the p-value was used to measure linear association. SE for the β estimate were not provided. ANOVA was used to test the difference among the 3 groups. No adjustment for multiple comparisons were performed. BPA < LOD were excluded.	Urinary BPA levels were detected in 76% of subjects. Geometric means of urinary BPA were similar among men, premenopausal and postmenopausal women, i.e., 0.53, 0.61, 0.58 µg/g cr respectively. Geometric means of oxidative stress and inflammation biomarkers were similar among men, premenopausal and postmenopausal women, except for WBC count, which was higher in men than pre- or post-menopausal women. Authors concluded that in postmenopausal women, urinary BPA was associated with MDA, 8-OHdG and CRP.(adjusted β and p-value, 0.066,p=0.007; 0.103, p=0.008; 0.113, p=0.029; 0.113, p=0.029) SE for the β were not provided. The authors concluded that postmenopausal women may be more susceptible to BPA-induced detrimental health effects based on the significant p-value of the β estimate. The interpretation of β is the expected change in BPA for a one-unit change in the inflammatory markers when the other covariates are held constant (or adjustment for confounders). It appears that the authors based their conclusions on the p-value of the β estimates. However, the p-value were not adjusted for multiple comparisons, and there was no difference in the geometric means of urinary BPA, or in the difference in the geometric means of the biomakers (except WBC, which didn't have a stat sig β in MLR for post-menopausal women anyway). For CRP, the magnitude of the β between pre- and post-menopausal women are not materially different. The significant p-value of the β in post-menopausal women could be an artifact due to the larger sample size. The formal test of difference of the mean geometric BPA between pre- and post- menopausal women was not statistically significant and dos not support the authors' conclusions. No associations were observed in men or pre-menopausal women. No association observed between urinary BPA and WBC count in post-menopausal women. 2. The authors also speculated that their results may be influenced by estrogen levels and receptor occupancy. This speculation of hormone receptor involvement will not be evaluated in this review because there wasn't any data presented to support this speculation, e.g., trend analyses of the association between BPA and inflammatory markers by increasing levels of estrogen.	

Reference:	Study Design	Study Population	Exposure	Outcome(s)	Analysis Method	Effects Measured	Utility
You, et al. 2011	Cross-sectional	2573 adults (20 years and older). Flowchart of study population: 1/3 random subsample of those 6 years and older (N=5250) where following were excluded:185 with missing BPA and AP, 953 with missing creatinine measurements, 61 with renal diseases (self-reported weak/failing kidneys or dialysis in past 12 months), 1302 participants < 20 years old because they lacked data renal disease history, 176 pregnant or lactating females	BPA exposure Spot urine Measurement online solid-phase extraction coupled with HPLC-isotope dilution and tandem-MS Duration Single timepoint measurement	Endpoints: insufficient renal function: eGFR. Serum creatinine was measured using a kinetic rate Jaffe method and recalibrated with standards from the Cleveland Clinic. Serum creatinine-based GFR was estimated by using i. modified 4-variable Modification of Diet in Renal Disease Study (MDRD) equation ii. Chronic Kidney Disease Epidemiology Collaboration equation /CKD-EPI (Levey et al. 2009). Outcome of insufficient renal function was categorized in the analyses as: i. normal renal: eGFR≥90 mL/min/m2; ii. mildly decreased renal function: 90>eGFR≥60 mL/min/m2; iii. chronic kidney disease (CKD): eGFR<60 mL/min/m2	A dose-response relationship was estimated with trend analyses in regression models and adjusted for covariates. BPA<LOD=values equal to LOD divided by the square root of 2. Confounders adjusted in the analyses: age, sex, education level, annual household income, BMI, waist circumference, dietary intake of energy, cigarette smoking status; alcohol drinking status, and daily activities. Creatine adjustment was used to correct for urine dilution.	41.7% had normal renal function, 47.8% had mildly decreased renal function and 10% had undiagnosed CKD. Marginally significant dose response where adjusted geometric means for urinary BPA excretion decreased with decreasing levels of eGFR using MDRD equation that tends to underestimate measured GFR at higher values (P for trend, 0.04). The trend was not supported when using the CKD-EPI equation that is reported to be a more accurate measure of GFR at higher values than MDRD (P for trend, 0.4). No evidence of effect modification of BPA on renal diseases by age or gender. Authors concluded that "… possibly, urinary BPA, decreased with decreasing renal function." However, while the trend test using the MDRD measurement of GFR was marginally significant, indicating a linear dose response of BPA and renal function, this was not supported by another measure of GFR that is reported to have less measurement error at higher ranges. Although the authors concluded that "The associations appeared primarily in females (P for trend, 0.03)", the formal test of interaction for association of BPA and renal diseases by gender was not significant.	