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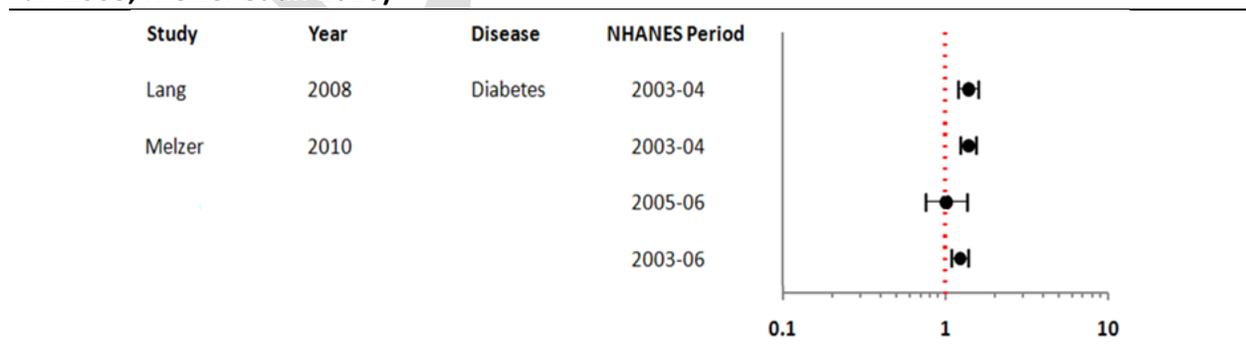
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1.1 Epidemiology Studies of Bisphenol A

Two cross-sectional analyses of the association between urinary BPA concentrations and diabetes using National Health and Nutrition Examination Survey (NHANES) data have been published. The first study, by Lang et al. (2008), analyzed data from 2003-2004 NHANES and reported that a 1-SD increased in urinary BPA concentrations was associated with an increased OR of reporting diabetes (OR and 95% CI = 1.39; 1.21–1.60) (Figure 1). The same research group then conducted a follow-up study using NHANES data from 2005-2006 and pooled data from 2003-2006 NHANES (Melzer *et al.* 2010). The association between urinary BPA concentration and diabetes remained significant when data from 2003-2006 was pooled (OR and 95% CI = 1.24; 1.1–1.40), but not when data from 2005-2006 data were considered separately (OR and 95% CI = 1.02; 0.76–1.38) (Figure 1).

Figure 1. Association between urinary BPA concentrations and diabetes prevalence (Lang *et al.* 2008; Melzer *et al.* 2010)



ORs calculated based on per 1-standard deviation increase in BPA concentrations.

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Melzer et al. (2010) also reported that that urinary BPA concentrations in 2005-2006 were substantially lower (geometric mean 1.79ng/ml) than in 2003-2004 (2.49ng/ml, difference p-value=0.00002) resulting in reduced power to detect an association. The power to detect an unadjusted odds ratio of similar magnitude to what was reported for the 2003-2004 NHANES reporting period decreased from 80% power to 73% power in 2005-2006. There is no clear explanation for the reduction in urinary BPA concentrations in 2005-2006. The high profile evaluations of BPA that led to market based changes in the use of polycarbonate plastics did not began to appear until 2007 and 2008 (Health Canada 2008; National Toxicology Program 2008; vom Saal *et al.* 2007). A more general factor that complicates conducting epidemiology studies of BPA is that a single urine sample from an individual does not appear to be strong predictor of a subject's exposure category. Mahalingaiah et al. (Mahalingaiah *et al.* 2008) analyzed samples from at least six repeat urinary BPA measurements from eight subjects. The sensitivity, specificity, and positive predictive value of a single urine sample to predict the highest BPA tertile were 0.64, 0.76, and 0.63, respectively. The positive predictive value increased to 0.85 when two samples were used to predict those individuals in the highest BPA tertile.

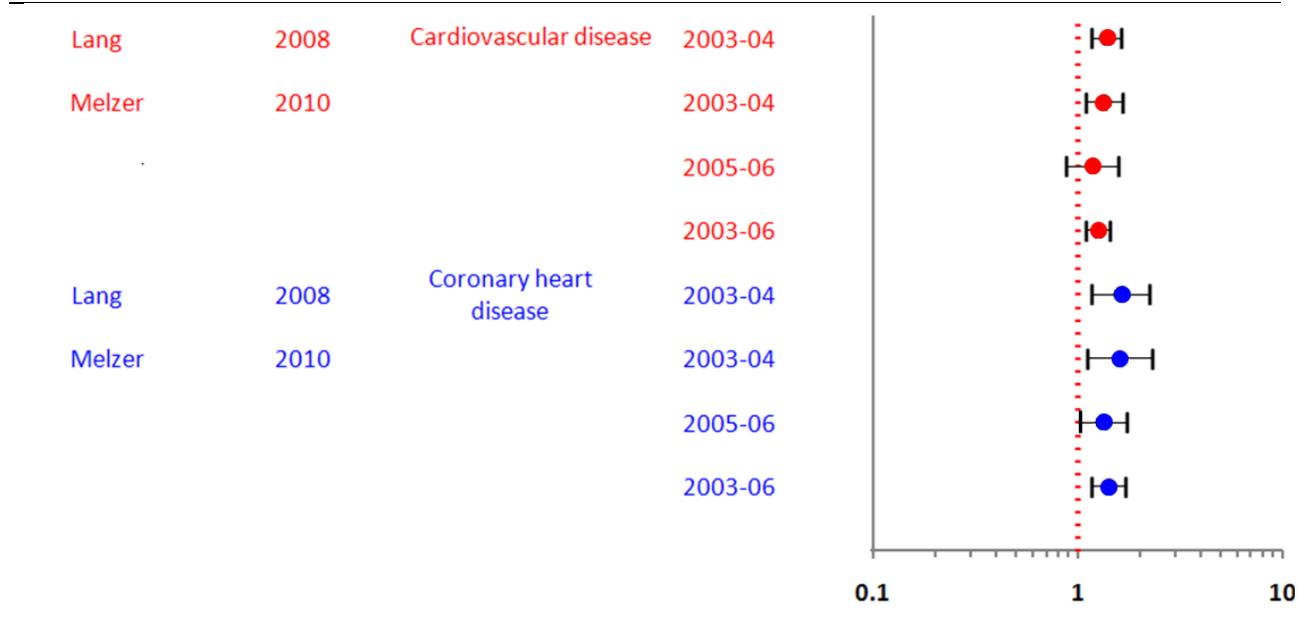
In contrast to the results for diabetes, a 1-SD increased in urinary BPA concentrations was associated with an increased OR of reporting coronary heart disease regardless of the time frame, i.e., 2003-2004, 2005-2006, 2003-2006 (Figure 2). Neither Lang et al. (2008) or Melzer et al. (Melzer *et al.* 2010) observed any significant associations between urinary BPA concentration and other common conditions, i.e., arthritis, asthma, cancer, chronic bronchitis, emphysema, liver disease, stroke, or thyroid disease.

The Lang et al. (2008) study was the subject of several commentaries that included (1) suggestions to eliminate participants with borderline diabetes and to report the mean concentrations of BPA in groups classified as diabetics, borderline diabetics, and non-diabetics (Wei 2009); (2) a suggestion to include assessment of both Type 1 and Type 2 diabetes in future analyses (Howard and Howard 2009); and a comment that analysis of NHANES data may lend itself to detecting false positives given the total possible number of questions that can be addressed based on the number of environmental chemicals measured (n=275) and the number of health outcomes included (n=32) (Young and Yu 2009).

Three studies have looked at the association between BPA and growth, based on either birth weight (Padmanabhan *et al.* 2008; Wolff *et al.* 2008) or BMI at 6-8 years of age in girls (Wolff *et al.* 2007). Padmanabhan et al. (2008) concluded there was no association between maternal serum BPA collected at time of delivery and birth weight in a cross-sectional study of 40 mother-infant pairs in Ann Arbor, Michigan. Birth weights in infants born to mothers with serum BPA levels >5ng/ml compared to ≤ 5ng/ml were similar, ~3.2 versus ~3.3 kg respectively. The other birth weight assessment was obtained as part of a prospective birth cohort study of 367 women in New York City. Wolff et al. (2008) looked at the association of maternal urinary BPA measured in the 3rd trimester of pregnancy (25-40 weeks) and found modestly positive, but non-statistically significant, associations with increases in birth weight (38 grams; 95% CI for

β -coefficient: -6.0 to 82 grams), length (0.11 cm; 95% CI for β -coefficient: -0.14 to 0.36 cm), and head circumference (0.08 cm; 95% CI for β -coefficient: -0.09 to 0.25cm). The third study reported lower urinary BPA concentrations in girls >85th BMI percentile compared to girls < 85th BMI percentile (2.2 versus 3.7 $\mu\text{g/g}$) in a cross-sectional study of 90 girls ages 6- to 8-years from Cincinnati OH, San Francisco CA, and New York NY (Wolff *et al.* 2007).

Figure 2. Association between urinary BPA concentrations and prevalence or cardiovascular and coronary heart disease (Lang *et al.* 2008; Melzer *et al.* 2010)



ORs calculated based on per 1-standard deviation increase in BPA concentrations.

1.2 Animals Studies of Bisphenol A

1.2.1 Effects on body weight following developmental exposure

There have been several reports of increased post-natal growth in laboratory rodents following exposure to low administered doses of BPA during gestation, early post-natal life, or throughout gestation and lactational leading to the suggestion that it may be acting as a developmental “obesogen” (Ben-Jonathan *et al.* 2009; Grun and Blumberg 2007; Newbold *et al.* 2009; Rubin and Soto 2009). There is a very large literature evaluating the effects of BPA on body weight, and surveys of these studies do not indicate that developmental exposure to BPA causes “obesity” as defined by a consistent reporting of increased body weight or growth (National Toxicology Program 2008).¹ This conclusion remains true when the analysis is restricted to only those studies that tested low doses of BPA, defined as less than 5000 $\mu\text{g/kg bw}$ (Appendix Table

¹ There are also a large number of studies of animals treated with BPA during adulthood that report body weight. In general, these studies involved treatment with doses that are considered high and no effects or decreases in body weight were observed. The focus of this chapter is on the concept of BPA as a developmental “obesogen.”

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A), and reported some health outcome, typically unrelated to growth and most often describing effects on reproductive tissues or neurodevelopment. This strategy was used to avoid the issue of considering studies in the analysis that have been criticized as being insensitive to detect low dose effects (Myers *et al.* 2009). Many of the studies did not detect an effect on body weight, or else did not report body weight findings past the period of weaning. The magnitude of the effect in cases where an increase in body weight was observed ranged from 3% to 50%, with most reporting increases of 10% or less (Akingbemi *et al.* 2004; Alonso-Magdalena *et al.* 2010; Howdeshell *et al.* 1999; Kubo *et al.* 2003; Miyawaki *et al.* 2007; Nikaido *et al.* 2004; Okada and Kai 2008; Patisaul and Bateman 2008; Rubin *et al.* 2001; Ryan *et al.* 2010; Salian *et al.* 2009; Somm *et al.* 2009).

The ability to consistently detect a relatively subtle effect of $\leq 10\%$ change is perhaps unrealistic given the wide range of variation in experimental designs used in the studies summarized in [Appendix Table A](#), including variation in sample size (and thus statistical power to detect an effect if present), control for litter effects, rodent species and strain, and other factors such as diet. Variation in the phytoestrogen content of laboratory animal diets has been suggested as a contributing factor to conflicting or inconsistent findings reported in experimental animal studies, especially for “low dose” studies of BPA and other estrogenic compounds (Heindel and vom Saal 2008; Jensen and Ritskes-Hoitinga 2007; Thigpen *et al.* 2007). As a further complication, phytoestrogen content of the same rodent diet can vary from batch-to-batch (Jensen and Ritskes-Hoitinga 2007; Thigpen *et al.* 2007), potentially altering experimental outcomes and contributing to variability in response observed within a laboratory or between laboratories. For example, Thigpen *et al.* (2007) assessed the isoflavone content of genistein + daidzein in the three batches, i.e., different mill dates, of PMI 5002. The isoflavone content of genistein + daidzein in the three batches were 98, 223, and 431 $\mu\text{g/g}$ diet. It is also worth noting that the early studies on the use of DES as a growth promoter in livestock did not always detect a growth promoting effect of the hormone, even at doses that were later considered optimal for weight gain within the industry [reviewed in Raun and Preson (2002)].

In some cases, divergent results were reported by the same laboratory in separate experiments using the same rodent strain under similar experimental protocols. For example, Nagel *et al.* (1997) reported that adult 6-month old male CF-1 mice treated with 2 $\mu\text{g/kg}$ bw/day of BPA *in utero* from GD11-17 were significantly lighter than control males (34.6 versus 37.9 grams) while there was no effect on body weight at a higher BPA dose of 20 $\mu\text{g/kg}$ bw/day (36.7 versus 37.9 grams). Using this same strain of mouse and very similar dosing regimen, Howdeshell *et al.* (1999) reported a statistically significant increase in body weight at weaning in male and female offspring of dams treated with 2.4 $\mu\text{g/kg}$ bw/day of BPA during pregnancy, e.g., ~ 9.5 g versus ~ 10.5 g in control and BPA treated females. The effects on body weight were most apparent in female² mice situated *in utero* between 2 female littermates (“0M” position, ~ 9 g versus ~ 11 g) and there was no effect in animals situated between 2 males (“2M” position, ~ 9 g versus ~ 9.5 g) suggesting that small differences in prenatal hormone exposure may be one source of

² Data were not presented for male offspring, but findings were characterized as “virtually identical for male siblings.”

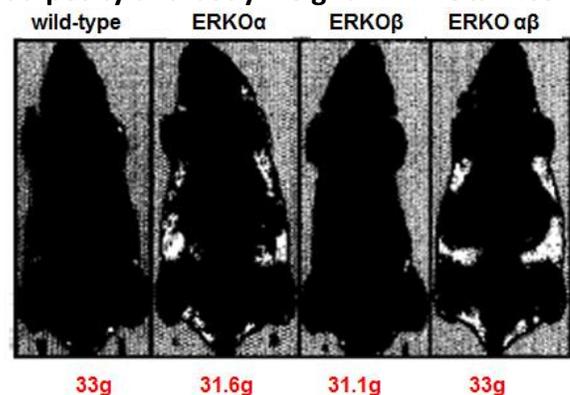
variability in response. Both of these studies utilized models considered to be sufficiently sensitive to detect low dose effects of BPA based on findings of increase prostate weight in Nagel et al. (1997) and indications of earlier sexual maturation in females in Howdeshell et al. (1999). One difference may be in the age at assessment, 6-months in Nagel et al. (1997) and weaning in Howdeshell et al. (1999).

Some of the studies cited in [Appendix Table A](#) describe non-monotonic effects on growth (Alonso-Magdalena *et al.* 2010; Okada and Kai 2008; Rubin *et al.* 2001; Talsness *et al.* 2000; Xu *et al.* 2010) where body weight was increased at a low dose and not affected or decreased at a higher dose. In toxicology, interpretation of non-monotonic dose responses is challenging but is perhaps less complicated for body weight. Decreases in body weight are common at dose levels considered “high,” based on the detection of other adverse health effects at those same dose levels. From this perspective, the “high” dose studies may be of limited utility to understand relatively subtle effects on growth that may be occurring at low doses. A complicating factor in the studies with gestational exposure is that treatment with BPA, or other test compound, may alter aspects of maternal metabolism such as leptin, insulin, glucose or triglycerides that may act or cross the placenta (Alonso-Magdalena *et al.* 2010). There is also precedent for non-monotonic dose response in some of the early studies characterizing optimal DES dosing regimens to use in livestock for growth promotion [reviewed in Garrigus (1969). Garrigus et al. (1969) reported increased growth response in post-weaning aged bulls implanted with 36 mg DES for 84 days and no effect in bull implanted with 72 mg DES. Weight gain was reduced in animals that were implanted with 36 mg DES at weaning, 84 days post-weaning, and 154 days post-weaning (0.89 kg/day) compared to bulls receiving a single 36 mg DES implant at 154 days post-weaning (1.14 kg/day).

1.2.2 Adiposity and effect of consuming a high fat diet

A major caveat to the analysis described above is the general acceptance that body weight is a relatively crude indicator of internal body fat in rodent models. For example, no changes in body weight were observed by Ohlsson et al. (2000) in ER α mice compared to wild-type controls despite having visibly greater amounts of adipose tissue ([Figure 3](#)). Similarly, in ArKO males, increases in gonadal and infrarenal fat pad mass were apparent by 4 months of age, but body weight was not significantly greater in these animals until later, at 1-year of age (Jones *et al.* 2001). In humans, low birth weight is increasingly recognized as a risk factor later in life for higher percent body fat and detrimental fat distribution (more central, abdominal, and visceral) (Remmers

Figure 3. Lack of relationship between adiposity and body weight in ERKO α mice.



Reprinted from Ohlsson et al. (2000) with permission from publisher.

and Delemarre-van de Waal 2010). A phenotype referred to as the “thin-fat” phenotype has been described in babies of Southeast Asian descent where the babies are smaller in size at birth compared to babies born in other regions (such as Southampton UK) but are more similar on measures of skin fold thickness, especially subscapular skin. The interpretation is that the “thin-fat” babies have signs of truncal adiposity despite being low birth weight (Krishnaveni *et al.* 2005; Kulkarni *et al.* 2009). This larger literature on adiposity suggests studies of BPA and body weight have very limited utility for addressing the issue of whether BPA might be altering aspects of adiposity, which requires more refined assessments of fat mass or distribution.

Four studies in laboratory animals have been published since 2007 that most directly address the issue of whether developmental exposure to BPA can affect adiposity, glucose or insulin regulation, lipid profiles, or other endpoints related to diabetes or metabolic syndrome (Alonso-Magdalena *et al.* 2010; Miyawaki *et al.* 2007; Ryan *et al.* 2010; Somm *et al.* 2009). These studies are summarized in [Table 1](#) and specific findings that support the hypothesis of BPA as a potential risk factor for diabetes, “obesity,” or metabolic syndrome are highlighted in red. No published studies have assessed whether BPA can cause hypertension, another risk factor for metabolic syndrome.

With respect to body weight, all four of these studies reported increases in body weight early in life although this pattern did not always persist until adulthood (Somm *et al.* 2009) and was not necessarily observed in both sexes and at all dose levels tested. In Alonso-Magdalena *et al.* (2010), a 3 – 7% increase in body weight at birth and weaning was observed in the offspring of pregnant OF-1 mice treated from GD9-16 with 10 µg/kg bw/d BPA by sc injection, but a 5% decrease in body weight was observed in pups in the 100 µg/kg bw/d treatment group. Miyawaki *et al.* (2007) reported an increase in female pup weight on PND31 following maternal treatment with 260 µg/kg bw/d BPA in drinking water during gestation and lactation (pups were weaned to the drinking water treatment of the dam). There was no effect on female body weight in the 2,600 µg/kg bw/d treatment group and body weight in males was not altered at any dose level.

Somm *et al.* (Somm *et al.* 2009) found that PND1 body weights in male and female offspring were 6% and 8%-times larger than control animals [male: 7.33 versus 6.91 g ($p < 0.05$); females 7.03 versus 6.47 g, $p < 0.001$). The authors also noted a non-statistically significant reduction in litter size in the BPA animals compared to controls (12.7 versus 14.1 pups per litter) and conducted analyses on size-matched litters and a regression analysis adjusted for litter size to see if the PND 1 differences in body weight could be an indirect effect of reduced litter size. The body weight remained significant in both cases leading the authors to conclude that BPA was exerting a direct effect on body weight at PND1. Body weight remained significantly higher in BPA-treated females but not males on PND 21. Ryan *et al.* (2010) also reported indications of shifted growth curve during early life in animals treated with BPA, but no lasting effects on body weight. No shift in growth pattern was noted in animals treated with a positive control DES dose of 1 µg/kg bw/day. The authors also reported that cages containing mothers and pups exposed to BPA-supplemented diet consumed more calories from p14 to p21 compared with

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both the control and DES groups. It should be noted that the dose level used by Ryan et al. (2010), 0.25 µg/kg bw/day to the dam via the diet, is a dose level of BPA that is similar to current estimates of daily intake in the general population and lower than estimated intakes in infants. The estimated daily intake based on back-calculating from urine biomonitoring data in NHANES for adults at the 95th percentile is 0.223 µg/kg bw/day (Lakind and Naiman 2010).). The estimated intake from food contact material is higher in infants, 2.25 to 2.42 µg/kg bw/day for a 1-2 month old male and female infants, respectively (FDA 2008).

The Somm et al. (2009) and Ryan et al. (2010) studies included experimental components where groups of animals that were exposed to BPA during gestation and lactation were subsequently “challenged” with a high-fat diet later in life, either during 4-14 weeks or 9-14 weeks of age, respectively. No effects on adult body weight were reported by Ryan et al. (2010) in male or female mice that consumed either the low fat or high butter fat diet (40% fat by weight). Consumption of the high butter fat diet caused the expected increase in body fat in control mice but did not alter glucose tolerance.³ Somm et al. (2009) found that BPA-treated animals of both sexes grow more on the high fat diet (40% of calories from fat) compared to animals not treated with BPA during development. The authors measured food intake during this period and did not see any differences in food intake in any of the standard chow (a low phytoestrogen diet, KLIBA NAFAG 3250 with 7% of calories from fat) or high-fat diet groups. This same growth pattern was observed in BPA-treated female mice consuming the standard chow, but not males. It is unknown whether the differences in dose levels of BPA used might account for the different results, i.e., the Somm et al. administered an oral dose level that was almost 300-times higher (70 versus 0.25 µg/kg bw/day).

Findings for other endpoints from these studies are generally less consistent than the increases in growth early in life described above, which may be also be the result of variation in the dose levels tested, route of administration (oral and sc injection), strategies used to measure the end-points (i.e. fasting glucose versus glucose tolerance test) and other aspects of experimental design. The most notable findings for non-body weight effects are:

- Glucose intolerance, hyperinsulinaemia and increased serum glycerol in the 6-month-old male offspring of OF-1 mice treated with BPA at 0.01 or 0.1 mg/kg bw per day by subcutaneous injection from gestational day (GD) 9 to GD 16 (Alonso-Magdalena *et al.* 2010). In dams, the treatment with BPA aggravated the insulin resistance which is characteristic of pregnancy, producing an impairment of glucose tolerance and higher plasma insulin, triglyceride and leptin levels compared to control pregnant mice. Insulin-

³ A couple of factors may account for a lack of effect on glucose tolerance in control animals. First, is timing as the animals were only on HFD for 5-6 weeks prior to the second GTT; detecting a difference in glucose tolerance may require additional weeks of HFD. Second, the control diet used was a micro-nutrient matched 10% butter-fat diet rather than standard rat chow. Typically this research group finds less separation between groups on metabolic endpoints when matched HFD and LFD are compared rather than HFD and chow. However, using the matched HFD and LFD has the advantage that the only difference between the diets is fat content whereas chow content can vary substantially from batch to batch.

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stimulated Akt phosphorylation was reduced in skeletal muscle and liver of BPA-treated pregnant mice.

- Adipocyte hypertrophy and increased mass of parametrial white adipose (95 versus 33 mg in controls) and brown adipose tissue (178 versus 116 mg in controls) on postnatal day (PND) 21 in female offspring of Sprague-Dawley rats orally treated with BPA at 0 or approximately 0.07 mg/kg bw per day in drinking-water from GD 6 to PND 21 in drinking-water (Somm *et al.* 2009).
- Increased cholesterol on PND31 in female offspring of ICR mice orally treated with BPA at approximately 0.26 or 2.60 mg/kg bw per day in drinking-water from GD 10 to weaning via the dam and then after weaning with the same drinking-water treatment as the dam (Miyawaki *et al.* 2007).

Similar results have been observed in shorter-term *in vivo* assays. Experiments performed in male OF-1 mice showed that a single sc injection of 10 µg/kg of BPA produces a rapid decrease of blood glucose that occurs in parallel to an increase in plasma insulin levels. The insulin content in pancreatic β-cells was increased compared to vehicle-treated mice following a 4-day treatment with a higher dose level, 100 µg/kg/day by sc injection (Alonso-Magdalena *et al.* 2006). The BPA treatment did not have an effect on β-cell survival or β-cell mass in the *in vivo* study, but it affected insulin gene transcription in islet cells that were cultured with BPA for 48 hours, provoking an up-regulation of the gene in an extracellularly initiated ERα-dependent manner (Alonso-Magdalena *et al.* 2008). This 4-day treatment with BPA at a dose of 100 µg/kg BPA administered by sc injection also generates a post-prandial hyperinsulinemia. Results of glucose tolerance tests showed impaired glucose tolerance suggestive of insulin resistance. This was confirmed by an insulin tolerance test that showed a less hypoglycemic response to a challenge of insulin in those animals treated with BPA (Alonso-Magdalena *et al.* 2006).

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Table 1. Summary of study findings related to body weight, adiposity, and serum lipids following developmental exposure to BPA

Reference	Study Design	Dose (mg/kg bw/day)	Endpoint	Findings		
Alonso-Magdalena et al. (2010)	OF-1 pregnant mice treated with 0 (tocopherol-stripped corn oil), 10, or 100 µg/kg bw/day BPA by sc injection from GD9-16 (6-13 male F1 offspring assessed at 6 months of age depending on endpoint) <u>feed</u> : 2014 Teklad Global 14% Protein Rodent Maintenance Diet animals (does not contain alfalfa or soybean meal) <u>control for litter effects</u> : dam number/group not specified; pups of same treatment group were pooled and then placed in equal numbers with foster mothers of the same treatment group (11 pups/group); pups housed together were of same sex	0.01& 0.1 (sc injection)	insulin (µg/L)	0.01 dam, GD18 (fasting)	↑ (1.4-fold)	
				F1 ♂, 6 months (fasting)	↑ (2.0-fold)	
			0.1	dam, GD18 (fasting)	↑ (2.4-fold)	
				F1 ♂, 6 months (fasting)	↑ (1.6-fold)	
			glucose (units)	0.01	dam, GD18 (AUC, ipGTT)	↑
					dam, GD18 (ipITT)	↔
				0.1	F1 ♂, 6 months (AUC, ipGTT)	↑
					F1 ♂, 6 months (ipITT)	insulin resistance
					dam, GD18 (AUC, ipGTT)	↔
					dam, GD18 (ipITT)	insulin resistance
			leptin (ng/mL)	0.01	dam, GD18 (4 hour fasting)	↔
					F1 ♂, 6 months (4 hour fasting)	↔
			0.1	dam, GD18 (4 hour fasting)	↑ (1.9-fold)	
				F1 ♂, 6 months (4 hour fasting)	↔	
			triglycerides (mg/mL)	0.01	dam, GD18 (4 hour fasting)	↔
					F1 ♂, 6 months (4 hour fasting)	↔
				0.1	dam, GD18 (4 hour fasting)	↑ (1.9-fold)
					F1 ♂, 6 months (4 hour fasting)	↔
			glycerol (mg/mL)	0.01	dam, GD18 (4 hour fasting)	↔
					F1 ♂, 6 months (4 hour fasting)	↑ (1.5-fold)
0.1	dam, GD18 (4 hour fasting)	↔				
	F1 ♂, 6 months (4 hour fasting)	↑ (2.6-fold)				
body weight (g)	0.01	PND1, ♂ and ♀ combined	↑ (1.03-fold)			
		PND22, ♂ and ♀ combined	↑ (1.07-fold)			
		11-25 weeks	↔ ♂, ↓ ♀			

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Table 1. Summary of study findings related to body weight, adiposity, and serum lipids following developmental exposure to BPA

Reference	Study Design	Dose (mg/kg bw/day)	Endpoint	Findings	
			0.1	PND1, ♂ and ♀ combined PND22, ♂ and ♀ combined 11-25 weeks	↓ (5%) ↓ (5%) ↔ ♂, ↓ ♀
Miyawaki et al. (2007)	ICR mice (pregnant); 0, 1 [~0.26 mg/kg bw/d], or 10 µg/mL [~2.72 mg/kg bw/d], BPA in drinking water from GD 10-throughout the lactating period, offspring weaned to drinking water treatment of dam (♂ and ♀ on PND 31; n=16-25 per group). <u>feed</u> : 30% high fat diet mentioned but no specific brand- dietary composition was described <u>control for litter effects</u> : 3 dams/group; no control for litter effects	0.26 & 2.60 (oral)	adipose tissue (mg) body weight (g) glucose (mg/dL), fasted leptin (ng/mL), fasted cholesterol (mg/dL), fasted triglycerides (mg/dL), fasted NEFA (mEq/L), fasted	0.26 PND31 2.6 PND31 0.26 PND31 2.6 PND31 0.26 PND31 2.6 PND31 0.26 PND31 2.6 PND31	↔ ♂, ↑ ♀ ↑ ♂, ↔ ♀ ↔ ♂, ↑ ♀ ↑ ♂ and ♀ ↓ ♂, ↔ ♀ ↔ ♂ and ♀ ↔ ♂, ↑ ♀ ↔ ♂ and ♀ ↔ ♂, ↑ ♀ ↔ ♂, ↑ ♀ ↑ ♂, ↔ ♀ ↔ ♂ and ♀ ↔ ♂ and ♀ ↑ ♂, ↔ ♀ ↔ ♂ and ♀
Somm et al. (2009)	Sprague Dawley rats (♂ and ♀ F1 offspring); 0 or 1 mg/L BPA in drinking water to dam from GD6 to PND21 (authors estimated intake as ~0.07 mg/kg bw/day BPA at end of gestation). After weaning F1 offspring were fed either a standard or high fat diet. <u>feed</u> : Low isoflavone diet used (KLIBA NAFAG 3250); <u>control for litter effects</u> : litter used as the fundamental unit of comparison “when appropriate and animals	0.07 (oral)	adipose tissue (mg) glucose (mg/dL) triglycerides (mg/dL) cholesterol (g/L) NEFA (mmol/L) body weight/growth (g)	PND21 eWAT/pWAT PND21 BAT PND21, non-fasted (♂ not assessed) adulthood, fasting, HFD (♀ not assessed) adulthood, AUC ipGTT, HFD (♀ not assessed) PND21 (♂ not assessed) PND21 (♂ not assessed) PND21 (♂ not assessed) PND1 PND21	↔ ♂; ↑ ♀ ↔ ♂; ↑ ♀ ↔ ↔ ↔ ↔ ↔ ↔ ↔ ↑ (~1.08 -fold; ♂ and ♀) ↔ ♂; ↑ ♀

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Table 1. Summary of study findings related to body weight, adiposity, and serum lipids following developmental exposure to BPA

Reference	Study Design	Dose (mg/kg bw/day)	Endpoint	Findings
	originated from at least three different litters in each group (eight litters for each group for data at birth)." Litter also considered in regression model to look at impacts on birth and weaning weight.		4-14 weeks, standard 4-14 weeks, HFD	↔ ♂; ↑ ♀ ↑ ♂ and ♀
Ryan et al. (2010)	CD-1 mice (pregnant); 0 or 0.25 µg/kg bw/day BPA via diet during gestation through lactation. 1 µg/kg bw/day DES used as positive control. F1 offspring consumed a low- or high-butter fat diet from 9 to 14 weeks of age <u>feed:</u> AIN93G diet used <u>approach to control for litter effects:</u> use of only one offspring per sex from each litter (animals from ~ 90 litters included in post-weaning analyses); litter size included as a covariate in ANCOVA 30 litters x 3 treatments = 90 litters x 2 sexes = 180 mice	0.00025 (oral)	fat mass (g) lean mass (g) glucose, ipGTT (mg/dL and AUC) body weight/growth (g)	7 weeks ↔ 14 weeks, LFD ↔ 14 weeks, HFD ↔ ♂; ↓ ♀ 7 weeks ↔ 14 weeks, LFD ↔ 14 weeks, HFD ↔ 7 weeks ↔ 14 weeks, LFD ↔ 14 weeks, HFD ↔ weaning ↑ (~1.1-fold; ♂ and ♀) 3-9 weeks ↔ 9-14 weeks, LFD ↔ 9-14 weeks, HFD ↔

1.3 Mechanistic Findings

In vitro studies, summarized in [Appendix Table C](#), show that BPA can alter aspects of islet cell function and adipocyte differentiation and function. These effects are reported at lowest effect concentrations (LOECs) that range from 0.0001-40 μM . BPA appears to have direct effects on at least two of the four cell types found in islet of Langerhans, α -cells and β -cells [the other cell types are somatostatin (δ -cells) and pancreatic polypeptide (PP-cells)]. *Ex vivo* studies show that BPA at a concentration of 1 nM suppresses low glucose-induced intracellular calcium oscillations on α -cells (Alonso-Magdalena *et al.* 2005). The hormone glucagon is secreted from the α -cells in response to low glucose concentration to enhance the synthesis and mobilization of glucose in the liver. Glucagon also has many extrahepatic effects such as to increase lipolysis in adipose tissue and plays a role in the satiety control in the central nervous system among others. Thus the effect on α -cells described above suggests that BPA may alter both glucose and lipid metabolism (Alonso-Magdalena *et al.* 2005). In β -cells BPA has the opposite effect and enhances the frequency of glucose-induced intracellular calcium oscillations at the same low concentration of 1 nM (Alonso-Magdalena *et al.* 2006; Nadal *et al.* 2000). It has been shown that BPA is able to increase the activation of the transcription factor CREB in a rapid manner (Quesada *et al.* 2002). This effect may be of great importance for β -cell physiology, since CREB activation induces insulin gene expression (Oetjen *et al.* 1994) and is implicated in β -cell survival (Jhala *et al.* 2003). Other studies report increased insulin secretion from isolated rat or mouse pancreatic islet cells after incubation with BPA (Adachi *et al.* 2005; Alonso-Magdalena *et al.* 2008). In some cases, these effects appeared to be mediated via classic ERs (Adachi *et al.* 2005; Alonso-Magdalena *et al.* 2008) and others suggested to be mediated through non-classic ERs (Alonso-Magdalena *et al.* 2005; Nadal *et al.* 2000; Quesada *et al.* 2002).

BPA at concentrations of 10-80 μM has been reported to stimulate adipogenesis in 3T3-L1 adipocytes in most (Masuno *et al.* 2005; Masuno *et al.* 2002; Phrakonkham *et al.* 2008; Wang *et al.* 2010) but not all (Wright *et al.* 2000) studies that assessed this effect. Sargis *et al.* (2010) reported that 0.1 μM BPA increased lipid accumulation in differentiating adipocytes and up-regulated the expression of adipocyte proteins, presumably through activation of the glucocorticoid receptor (Sargis *et al.* 2010). Other studies report that BPA at 1 and 10 nM suppresses adiponectin release from breast adipose and abdominal subcutaneous adipose explants (Ben-Jonathan *et al.* 2009; Hugo *et al.* 2008) or decreases intracellular adiponectin or adiponectin mRNA expression in 3T3-L1-cells (Kidani *et al.* 2010; Kwintkiewicz *et al.* 2010). Adiponectin is an important adipokine that is considered protective in the development of metabolic syndrome.

It is an important point that simply inducing adipogenesis, in the absence of changes in energy balance, does not lead to changes in total adiposity (Rosen and Spiegelman 2006). Also, Somme *et al.* (2009) found that the increase in perigonadal fat pad weight was associated with increased cell size rather than cell number, which does not support increased adipogenesis.

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Masuno *et al.* (2005) suggested the acceleration of differentiation may be mediated through the phosphatidylinositol 3-kinase (PI 3-kinase) and Akt kinase pathways. BPA can also affect glucose transport in adipocytes; it provokes an increase in basal and insulin-stimulated glucose transport due to an increased amount of GLUT4 (Sakurai *et al.* 2004).

The following draft text was primarily prepared by GeneGo to support the schematic in [Figure 4](#) – this schematic has not been reviewed by the breakout group prior to the meeting and will be reviewed and discussed during the January workshop. However, the mechanism by which BPA may stimulate fat cell differentiation is enigmatic ([Figure 4](#)). BPA may stimulate fat cell differentiation via stimulation of PI3K reg class IA/AKT(PKB), activation of ESR1, ERR3, glucocorticoid receptor alpha (GCR-alpha) and/or inhibition of androgen receptor. Modulation of expression of these genes by BPA leads to activation of adipogenic marker proteins including PPARGC1 (PGC1-alpha), PERC, PPAR γ and A-FABP, lipogenic proteins (LPL, GPD1, SCD, FASN, leptin, SREBP1 precursor) as well as the glucose transporter, GLUT4.

BPA stimulates expression of LPL and A-FABP in a PI3K reg class IA/AKT(PKB)-dependent manner (Masuno *et al.* 2005). AKT(PKB) may also activate expression of LPL and A-FABP via pathways leading to the regulation of the transcription factors GATA-2 (Menghini *et al.* 2005), FKHR (Nakae *et al.* 2003), and CREB1 (Reusch *et al.* 2000). GATA-2 is phosphorylated and blocked by the PI3K reg class IA/AKT(PKB) signal transduction pathway (Menghini *et al.* 2005). It eliminates GATA-2-dependent inhibition of C/EBP-beta and C/EBP-alpha transcription activity (Tong *et al.* 2000) and PPAR- γ expression (Menghini *et al.* 2005; Schupp *et al.* 2009; Tong *et al.* 2000; Tong *et al.* 2005). FKHR is phosphorylated and blocked by AKT(PKB). It eliminates FKHR-dependent inhibition of PPAR- γ transcription activity (Nakae *et al.* 2003).

CREB1 is phosphorylated and stimulated by AKT(PKB). It was suggested that activated CREB1 increases PPAR- γ , C/EBP beta, LPL, SCD, FASN, leptin and A-FABP expression (Fox *et al.* 2006; Kim *et al.* 2010; Phrakonkham *et al.* 2008; Reusch *et al.* 2000; Zhang *et al.* 2004). C/EBP β is an important adipogenic transcription factor which stimulates transcription of C/EBP α and PPAR- γ . C/EBP β , C/EBP α and PPAR- γ form a network of transcription factors that coordinate expression of proteins responsible for establishing the mature fat-cell phenotype including, LPL, SCD, FASN, Leptin and A-FABP and others (Farmer 2006; Tang *et al.* 2004).

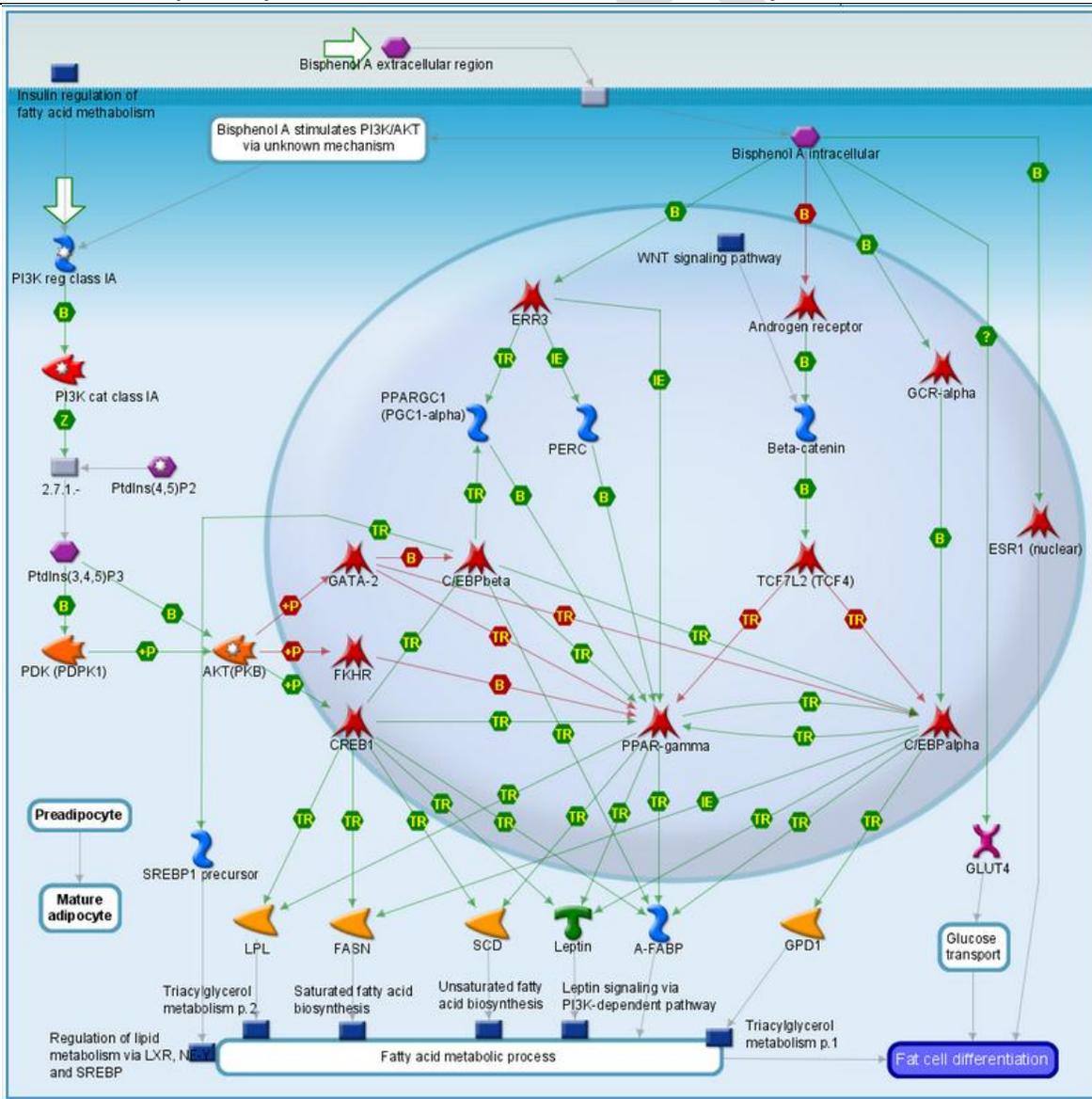
BPA is equipotent to estradiol in some of its effects. It is possible that BPA stimulates fat cell differentiation via estrogen receptors, most likely, ESR1 (Dieudonne *et al.* 2004; Hong *et al.* 2006; Inoshita *et al.* 2003) and ERR3 (Kubo *et al.* 2009; Okada *et al.* 2008). The mechanism of action of BPA-stimulated ESR1 on fat cell differentiation is unknown. ERR3 stimulates transcription of adipogenic marker genes including PPAR- γ co-activators PPARGC1 (PGC1-alpha) (Wang *et al.* 2005) and PERC, PPAR- γ and A-FABP (Kubo *et al.* 2009). BPA may also be capable of promoting adipogenesis through activation of the GCR-alpha (Prasanth *et al.* 2010; Sargis *et al.* 2010). The activated GCR-alpha increases lipid accumulation (Sargis *et al.* 2010), possibly, via C/EBP α -dependent stimulation of leptin expression (De Vos *et al.* 1998).

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In addition, BPA may stimulate fat cell differentiation via inhibition of androgen receptor (Tamura *et al.* 2006). BPA is likely to eliminate nuclear translocation of the androgen receptor complex with Beta-catenin and TCF7L2 (TCF4). It elevates TCF7L2 (TCF4)-dependent inhibition of translation of C/EBP α and PPAR γ (Singh *et al.* 2006). LPL and GPD1 expression may be stimulated via this pathway (Dieudonne *et al.* 2000; Masuno *et al.* 2002; Ramirez *et al.* 1997). Lipogenic proteins (LPL, GPD1, SCD, FASN, leptin, SREBP1 precursor and A-FABP) stimulate fatty acid metabolic pathways, thus contributing to the establishment of the mature fat-cell phenotype (Rosen and MacDougald 2006). BPA-dependent fat cell differentiation may be realized at least partially via stimulation of glucose metabolism. BPA affects glucose transport in adipocytes by increasing GLUT4 protein level (Sakurai *et al.* 2004).

Figure 4. Putative pathways for stimulation of fat cell differentiation by BPA



Draft text and schematic prepared by GeneGo. See background document "MCLegend.pdf" for figure legend

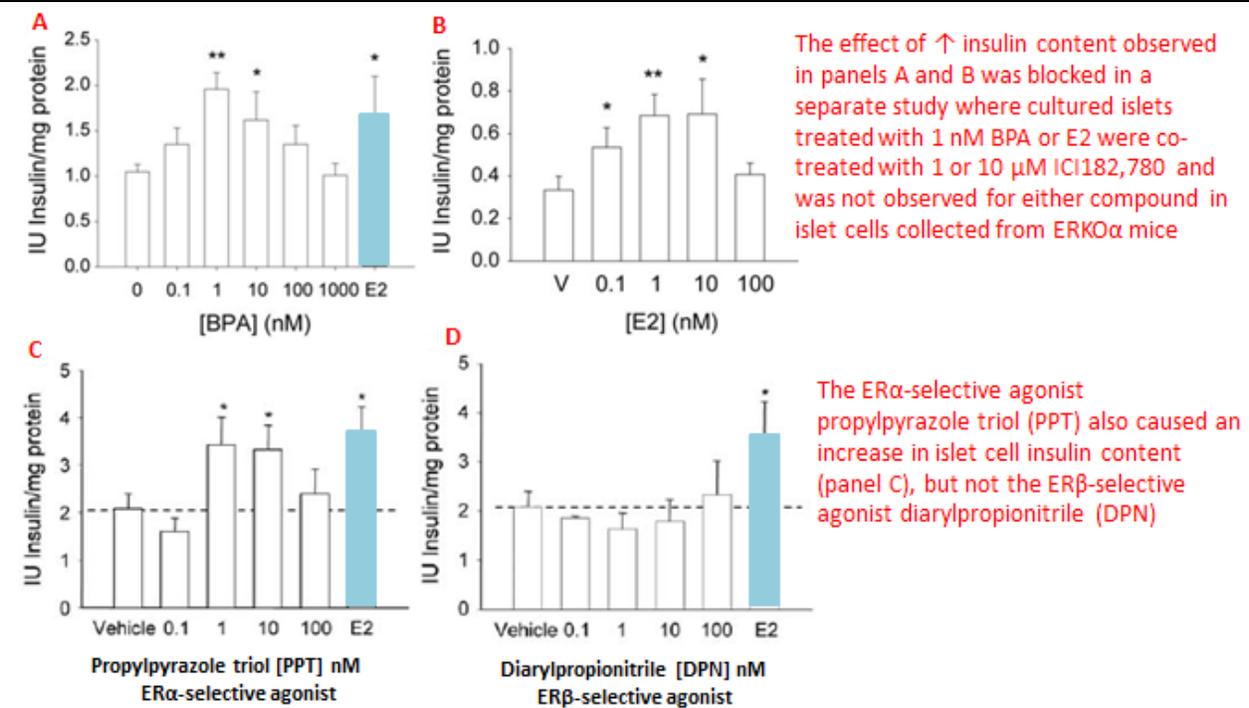
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1.3.1 Role of estrogen receptors and other biochemical or molecular interactions

In some cases, the effects of BPA seem to be mediated through the classic ER α pathway although with an apparent potency that is greater than predicted based on BPA's relative binding affinity for ER α (~1000 less potent than estradiol) and ER β . One example comes from a study by Alonso-Magdalena et al. (2008) where the insulin content of pancreatic islet cells increased with treatment of 1 or 10 nM BPA and 0.1, 1, or 10 nM estradiol (panels A and B, Figure 5; the non-monotonic nature of the dose-response is discussed below). A similar response was seen for the ER α -agonist propylpyrazole triol (PPT) but not the ER β agonist diarylpropionitrile (DPN) (panels C and D, Figure 5). The authors conducted a number of other experiments to determine the role of ERs in mediating the effects and reported that the effect of 1 nM E2 or BPA on increasing insulin content from islet cells could be completely blocked by co-incubation with 1 or 10 μ M of ICI182,780. Also the effect of BPA and E2 was not observed in islet cells collected from ERKO α mice but was still present in islets collected from ERKO β animals.

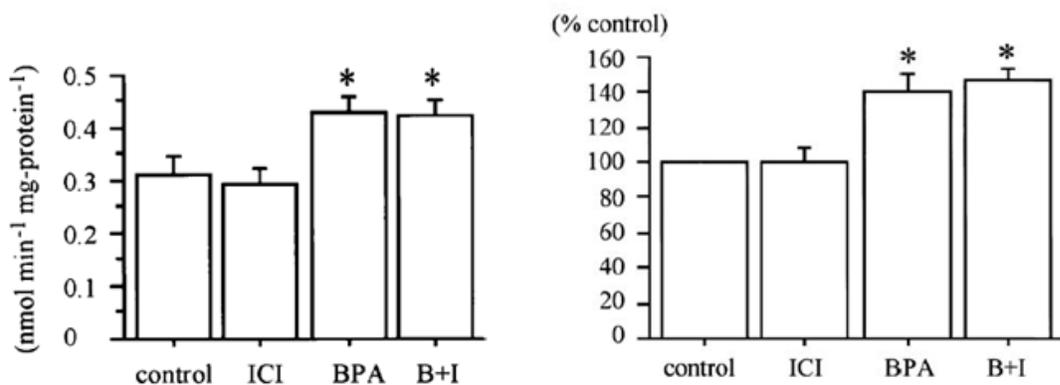
Figure 5. Effect of BPA and E2 on insulin content in cultured islet cells



From Alonso-Magdalena et al. (2008) an open access article.

In other cases, BPA seems to be causing effects that are unrelated to classic ER. Figure 6 presents data presented in Sakurai et al. (2004) showing that 100 μ M BPA can increase glucose uptake and Glut4 expression in 3T3-F442A adipocytes. These effects were not altered when the adipocytes were co-treated with 1 μ M ICI182,780 (1 μ M). However, it is unclear how physiologically relevant this response is given the high concentration of BPA used (100 μ M)

Figure 6. ICI182,780 (1 μ M) did not inhibit effects of 100 μ M BPA on glucose uptake or Glut4 expression in 3T3-F442A adipocytes



Glucose uptake: [³H]-2-deoxy-glucose uptake in 3T3-F442A adipocytes in the presence of 100 nM insulin

GLUT4: Glut4 expression in 3T3-F442A adipocytes

From Sakurai et al. (2004)

In addition to nuclear hormone receptor activity, BPA also impacts cellular physiology through rapid signaling mechanisms to modify the activities of various intracellular signaling networks [reviewed in Wetherill et al. (2007)]. The rapid signaling effects of BPA are independent of nuclear hormone receptor activity and occur within minutes or seconds of exposure, initiated by membrane associated or intracellular receptor systems. Concentration response analysis in different experimental models has found that BPA is able to stimulate rapid signaling effects at concentrations in the pM to nM range. Maximal rapid signaling effects for BPA and 17 β -estradiol are often observed at similar concentrations. The rapid signaling actions characterized by increased effects at lower concentrations, with decreased or loss of efficacy at higher concentrations that results in non-sigmoidal (inverted-U shaped) concentration response curves (reviewed in Wetherill et al. (2007)). It is considered likely that rapid estrogen-like signaling effects and “classical” nuclear hormone activity interact to coordinately impact physiological responses to estrogens. This type of rapid signaling effect was observed for the finding described above where 1nM BPA suppressed low glucose-induced intracellular calcium oscillations on cultured α -cells (Alonso-Magdalena *et al.* 2005). This action is characterized by rapid onset and involves a pertussis toxin sensitive G-protein, nitric oxide synthase, guanylatecyclase and cGMP-dependent protein kinase (PKG). A similar effect occurred following treatment with 1 nM DES. The effect of BPA and DES on Ca²⁺ oscillations was not blocked by ICI182,780 and could be reproduced by treatment with impermeable E2 conjugated with horseradish peroxidase, leading the authors’ to conclude that the effect was mediated through a non-classical membrane ER. In beta cells ICI independent effect was demonstrated on CREB phosphorylation and *in vivo* in plasma insulin in Quesada et al. (2002) and Alonso-Magdalena et al. (2006).

As discussed more fully in [Appendix C](#) (a separate document), an increasing number of *in vivo* and *in vitro* studies conducted over the past several years have reported health effects or

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implicated the involvement of cellular/physiological systems that extend beyond BPA's historical reputation as an environmental estrogen. It is not currently known the extent to which these non-nuclear estrogen receptor interactions account for some of the complexities encountered in the *in vivo* studies of BPA. However, they can complicate easy interpretation of findings when a response to BPA is compared to the response seen for the positive control estrogens, usually 17 β -estradiol (E2), estradiol benzoate (EB), diethylstilbestrol (DES), or ethinyl estradiol (E2). This is especially true in "low dose" studies where BPA does not cause an effect predicted based on the positive control estrogen or elicits a response with a greater potency than predicted based on relative binding affinities or transcriptional activation of ER α or ER β .

BPA has been reported to act as an anti-androgen with IC₅₀ values ranging from ~0.8 to 19.6 μ M based on studies that assess the ability of BPA to reduce the binding of 5 α -dihydrotestosterone (DHT) to the androgen receptor. The results vary depending on the model system and a study in HepG2 cells did not demonstrate any anti-androgenic activity [reviewed in Wetherill et al. (2007)]. The NTP recently conducted a reporter gene assay using a transient transfection of human full-length ER α or AR with a 3xERE-Luc or MMTV-Luc reporter, respectively into CV-1 cells. BPA was an androgen receptor antagonist with an IC₅₀ of 2.34 μ M, a concentration that was about 10-fold higher than the EC₅₀ for ER α agonistic activity of 0.272 μ M. In this study, the antagonistic effect of BPA for the androgen receptor occurred at a concentration that was about 140-times higher compared to hydroxyflutamide (IC₅₀ of 0.0171 μ M) and 7-times higher compared to the anti-androgen Casodex (IC₅₀ of 0.327 μ M) while the agonist effect on ER α occurred at a concentration that was ~2,000-times higher compared to estradiol (EC₅₀ of 0.000126 μ M) (personal communication with Dr. Tina Teng, September 9, 2010). These concentrations are lower than the activity of BPA for these receptors reported in Tox21 assays that use partial androgen and ER α receptors, where active concentrations, or AC₅₀ values, were reported of 17.9 to 73 μ M for androgen receptor⁴ and 0.64 to 1.723 μ M for ER α .⁵ Once bound to androgen receptor, the receptor-BPA complex may alter the ability of endogenous androgens to regulate androgen-dependent transcription [reviewed in Wetherill et al. (2007)].

There are also reports that BPA can interact with non-classic estrogen receptor systems at similar or lower concentrations than interactions with ER α and ER β . BPA has a high binding affinity to estrogen-related receptor- γ (ERR γ), an orphan receptor that shares a sequence homology with ER α and ER β but is not activated by estradiol (Coward *et al.* 2001; Greschik *et al.* 2004; Okada *et al.* 2008; Takayanagi *et al.* 2006). Takayanagi *et al.* (2006) reported that the IC₅₀ of BPA for ERR γ (13.1 nM) was ~80 to 100-times lower than the IC₅₀ values of BPA for ER α (IC₅₀ = 1040 nM) or ER β (IC₅₀ = 1320 nM).⁶ Also, BPA had a stronger binding affinity for ERR γ

⁴ Based on these ToxCast™ assays: NCGC_AR_Antagonist and NVS_NR_hAR

⁵ Based on these ToxCast™ assays: ATG_ERE_CIS; NVS_NR_hER; ATG_ERa_Trans; and NCGE_ERalpha_Agonist. The NCGC also includes an assay for ER α antagonism, NCGC_ERalpha_Antagonist, and BPA had a AC₅₀ value of 17 μ M in that assay.

⁶ An Attagene reporter gene assay for ERR γ in HepG2 cells is included in ToxCast and BPA was not considered active in this assay. However, the Attagene assay appears to have relatively low sensitivity, potentially due to low cellular expression (personal communication with Dr. Keith Houck, EPA, September 15, 2010).

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compared to DES ($IC_{50} = 13.1$ nM versus 54.3 nM) but in contrast to DES or 4-hydroxy tamoxifen did not impact ERR γ 's high basal transcriptional activity in report assays (Greschik *et al.* 2004; Takayanagi *et al.* 2006). ERRs can bind to both functional estrogen response elements (EREs) and ERR-response elements (ERREs), providing a mechanism through which ERR γ and ER α systems may cross-talk to mediate BPA's activities. In this model, a BPA-ERR γ complex may bind DNA together with E2-ER α via an ERE to create heterodimers that could interfere or cooperate to regulate overall estrogenic response in a particular cell type (Takayanagi *et al.* 2006). ERR γ can be found in the heart, brain, placenta, pancreas, kidney, prostate, brown adipose, breast and visceral adipose tissue, and muscle (Hugo *et al.* 2008; Kubo *et al.* 2009; Takayanagi *et al.* 2006; Takeda *et al.* 2009) and is implicated in regulating mitochondrial functions such as mitochondrial biogenesis, oxidative phosphorylation, adipocyte differentiation, and β -oxidation of fatty acids (Kubo *et al.* 2009) and behavior in zebrafish (Kubo *et al.* 2009; Schletz Sali 2010) reported that ERR γ has a promoting role in regulating adipocyte differentiation. Gene knockdown by ERR γ -specific siRNA resulted in a decreased of mRNA levels for adipogenic marker genes including fatty acid binding protein 4, PPAR γ , and PGC-1 β in a preadipocyte cell line 3T3-L1 preadipocytes and mesenchymal ST2 and C3H10T1/2 cells in the adipogenesis medium. In contrast, stable expression of ERR γ in 3T3-L1 cells resulted in lipid accumulation during 3T3-L1 differentiation and up-regulation of the adipogenic marker genes under adipogenic condition.

BPA was reported to bind to G-protein-coupled receptor referred to as GPER (formerly GPR30) with an IC_{50} of 0.630 μ M, a binding affinity that was 2-3% of the IC_{50} value for estradiol (0.0178 μ M) (Thomas and Dong 2006). DES did not bind to this receptor at concentrations up to 10 μ M and ER α antagonist ICI182,780 and tamoxifen acted as agonist for GPER. While there is debate on whether GPER should be considered an estrogen receptor, it appears to contribute to some of the early actions of estrogen including rapid non-genomic signaling events that take place on the membrane (Olde and Leeb-Lundberg 2009). Understanding the physiological role of GPER is an area of active research and early studies suggest it may play a role in pancreatic islet cell function and glycemic control, bone growth, and immune and cardiovascular function (Balhuizen *et al.* 2010; Liu *et al.* 2009; Maggiolini and Picard 2010; Martensson *et al.* 2009; Mizukami 2010; Nadal *et al.* 2009; Olde and Leeb-Lundberg 2009; Prossnitz and Barton 2009).

BPA has recently been described as an agonist for the glucocorticoid receptor (Prasanth *et al.* 2010; Sargis *et al.* 2010). Sargis *et al.* (2010) reported that 1 μ M of BPA (the only concentration tested) could increase luciferase expression in 3T3-L1 preadipocytes that were transfected with a glucocorticoid response element. Prasanth *et al.* (2010) used *in silico* molecular modeling and docking techniques to evaluate BPA's ability to bind to GR. BPA could be docked to the receptor and the nature of the interaction was described as similar to dexamethasone and cortisol and very different from the known GR antagonist RU-486. In ToxCast[™], the AC_{50} value for BPA binding to the glucocorticoid receptor (gene symbol NR3C1) was 10.5 μ M.

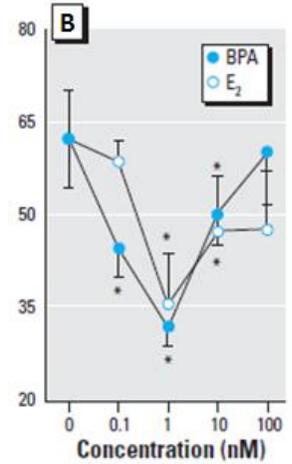
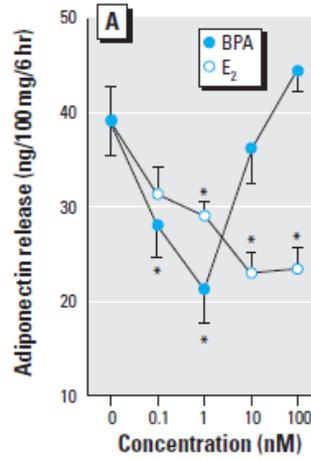
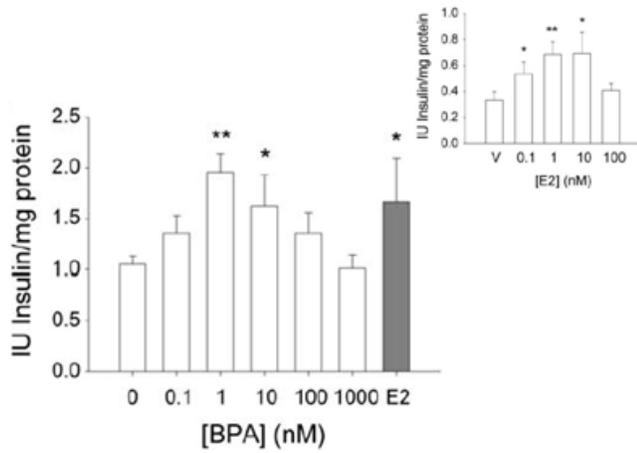
1.3.2 Non-monotonic dose responses.

The *in vitro* literature on BPA contains some examples of non-monotonic dose response (Figure 7). Panel A shows the finding discussed above where BPA increased the insulin content of cultured mouse islet cells at 1 or 10 nM, but had no effect at lower or higher concentrations (Alonso-Magdalena *et al.* 2008). Panel B shows data from Hugo *et al.*, 2008 (2008) on adiponectin secretion from adipose specimens collected from people undergoing surgery for breast reduction (breast adipose), abdominoplasty (subcutaneous abdominal adipose), or gastric bypass surgery (visceral adipose). Adiponectin is an adipocyte-specific hormone that increases insulin sensitivity and reduces tissue inflammation. Incubation with 0.1 to 1 nM BPA suppressed adiponectin release from breast and abdominal subcutaneous adipose explants, but the effect was either less pronounced or not observed at higher concentrations (Hugo *et al.* 2008). Similar non-monotonic dose response curves have been reported in other BPA studies, mostly *in vitro*, focusing on a range of endpoints, including neurological or reproductive system related [reviewed in (vom Saal *et al.* 2007)]. Collectively, when considered across the entire body of BPA literature, it is becoming more accepted that the non-monotonic dose response curves are “real” findings. Nevertheless, they remain difficult to interpret as they are generally reported in *in vitro* studies.⁷ It is also not clear to what extent the findings are generalizable for a given cell or tissue type. For example, Adachi *et al.*, 2005 did not observe a non-monotonic dose-response for insulin secretion in rat pancreatic islets treated with BPA concentrations of 0.1 – 100 µg/L (0.438 – 438 nM) for 24 hours. In this study, insulin secretion in the presence of 16 mM glucose was significantly increased only at the 2 highest concentrations of 10 and 100 µg/L (43.8 – 438 nM), BPA concentrations that are higher than those reported to cause non-monotonic increases in the insulin content of mouse islet cells (1 and 10 nM with 8mM glucose, Alonso-Magdalena *et al.*, 2008)⁸ or suppression of adiponectin in explants of human adipose tissue (0.1 – 10 nM, Hugo *et al.*, 2008) (Figure 7).

⁷ In 2008 the NTP conducted an evaluation of the potential reproductive and developmental effects of BPA that included consideration of a large body of laboratory animal studies. This literature did not lend itself to reaching conclusions on the frequency of non-monotonic dose response curves from *in vivo* studies because most of the studies reporting effects only tested 1 or 2 dose levels and those that tested more dose levels often did not report an effect.

⁸ The finding by Alonso-Magdalena *et al.* (2008) was reported in the presence of 8 mM glucose which may explain the differences in dose response. 8 mM is a glucose level considered relevant to *ad libitum* fed mice and 16 mM is considered to be relatively high, more similar to glucose load after eating a large meal or in diabetics.

Figure 7. Examples of non-monotonic dose-response relationships



A. Insulin content of cultured islets treated with BPA or E2 for 48 hours (n = 4-7 mice). Modified from Figures 1 and 3 of Alonso-Magdalena et al., 2008 (Alonso-Magdalena *et al.* 2008) an open access article.

B. Suppression of adiponectin release from breast (A) and abdominal subcutaneous adipose explants (B) by E2 and low concentrations of BPA Modified from Figures 1 and 2 in Hugo et al., 2008 (Hugo *et al.* 2008) an open access article.

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1.4 Appendix Tables

Appendix Table A. Body weight in studies reporting developmental effects at “low” dose of BPA ($\leq 5,000 \mu\text{g}/\text{kg bw}/\text{day}$)

Reference	Animal Model	Route of Administration	Treatment period	Diet	Dose ($\mu\text{g}/\text{kg bw}/\text{d}$)	Sex	up to weaning	adult
Gestation only treatment								
Alonso-Magdalena et al. (2010)*	OF-1 mice (n=8-11 litters/group)	Subcutaneous injection	Dams: GD9–16	Chow without alfalfa or soybean meal (Harlan Laboratories)	10	male	[male and female pups were tabulated together until weaning] \uparrow PND 1 (1.03-fold) \uparrow PND 22 (1.07-fold)	\leftrightarrow
						female		\downarrow at 11-25 weeks of age (~97% of control)
					100	male	[male and female pups were tabulated together until weaning] \downarrow PND1 (95% of control)	\leftrightarrow
						female		\downarrow at 11-25 weeks of age (~97% of control)
Betancourt et al.(2010)* (positive control, DES)	Sprague-Dawley rats (n=29-33 litters/group)	Oral gavage	Dams: GD10-21	Phytoestrogen-free AIN-93G	25	female	\leftrightarrow	\leftrightarrow
					250	female	\leftrightarrow	\leftrightarrow
Durando et al. (2007)*	Wistar Rats (n=11-14 dams/group)	Osmotic pump (0.25 $\mu\text{L}/\text{hour}$)	Dams: GD7–22 ^a	Standard laboratory chow (Cooperación)	~0.25	female	\leftrightarrow	\leftrightarrow
Honma et al. (2002)* (positive control, DES)	ICR/Jc1 mice (n=10 mated females/group)	Subcutaneous injection	Dams: GD11-17	Commercial diet (CE-2, CLEA)	2	male	\leftrightarrow	\leftrightarrow
						female	\downarrow PND23 ^a (~90% of control value)	\leftrightarrow
					20	male	\downarrow PND1 ^a (~95% of control value)	\downarrow PND61 ^a (~94% of control value)
						female	\downarrow PND23 ^a (~93% of control value)	\downarrow PND61 ^a (~96% of control value)
Howdeshell et al. (1999)*	CF-1 mice (n=21 dams/group)	Oral, micropipette	Dams: GD11-17	[not reported]	2.4	male ^b	\uparrow PND22 (~1.1-fold)	NA
						female	\uparrow PND22 (~1.1-fold)	NA
Kubo et al. (2003)	Wistar rats	Oral, drinking	Dams: "During	[not reported]	~30	male	\leftrightarrow	\leftrightarrow

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Appendix Table A. Body weight in studies reporting developmental effects at “low” dose of BPA ($\leq 5,000 \mu\text{g}/\text{kg bw}/\text{day}$)

Reference	Animal Model	Route of Administration	Treatment period	Diet	Dose ($\mu\text{g}/\text{kg bw}/\text{d}$)	Sex	up to weaning	adult
positive control, DES and resveratrol)	(n=6 mated females/group)	water	the fetal and suckling periods"		~300	female	↔	↔
						male	↔	↔
						female	↔	↑ at vaginal opening during week 6 (1.1-fold)
Laviola et al.(2005)**	CD-1 mice (n=10-12 mated females/group)	Oral	Dams: GD11-18	[not reported]	10	male	↔	↔
						female	↔	↔
Nagel et al. (1997)*	CF-1 mice (n=7 adult males/group)	Oral, micropipette	Dams: GD11-17	Purina Laboratory Chow #5001	2	male	NA	↓ at ~6months of age (~91% of control value)
					20	male	NA	↔
Nakagami et al. (2009)	Cynomolgus monkeys (n=18-19 pregnant females/group)	Subcutaneous osmotic pumps	Dams: GD20-160	"food pellets" (Harlan)	10	male	↔	NA
						female	↔	NA
Nikaido et al. (2004)* (positive control, DES)	Crj:CD-1 (ICR) mice [group size not reported]	Subcutaneous injection	Dams: GD15-18	Low phytoestrogen (NIH-07 PND)	500	female	↔	↑ at 16 weeks (~1.5 fold) (n=6)
					10,000	female	↔	↑ at 16 weeks (~1.2 fold) (n=6)
Palanza et al.(2002)*	CD-1 mice (F1: n=15/sex/group)	Oral – trained to drink from a micropipette	F0: GD14-18 and/or F1: GD14-18	Purina 5008 (soy-based) breeder chow OR Purina 5001 (soy-based) Chow	10 (prenatal exposure only)	male	↔	NA
						female	↔	NA
					10 (adult exposure only)	male	↔	NA
						female	↔	NA
					10 (prenatal and adult exposure)	male	↔	NA
	female	↔	NA					
Takai et al. (2001)	B6C3F1 mice EMBRYOS (n=29-34 embryos/group)	Cultured embryos	From the 2-cell stage to the blastocyst stage (48 hours)	[not reported]	0.001 (1 ng/mL)	male and female	↑ PND 21 (1.4-fold)	NA
					100 $\mu\text{g}/\text{mL}$	male and female	↑ PND 21 (1.3-fold)	NA
Talsness et al.(2000)* (positive control,	Sprague-Dawley Rat (n=20 or 18 dams/group)	Oral gavage	Dams: GD6-21	[not reported]	100	male	↔	NA
						female	↔	↓ at ~4 months of age (~85% of control value)

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Appendix Table A. Body weight in studies reporting developmental effects at “low” dose of BPA ($\leq 5,000 \mu\text{g}/\text{kg bw}/\text{day}$)

Reference	Animal Model	Route of Administration	Treatment period	Diet	Dose ($\mu\text{g}/\text{kg bw}/\text{d}$)	Sex	up to weaning	adult
EE)					50,000	male	↔	NA
						female	↔	↔
Gestation and lactation treatment								
Akingbemi et al. (2004)**	Long Evans rat (7 dams/group)	Oral gavage (dam)	Dams: GD12–PND21	Purina rodent chow	2.4	male	↔	↑ PND90 (~1.1-fold)
	Long Evans rat (20-24 pups/group)	Oral gavage	Pups: PND21–PND90	Purina rodent chow	2.4	male	NA	↔
Gioiosa et al.(2007)*	CD-1 mice (n=14/sex/group)	Oral – trained to drink from a syringe	Dams: GD11-PND8	Standard mouse chow (4RF21)	10	male	NA	NA
						female	NA	NA
Kobayashi et al.(2010)*	C57BL/6J Mice (n=20/generation/group)	Oral, diet (0.33, 3.3, or 33 ppm)	Dams: GD6 for the F0 generation through termination of the F2 generation	Standard laboratory chow (CE-2)	~50	male	↔	↔
						female	↔	↔
					~500	male	↔	↔
						female	↔	↔
					~5,000	male	↔	↔
						female	↔	↔
Martini et al.(2010)*	CD-1 mice (n=6 pups/group)	Oral – trained to drink from a syringe	Dams: GD11-PND8	Standard mouse chow (4RF21)	10	male	"A tendency to a significant effect of exposure was found for litters' body weight at birth. However, no differences were found for growth rates and body weight at weaning" [data not shown]	↔
						female		↔
					20	male		↔
						female		↔
					40	male		↔
						female		↔
Midoro-Horiuti et al.(2010)*	BALB/c mice (n=12-16 pups from 6-7 litters/group)	Oral, drinking water (10 $\mu\text{g}/\text{mL}$)	Dams: "one week before mating through pregnancy and lactation"	Casein-based diet (Research Diet)	~2,000	male	↔	NA
						female	↔	NA

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Appendix Table A. Body weight in studies reporting developmental effects at “low” dose of BPA ($\leq 5,000 \mu\text{g/kg bw/day}$)

Reference	Animal Model	Route of Administration	Treatment period	Diet	Dose ($\mu\text{g/kg bw/d}$)	Sex	up to weaning	adult
Miyawaki et al. (2007)*	ICR mice (3 dams/group)	Oral, drinking water (1 mg/L or 10 mg/L)	Dams: GD10–PND31	30% fat diet	~260	male	NA	↔
						female	NA	↑ PND31 (~1.1-fold)
					~2,720	male	NA	↑ PND31 (~1.2-fold)
						female	NA	↑ PND31 (~1.1-fold)
Murray et al. (2007)*	Wistar-Furth rats [group size not reported]	Osmotic pump	Dams: GD8 ^a -PND1	Harlan Teklad 2018	2.5	male	↔	↔
						female	↔	↔
					25	male	↔	↔
						female	↔	↔
					250	male	↔	↔
						female	↔	↔
					1,000	male	↔	↔
						female	↔	↔
Negishi et al. (2003)*	F344 rats (n=8-9 pregnant females/group)	Oral	Dams: GD10 – PND20	[not reported]	4,000	male	↔	↔
						female	↓ PND7 (96% of control) and PND28 (97% of control)	↔
					40,000	male	↓ PND7 and PND28 (96% of control)	↔
						female	↓ PND7 (95% of control) and PND28 (96% of control)	↔
					400,000	male	↓ PND7 (91% of control), PND 21 (90% of control), and PND 28 (93% of control)	↓ PND56 (94% of control)
						female	↓ PND7 (93% of control), PND 21 (92% of control), and PND 28 (96% of control)	↔
Negishi et al. (2004)*	F344/N rats (n=10-11 mated females/group)	Oral gavage	Dams: GD3–PND21	[not reported]	100	male	↔	↔

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Appendix Table A. Body weight in studies reporting developmental effects at “low” dose of BPA ($\leq 5,000 \mu\text{g}/\text{kg bw}/\text{day}$)

Reference	Animal Model	Route of Administration	Treatment period	Diet	Dose ($\mu\text{g}/\text{kg bw}/\text{d}$)	Sex	up to weaning	adult
Okada et al. (2008)* (positive control, E ₂)	ICR mice (n=5-7 litters/group)	Type B Tubes implanted subcutaneously (1.2 or 60 $\mu\text{g}/\text{day}$)	Dams: 3 days before being paired for mating through weaning	LABO MT STOCK	100	male	↑ at weaning (~1.1-fold)	NA
					5,000	male	↔	NA
Poimenova et al.(2010)*	Wistar rats (n=5 mated females/group)	Oral – each daily dose was pipetted onto one corn flake	Dams: "During the entire period of pregnancy and lactation"	Normal chow	40	male	NA	NA
						female	NA	NA
Rubin et al. (2001)*	Sprague-Dawley rat (6 mated females/group)	Oral, drinking water (1 mg/L or 10 mg/L)	Dams: GD6–PND21	Purina rodent chow	~100	male	↑PND4-11 (~1.2-fold)	↔
						female	↑PND4-11 (~1.2-fold)	↑PND22-110 (~1.1-fold)
					~1200	male	↑PND4-11 (~1.1-fold)	↔
						female	↑PND4-11 (~1.1-fold)	↔
Ryan et al. (2006)* (positive control, EE)	C57/B1-6 mice [group size not reported]	Oral gavage	Dams: GD2 ^a – PND21	Purina Rodent Chow #5001	2	female	↔	NA
					200	female	↔	NA
Ryan et al. (2010)** (positive control, DES)	CD-1 mice (n~30 litters/group)	Diet (1ppb)	Dams: GD0–PND21	AIN93G until weaning; low butter-fat diet after weaning	~0.25	male	↑ at weaning (~1.1-fold)	↔
						female	↑ at weaning (~1.1-fold)	↔
Salian et al. (2009) (positive control, DES)	Holtzman rats (n=8 pregnant F0 females/group; 24 male pups/generation/group)	Oral gavage	Dams: GD12-PND21 (only F0 females were dosed)	Soy-free, in-house prepared rat pellets	1.2	F1 male	NA	↑ (~1.1-fold)
						F2 male	NA	↑ (~1.3-fold)
						F3 male	NA	↑ (~1.2-fold)
					2.4	F1 male	NA	↔
						F2 male	NA	↑ (~1.3-fold)
						F3 male	NA	↑ (~1.2-fold)
Signorile et al.(2010)**	Balb-C mice (6 dams/group)	Subcutaneous injection	Dams: GD1-PND7	Mouse chow	100	female	NA	NA
					1,000	female	NA	NA
Somm et al. (2009)	Sprague-Dawley rat (n=6 litters/group)	Oral, drinking water	Dams: GD6–PND21	Gestation: Standard diet, low in	~70	male, standard diet	↑ PND1 (~1.3-fold)	↔

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Appendix Table A. Body weight in studies reporting developmental effects at “low” dose of BPA ($\leq 5,000 \mu\text{g}/\text{kg bw}/\text{day}$)

Reference	Animal Model	Route of Administration	Treatment period	Diet	Dose ($\mu\text{g}/\text{kg bw}/\text{d}$)	Sex	up to weaning	adult
		(1 mg/L)		phytoestrogens After weaning: ½ switched to a high-fat diet.		female, standard diet	↑ PND1 (~1.1-fold) ↑ PND21 (~1.1-fold)	↑ Weeks 4-6, 8, 9, 13 (~1.1-fold)
						male, high fat diet	NA	↑ Weeks 9-14 (~1.1-fold)
						female, high fat diet	NA	↑ Weeks 4, 5, 8-11 (~1.1-fold)
Tian et al.(2010)*	ICR mice (n=6 litters/group)	Oral [exact route not stated]	Dams: GD7-PND21 Pups: PND22-36	[not reported]	100	male	↔	↔
						female	↔	↔
					500	male	↔	↔
						female	↔	↔
Xu et al.(2010)*	ICR mice (n=10 dams/group)	Oral, gavage	Dams: GD7-PND21	Soy-free diet	50	male	↓ (~91% of control)	↓ (~93% of control)
						female	NA	NA
					500	male	↔	↓ (~93% of control)
						female	NA	NA
					5,000	male	↔	↔
						female	NA	NA
					50,000	male	↑ (~1.2-fold)	↔
						female	NA	NA
Zhou et al. (2009)	Sprague-Dawley rats (n=6 litters total)	Subcutaneous injection	Dams: GD8-PND21	[not reported]	20	male	NA	NA
Lactation only treatment								
Bosquiazzo et al.(2010)**	Wistar rats (n=8 pups/group)	Subcutaneous injection	Pups: PND 2, 4, 6, 8	Laboratory Chow (Nutrición Animal)	50	female	↔	↔
					20,000	female	↔	↔
Ceccarelli et al.(2007)* (positive control, EE)	Sprague-Dawley rats (84 male pups and 84 female pups divided into 3 dose groups)	Oral, micropipette	Pups: PND23-30	[not reported]	40	male	NA	NA
						female	NA	NA
Della Seta et al.(2006)* (positive control, EE)	Sprague-Dawley rats [group size not reported]	Oral, micropipette	Pups: PND23-30	[not reported]	40	male	NA	↔

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Appendix Table A. Body weight in studies reporting developmental effects at “low” dose of BPA ($\leq 5,000 \mu\text{g/kg bw/day}$)

Reference	Animal Model	Route of Administration	Treatment period	Diet	Dose ($\mu\text{g/kg bw/d}$)	Sex	up to weaning	adult
Fernandez et al. (2009)**	Sprague-Dawley rats (n=11-15 pups/group)	Subcutaneous injection (50 or 500 $\mu\text{g}/\mu\text{L}$) [dose volume not stated]	Dams: PND1-10	Commercial laboratory chow	2,500 – 6,200	female	NA	\leftrightarrow
					25,000-62,500	female	NA	\leftrightarrow
Ho et al.(2006)** (positive control, EB)	Sprague-Dawley rats (n=20-30 pups/group)	Subcutaneous injection	Pups: PND1 ^a , 3 ^a , 5 ^a	Soy-free phytoestrogen reduced diet (Ziegler Reduced Rodent Diet 2)	10	male	NA	NA
Jenkins et al. (2009)**	Sprague-Dawley rats [groups size not reported]	Oral gavage	Dams: PND2-20 (only dosed Monday-Friday; 15 doses)	Phytoestrogen-free AIN-93G	25	female	NA	\leftrightarrow
					250	female	NA	\leftrightarrow
Monje et al. (2009)**	Wistar rats (10 pups/group)	Subcutaneous injection	Pups: PND2 ^a -8 ^a	Laboratory chow	50	female	\leftrightarrow	NA
					20,000	female	\leftrightarrow	NA
Newbold et al. (2007)**	CD-1 (ICR)BR mice (24 pups/group)	Subcutaneous injection	Pups: PND1–5	NIH 31 mouse chow	10	female	NA	\leftrightarrow at 18 months
					100	female	NA	\leftrightarrow at 18 months
					1,000	female	NA	\leftrightarrow at 18 months
Patisaul & Bateman (2008) (positive controls, EB, DPN, and PPT)	Long Evans rat (n=6-8pups/group)	Subcutaneous injection	Pups: PND1 ^a –4 ^a	Phytoestrogen free diets: (AIN-93G from conception through weaning; Purina 5K96 after weaning)	50	male	NA	\uparrow PND69 ^a (~1.1-fold)

\uparrow = Statistically significant increase

\downarrow = Statistically significant decrease

\leftrightarrow = no statistically significant difference

NA= not applicable, not assessed, or not reported

^a Adjusted to GD0 as the day mating was detected, or PND1 as the day of parturition.

^bHowdeshell et al. (1999) The effect on body weight was greatest in pups adjacent in utero to 2 females (1.22-fold) compared to 1 female (1.09-fold) or no females (no effect).

* No apparent control for litter effects

** Strategy to control for litter effects:

- Akingbemi et al.(2004) = male pups were pooled within their treatment group, randomly assigned to a dam and then cross-fostered
- Bosquiazzo et al.(2010) = female pups were pooled within their treatment group and then cross-fostered so that siblings were not in the same litter

Bisphenol A – Appendix Tables

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Appendix Table A. Body weight in studies reporting developmental effects at “low” dose of BPA ($\leq 5,000 \mu\text{g}/\text{kg bw}/\text{day}$)

Reference	Animal Model	Route of Administration	Treatment period	Diet	Dose ($\mu\text{g}/\text{kg bw}/\text{d}$)	Sex	up to weaning	adult
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- Fernandez (2009) = Siblings were assigned to different dose groups (but remained with their dam)
- Ho et al.(2006) = pups within each litter were randomly assigned to treatment groups
- Jenkins (2009) = One offspring from each litter was used in each experiment
- Laviola et al.(2005) = litter was the 'block variable' for the *split-plot* analysis
- Monje (2009) = Pups were cross-fostered so that siblings were not in the same litter
- Newbold et al.(2007) = female pups were pooled within their treatment group, randomly assigned to a dam and then cross-fostered
- Ryan et al.(2010) = excluded litters with less than 8 pups or more than 15 pups; only 1 male and 1 female from each litter included in the study; included litter size as a covariate in the statistical analysis
- Signorile et al.(2010) = all pups were pooled within their treatment group and then cross-fostered so that siblings were not in the same litter

Bisphenol A – Appendix Tables

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Appendix Table C. Summary of BPA *in vitro* studies

Reference	Effect	Model	Endpoint	LOEC (µM)	Doses (µM) *p≤0.05)
(Adachi <i>et al.</i> 2005)	insulin secretion	primary rat pancreatic islet cells	↑ glucose-induced insulin secretion from isolated rat pancreatic islet cells incubated with BPA for 24-hours. Effect of 24-hour incubation was eliminated by co-treatment with Act-D (an inhibitor of RNA synthesis) and ICI182,780 (ER antagonist); no effect with the 1 hour (acute) incubation time.	0.04	0, 0.0004, 0.004, 0.04* and 0.44*
(Alonso-Magdalena <i>et al.</i> 2005)	Ca ²⁺ signaling	primary mouse pancreatic α cells	↓ glucose-induced intracellular Ca ²⁺ oscillations in pancreatic α-cells incubated with 1 nM BPA or 1 nM DES. The effect of BPA and DES on Ca ²⁺ oscillations was not blocked by ICI182,780 and could be reproduced by treatment with impermeable E2 conjugated with horseradish peroxidase, leading the authors' to conclude that the effect was mediated through a non-classical membrane ER	0.001	0.001*
(Alonso-Magdalena <i>et al.</i> 2008)	insulin content	primary mouse pancreatic islet cells	↑ insulin content of islets exposed to 1 or 10 nM BPA for 48 hours [non-monotonic dose-response]. A similar effect was observed <i>in vivo</i> . A main focus of the publication was to assess the role of ERs in regulating pancreatic insulin content and the authors conducted a number of <i>in vitro</i> experiments with ICI182,780 and ERα and ERβ agonists (PPT or and DPN) and islets collected from ERαKO/ERβKO mice. Overall, the pattern of response observed led the authors to conclude that ERα is involved in mediating response and that this activation involves ERK1/2.	0.001	0, 0.0001, 0.001*, 0.01*, 0.1, and 1
(Ben-Jonathan <i>et al.</i> 2009)	suppression of adiponectin release	human adipose tissue extract	↓ adiponectin release from	0.0001	0, 0.0001*, 0.001*, 0.01*, and 0.1*
			↑ IL-6 release and ↑ TNF-α release at 10 nM BPA [Similar pattern of response was observed for E2 and ICI182.780.]	0.01	0.01
(Hugo <i>et al.</i> 2008)	suppression of adiponectin release	human adipose tissue explants	↓ adiponectin release from breast adipose and abdominal subcutaneous adipose explants [non-monotonic dose response].	0.0001	0, 0.0001*, 0.001*, 0.01, and 0.1
(Kidani <i>et al.</i> 2010)	adiponectin DNA and protein production	3T3-L1 mouse fibroblast cell line	↓ intracellular adiponectin	40	20, 40*, 80*
			↓ adiponectin secretion into medium, ↓ protein expression of Akt, p-Akt, and adiponectin	80	80*
(Lee <i>et al.</i> 2008)	gene expression of adiponectin, leptin, and resistin	mouse 3T3-L1 fibroblast cell line	↓ adiponectin and ↑ leptin mRNA expression after 24 hours *no effect on resistin expression	10	0.1, 10*
(Masuno <i>et al.</i> 2002)	adipocyte differentiation	mouse 3T3-L1 fibroblast cell line	↑ LPL activity following 2-day incubation with BPA prior to 9-day treatment with insulin (5 µl/mL) alone	8.76	0, 8.76*, 4.38*, 87.6*

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Appendix Table C. Summary of BPA *in vitro* studies

Reference	Effect	Model	Endpoint	LOEC (μM)	Doses (μM) *p<0.05
			accelerated differentiation of 3T3-L1 fibroblast cells into adipocytes following 2-day incubation with BPA prior to 9-day treatment with insulin (5 μl/mL) alone, conclusion based on ↑TG content (150%), ↑LPL activity (60%), and ↑GPDH activity (500%); Similar pattern was observed with a 2-day incubation with BPA prior to 9-day co-treatment with BPA + insulin (5 μl/mL): ↑TG content (370%), ↑LPL activity (200%), and ↑GPDH activity (225%)	87.6	87.6*
(Masuno <i>et al.</i> 2005)	adipocyte differentiation	mouse 3T3-L1 fibroblast cell line	accelerate differentiation of 3T3-L1 fibroblast cells into adipocytes, conclusion based on ↑ triacylglycerol content of cultures, ↑ percentage of oil red O-staining cells; ↑ levels of LPL and aP2 mRNAs. *study also looked at the effects 8 BPA-related chemicals	20 80	0, 4, 20*, 40*, 80* 80*
(Phrakonkham <i>et al.</i> 2008)	adipocyte differentiation	mouse 3T3-L1 fibroblast cell line	altered mRNA expression of differentiation genes (Pref-1, C/EBPβ, PPARγ2, FAS) or ERβ in 3T3-L1 cells induced to differentiate into adipocytes in the presence of 80 μM BPA on day 3 or 8 post-induction [effects generally appeared 8 days post-induction]; ↑ leptin mRNA on day 8 post-induction (1.9-fold). *no effect on DNA content, intracellular TG levels, proportion of Oil Red O stained cells, concentration of leptin in culture media, or ERα mRNA expression	80	80*
(Sargis <i>et al.</i> 2010)	adipocyte differentiation	mouse 3T3-L1 fibroblast cell line	↑ luciferase expression in 3T3-L1 pre-adipocytes transfected with a glucocorticoid response element (~3-fold compared to vehicle control); ↑ lipid accumulation of ~1.7-fold of amount in cells treated with a low concentration of “differentiation cocktail” (no effect in cells not treated with the cocktail); some suggestion of ↑ in adipocyte protein expression (based on immunoblotting) *no effect on luciferase expression in cells transfected with PPARγ response element and a second plasmid expressing PPARγ	1 μM	1 μM*
(Sakurai <i>et al.</i> 2004)	glucose uptake	mouse 3T3-F442A adipocyte cell line	↑ basal glucose uptake (no insulin-stimulation) ↑ insulin-stimulated glucose uptake (100 nM insulin) ↑ GLUT4 protein uptake (effect not blocked by co-treatment with 10 ⁻⁶ M ICI182,780)	100 1 1	0, 0.001, 0.01, 0.1, 1, 100* 0, 0.001, 0.01, 0.1, 1*, 100* 0, 0.001, 0.01, 0.1, 1*, 100*

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Appendix Table C. Summary of BPA *in vitro* studies

Reference	Effect	Model	Endpoint	LOEC (μM)	Doses (μM) *p<0.05)
(Wada <i>et al.</i> 2007)	adipocyte lipid accumulation	mouse 3T3-L1 fibroblast cell line	↑ accumulation of triacylglycerol (visualized by Oil Red O stain) in mature adipocytes differentiated from 3T3-L1 pre-adipocytes. Author's note that BPA caused time- and dose-dependent increases in glycerol accumulation in HuH-7 cells, a human hepatocellular carcinoma cell line (data not shown) and that BPA up-regulated expression of genes involved in lipid metabolism, adipocyte differentiation, and inflammation (data not shown)	10	10*
Wang <i>et al.</i> (2010)	adipocyte differentiation	mouse 3T3-L1 fibroblast cell line	↑ adipocyte differentiation	100	100
	PPAR activity	Huh7-PPRE-Luc cells	↓ PPRE-luciferase activation	10	0.1, 1, 10*, 100*

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