

TEDX

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**In-Vitro Outcome Analyses
for the Low Dose Bisphenol A Spreadsheet
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The following analyses examine the data in the Low Dose Bisphenol A Spreadsheet. They are meant to compliment and more specifically enumerate the results of the data compilation included in the summary and charts. For a broader view of the data, please refer to the summary and charts.

In Vitro (n=148)

MALE REPRODUCTIVE SYSTEM OUTCOMES						FEMALE REPRODUCTIVE SYSTEM OUTCOMES					
	testes	sperm	prostate	testosterone	Leydig	ovaries	vagina	uterus	breast	♀ organs	eggs/ oocytes
Change	1	0	5	0	4	8	1	5	28	1	2
No change	1	1	0	2	1	0	0	1	0	0	3
Total	2	1	5	2	5	8	1	6	28	1	5
% Change	50%	0%	100%	0%	80%	100%	100%	83%	100%	100%	40%

Testes:

- induces expression of orphan nuclear receptor Nur77
- induces steroidogenic enzyme expression
- increases progesterone biosynthesis by Nur77

Sperm

- no change

Prostate:

- nuclear translocation of the tumor derived receptor (AR-T877A),
- increased levels of prostate-specific antigen expression
- alters 5alpha-dihydrotestosterone binding to AR-T877A
- induced S-phase progression in prostate cancer cell line LNCaP
- severe atypia with nuclear elongation and irregular size, cellular piling and adenoma formation
- changes to epithelium, increased squamous metaplasia
- induced permanent CK10 expression in basal cells of prostate

Testosterone:

- no change

Leydig:

- decreased cAMP and progesterone production in Leydig tumor cells
- upregulated AR
- induces Nur77 gene expression in a dose dependent manner and stimulates progesterone synthesis
- decreased steroidogenic enzyme gene expression

Ovaries:

- decreased viability of granulosa cells, induced apoptosis
- increased basal progesterone production
- amplified FSH-stimulated progesterone production
- decrease in FSH-induced estradiol production
- increased testosterone synthesis and mRNA expression of 17-alpha hydroxylase (P450c17), cholesterol side chain cleavage enzyme (P450scc) and steroidogenic acute regulatory protein (StAR)
- increase in progesterone levels and P450scc mRNA expression
- inhibited estradiol levels and the expression of P450arom mRNA
- increased proliferation of BG-1 ovarian cancer cells
- increased progesterone production in theca-interstitial ovarian cells, but not granulosa cells
- concentration-dependently increased MMP-9 output by GLCs
- higher concentrations (>100 ng) were cytotoxic
- increased proliferation of BG-1 cancer cells
- increased activation of ERK1/2 via a non-genomic pathway
- increased testosterone production by ovarian T-1 cells
- increased mRNA expression of P450c17 and P450scc in theca-interstitial cells
- increased progesterone production in granulosa cells
- increased estradiol production in granulosa cells

Vagina:

- increased transcriptional activity for Era and Erβ in HeLa cells

Uterus:

- stimulated AlkP activity in endometrial cancer cells
- increased levels of progesterone receptor mRNA
- decreased proliferation of human endometrial endothelial cells at all doses
- HOXA10 expression elevated
- stimulated ER-a and ER-b mediated transcription in COS-7 and endometrial cancer cells, significantly increased TRAP220

Breast:

- significantly changed more than 40 transcripts in ERα-HA cells

- modulated a least 15 genes in the ER-null C4-12 cell line
- stimulated cell growth in a dose dependent manner
- significantly upregulated Era and Erβ expression
- proliferation at ≤100 nM, increased pS2 mRNA expression at 1nM - 1 mM
- increase in pS2 gene transcription in the presence or absence of testosterone
- increased proliferation of MCF cells
- inverted u dose response between 10(-7) and 10(-4)M in MCF7 cells
- reduces apoptosis
- proliferated cancer cell lines
- activated ERα and ERβ receptors
- DNA damage at 10(-6) in the presence of CIC182780
- stimulate the proliferation of MCF7wt and MCF7SH cells
- estrogenic effects at 1ppm in MCF7 cells
- increased cell proliferation and cell cycle progression
- inhibited proliferation of MCF-7 cells at 250 ng/L
- increased lactate dehydrogenase leakage and decreased metabolic activity at 24 hrs incubation time
- upregulated expression of Bloom helicase in MCF-7 cells
- increased estrogenic activity in MELN & MELP
- increase in VEGF excretion in cells expressing endogenous Era
- gene expression patterns which facilitate apoptosis evasion
- cell cycle deregulation without a detectable increase in cell numbers
- reduced adiponectin release from breast explants in a "u" shaped response
- increased synthesis of RecQ helicase mRNAs: BLM, WRN, RTS, RecQ5
- protected T47D & MDA-MB-468 cells from drug-induced cytotoxicity & increased T47D cell viability
- action independent of classic ERα and ERβ receptors
- increased MCF7 BUS cell proliferation
- induced estrogenic activity in MCF-7 cells
- enhanced cell proliferation
- maximally stimulated cell cycle progression and proliferation at 10(-6)

♀ **organs:**

- increased transcriptional activity of Era and ERβ

Eggs:

- induced germinal vesicle breakdown at 4.38-438 nM in smaller oocytes and at 0.044 in larger oocytes
- formed multipolar spindles resulting in failed cytokinesis
- promoted microtubule polymerization and centrosome-based microtubule nucleation
- abnormal telophase I at all doses
- abnormalities due to unalignment of chromosomes suggesting congression failure highest at 3 nM in metaphase II, but also apparent at 30, 300 nM and 3 μM doses.

MISCELLANEOUS OUTCOMES								
	body weight	maturation	growth	LH	enzymes	proteins	DNA/ gene expression	hormones & receptor binding affinity
Change	2	1	0	1	4	3	17	13
No change	0	1	1	1	1	1	1	5
Total	2	2	1	2	5	4	18	18
% Change	100%	50%	0%	50%	80%	75%	94%	72%

Body weight:

- accelerate conversion of 3T3-L1 fibroblasts to adipocytes
- enhanced glucose uptake and amount of GLUT4 protein in adipocytes

Maturation

- increased rate of oocyte maturation

Growth

- no change

Lutenizing hormone

- decrease in serum levels

Enzymes:

- inhibits E2 sulfination in a primarily non-competitive manner
- exhibited potent antiandrogenic activity
- increased cAMP levels
- alters steroidogenic enzymes

Proteins:

- increased expression of chaperone protein HSP27 at 10(-6)
- irreversibly inhibited gap junction-mediated intercellular communication
- increase in VEGF protein excretion in cells expressing endogenous Era

DNA/gene expression:

- positive regulation of hTERT
- K-ras mutant DNA found at concentration 1x10-8 to 1x10-5
- increased Rsa mutations, 2.2-2.8 fold
- down-regulation of HOXC6 expression
- down-regulated BRCA 1, PIAS3, HER3, and 10 other genes
- inhibits replication
- up-regulation of an estrogen target gene like pS2
- reduced DNA methylation at CpGs 49-56
- PDE4D4 message areas markedly higher
- upregulated genes involved in cell cycle progression, oxidative phosphorylation, purine and pyrimidine metabolism
- DNA strand breaks in invertebrates
- expression of cell-cycle associated genes, cdc6, MCM5, MCM2, Myt1, PCNA and AuroraA were up-regulated
- activated reporter gene construct of HeLa cells
- upregulated AR at 10(-6) & 10(-8)
- 9 genes ↑, 17 ↓ in combination with human chorionic gonadotrophin
- changed regulation of 139 genes - 89 upregulated, 50 downregulated
- increased PSA mRNA expression
- increased WISP3
- downregulated ERβ and Erβ transcription copy number but only in cells expressing the AR-T877A mutant
- upregulated: Cadmodulin, Glutathione S-transferase, alpha-like
- downregulated: Compliment C3-H1, ZGC:56023 (fibrinogen, gamma polypeptide1)
- viltellogenin 5' end most increased at 96 hours
- induced expression of 47 genes that are involved in: development and reproduction, metabolism, metal ion transport and homeostasis, signal transduction, immune response, response from stress
- gene expression highly correlated with antiestrogen ICI
- gene expression patterns which facilitate apoptosis evasion
- upregulated *recA* & downregulated *grpE* E coli stress responsive genes
- increased mRNA expression of P450c17 at 10(-7)M, and P450scc at all doses in theca-interstitial cells

Hormones & Binding affinity:

- BPA competed with estradiol in binding to Type II EBS
- high binding capacity to Era and Erb which inhibits immunoreaction
- increased binding for Era, Erb, and AR
- competed with DHT for the binding of androgen receptors
- stimulated Era activity
- induced prostate AR binding
- in adult human male serum, BPA shows greater estrogenic activity than either octylphenol or nonylphenol
- stimulated ER-D351Y activity ~7 fold whereas wtERα was stimulated only ~4 fold.
- wtERα coactivator TIF2 significantly enhanced the agonistic properties of BPA
- interfere with estrogen signaling through GPR30 receptor
- binding affinity(IC50, nM) to ERRγ = 13.1 +/- 2.34
- 80% specific and exclusive binding to ERRγ
- KD affinity constant 5.5 nM
- receptor density 18.9 nM/mg
 - show the presence of a BPA specific hormone receptor in C. elegans, IC50= 2.3 μM
- binds to both Era and Erβ with a higher affinity for Erβ but was more active in Era (Ishikawa endometrial adenoma cells)
- increased ER E-luc activity in both cells line at 10(-6)

	EARLY OFFSPRING OUTCOMES		BRAIN & BEHAVIOR OUTCOMES				
	embryos	brain	dendrites	hippo	hypoth	pituitary	dopamine
Change	2	7	3	2	1	4	1
No change	0	3	0	3	1	3	0
Total	2	10	3	5	2	7	1
% Change	100%	70%	100%	40%	50%	57%	100%

Embryos:

- decreased calcein accumulation in trophoblast cells at 100 nM
- faster growth at 8 cell and blastocyte stages
- higher doses decrease #of blastocytes and increase in trophoblast areas

Brain:

- induced intracellular Ca⁺⁺ changes and prolactin secretion
- NB-1 cells showed limited but significant sulfotransferase activity
- inhibited differentiation of oligodendrocyte precursor cells
- GFAP (glial fibrillary acidic protein) expression significantly increased
- increased estrogen-like LDH release
- inverted u response curve for GFAP production from 0.1pg - 1 ng through excessive activation of STAT3 and Smad1
- increased cell proliferation at all concentrations except 10(-9)
- increased ration of oligodendrocytes generated to total cells

Dendrites:

- spine density and mossy fiber growth increased
- significantly inhibited dendritic development of Purkinje cells at all doses
- caused robust activation of midbrain astrocytes
- caused biphasic response in neurone/glia cocultures with activation at 100 fM, 1pM, 10pM, 100nm or 1µM and no activation at 10FM, 100pM, 1nM or 10nM.
- these effects were not reversed by tamoxifen or androgens
- highest concentrations induced neuronal cell death

Hippocampus:

- exacerbated glutamate induced neuronal damage
- 10-100nM induced a transient increase in the intracellular Ca²⁺ of NMDA-responsive neurons suggesting that a low dose BPA application rapidly drives the Ca²⁺ signaling system via activation of non-genomic pathway including estrogen receptors

Hypothalamus:

- increased MAP2, synapsin I positive areas & synaptic dentistry at 100 nM
- decreased MAP2, synapsin I positive areas & synaptic dentistry at 1 µM

Pituitary:

- max change in intercellular Ca⁺⁺ levels at nM levels and declined at higher concentrations
- rapid secretion of PRL at 10(-8)M in a bimodal response curve
- growth hormone content significantly reduced, cell numbers significantly reduced
- stimulates PRL release from anterior pituitary cells
- increases PRL release and cell proliferation in GH3 cells
- increased PRF activity
- noted significant differences in response between SD and F344 rats
- blocked T3 binding to TR, bound to TH receptors in non-competitive manner, suppressed production of prolactin

Dopamine:

- increased Ca²⁺ responses to dopamine at the lowest doses

ORGAN & SYSTEM OUTCOMES										
	adrenal	liver	thyroid	pancreas	kidney	spleen	bones	immune system	blood	other
Change	1	9	3	4	3	3	5	7	1	2
No change	0	1	2	0	1	0	0	2	0	3
Total	1	10	5	4	4	3	5	9	1	5
% Change	100%	90%	60%	100%	75%	100%	100%	78%	100%	40%

Adrenal:

- stimulated catacholamine synthesis.

Liver:

- metabolite of BPA, MBP, is much more estrogenically active than parent compound
- antagonist activity in +S9 test at about 780 nM
- stimulated [35S]GTPγS binding induced by dopamine
- increases production of Vtg at 1x10⁻⁷ M
- increased vitellogenin mRNA at 1 µM 18 degrees C
- did not reduced EROD activity induced by TCDD at less than 1ppm
- elevated induction of ER-mRNA
- VTG induction at all doses
- antagonist activity against E2 response
- BPA mainly glucuronidated by UGT2B15

Thyroid:

- weak ligand for TR and decreased gene transcription
- suppressed transcriptional activities mediated by TR alpha 1 and TR beta 1
- BPA recruits N-CoR
- blocked T3 binding to TR, bound to TH receptors in non-competitive manner, suppressed production of prolactin

- lowered the levels of TR β mRNA expression
- 10(-7)M T3 induced T α mRNA expression also suppressed in concentration dependent manner
- BPA has a greater effect on the expression of TR β than T α

Pancreas:

- activates CREB in a Ca²⁺- dependent manner
- insulin secretion following long-term exposure to BPA or NP for 24h in 16.7mM glucose was significantly higher than without exposure
- suppressed low glucose induced Ca²⁺ oscillations
- insulin content of islets increased at 1& 10 nM in vitro
- ER α involved in insulin increases

Kidney:

- increased transcriptional activity of Er α and ER β
- increase in luciferase activity at concentrations of 100 nmol/L upward.
- Induced estrogenic activity in CV-1MCF-7 cells

Spleen:

- decreased IgE level and enhanced the IgM level at all concentrations
- enhancement of Thy1- splenocytes
- upregulation proliferation of splenocytes stimulated with ConA
- stimulated spleen cell proliferation
- enhanced IL-4, (IFN)- γ , IgG production
- induced TNF- α and IL-1 α by macrophages
- further enhanced LPS-induced TNF- α , but not IL -1 α productions

Bones:

- increased ALP activity
- increased Ca content and similarly P content
- increased transcriptional activity of Er α and Er β
- increase in phagocytosis at 1 μ M
- reduces proliferation of osteoblast-like cells at all concentrations
- decreased activity of TRAP and ALP in scales

Immune system:

- production of superoxide anions by phagocytic cells increased
- phagocytic activity decreased
- interleukin (IL)-4 increased significantly,
- production of IL-4 and IL-10, but not that of IL-13, markedly increased in Ts-infected mice inoculated orally
- significantly increased OZ triggered superoxide production at very low concentrations, (0.1-10 m M)
- at 1 nM induced a significant shift in the peak channel of CD 18 fluorescence
- enhances the activity of transcription factor PU.1
- inhibited lipopolysaccharide (LPS)-induced NO and TNF- α production, and the levels of iNOS and TNF- α mRNA
- decreased IgE level and enhanced the IgM level at all concentrations
- enhancement of Thy1- splenocytes
- upregulation proliferation of splenocytes stimulated with ConA
- induced lymphocyte proliferation at 3 lowest doses.
- induced induction of macrophage proliferation at .054 and .54 mg/L
- stimulated respiratory burst of macrophages at all low doses.

Blood:

- showed significant association with the SCEs measured in treated and untreated lymphocytes

Other

- immobilization of invertebrates, EC(50) = 1.04 μ M
- reduced adiponectin release from abdominal explants from obese women in a "u" shaped response
- reduced adiponectin release in mature adipocytes from non-obese women but not in a dose response manner