

**TEDX**

The Endocrine Disruption Exchange

P.O. Box 1407, Paonia, CO 81428

970-527-4082

[tedx@tds.net](mailto:tedx@tds.net)

[www.endocrinedisruption.org](http://www.endocrinedisruption.org)

**Non-Mammalian 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. The studies are grouped by timing of exposure and the results are listed below. For a broader view of the data, please refer to the summary and charts.

## Eggs/Embryos (n=17)

MALE REPRODUCTIVE SYSTEM OUTCOMES				FEMALE REPRODUCTIVE SYSTEM OUTCOMES	
	testes	sperm	feminization	ovaries	eggs/ oocytes
Change	2	1	1	1	3
No change	2	0	1	1	1
Total	4	1	2	2	4
% Change	50%	100%	50%	50%	75%

### Testes:

- testes/ova
- abnormalities

### Sperm:

- fertilization success decline at all doses
- exposed sperm resulted in decreased offspring quality

### Feminization:

- induction of female specific proteins

### Ovaries:

- oviduct malformation.

### Eggs/oocytes:

- increases in germ cell production
- fluid accumulation (oedema) in yolk sac and haemorrhages in the front part of yolk sac
- delayed emergence time

MISCELLANEOUS OUTCOMES							
	body weight	maturation	growth	mortality	enzymes	DNA/ gene expression	hormones & receptor binding affinity
Change	1	3	2	1	1	1	1
No change	0	1	1	2	0	0	1
Total	1	4	3	3	1	1	2
% Change	100%	75%	67%	33%	100%	100%	50%

### Body weight:

- increased body weight (dry and wet)

### Maturation:

- emergence rates decreased
- arrest of differentiation at gastrula stage
- earlier hatching at 200 µg/L
- produced eggs earlier at 200 µg/L

### Growth:

- increase in larval malformations as skeletal deformities at pluteus stage
- smaller as embryos and as adults

### Mortality:

- increase in mortality

### Enzymes:

- catalase increased, peroxidase decreased, glutathion s-transferase increased

### DNA/gene expression:

- alterations across multiple genes.

EARLY OFFSPRING OUTCOMES				BRAIN & BEHAVIOR OUTCOMES		ORGAN & SYSTEM OUTCOMES		
	embryos	birth rate	sex ratio	brain	behavior	liver	bones	other
Change	4	0	2	0	1	1	0	1
No change	1	2	2	1	0	2	1	1
Total	5	2	4	1	1	3	1	2
% Change	80%	0%	50%	0%	100%	33%	0%	50%

### Embryos:

- short body length, microcephaly, flexure, edema, abnormal gut coiling, altered normal development
- growth reduction at early life stages
- increase in larval malformations as skeletal deformities at pluteus stage
- reduced normal development

- decreased body length & accelerated development of the eye
- earlier hatching at 200 µg/L

**Birth rate:**

- no change

**Sex ratio:**

- increased # of males

**Brain:**

- no change

**Behavior**

- phlegmatic behavior

**Liver**

- histological changes
- strongly stained fragments in nuclei of liver cells

**Bones:**

- no change

**Other:**

- decreased offspring quality with exposed sperm

**Immature Animals (n=15)**

MALE REPRODUCTIVE SYSTEM OUTCOMES					FEMALE REPRODUCTIVE SYSTEM OUTCOMES
	testes	sperm	testosterone	feminization	eggs/oocytes
Change	5	2	1	3	1
No change	0	0	0	0	0
Total	5	2	1	3	1
% Change	100%	100%	100%	100%	100%

**Testes:**

- testes/ova
- change in gonad development
- unregulated ER mRNA
- increased esterone levels in testes
- decreased weight and size at 10 weeks
- elevated expression of p450arom mRNA
- upregulated GSTM transcript at 12 & 24 hours in juveniles
- upregulated GSTM mRNA in adult gonads with higher expression in secondary males than hermaphrodites.

**Sperm:**

- decreased spermatogonias, spermatids
- decrease in size of seminiferous tubes
- fertilization success declined at all doses
- exposed sperm resulted in decreased offspring quality

**Testosterone:**

- decrease in biliary testosterone below female levels
- decreased 11-ketotestosterone levels
- relationship of androgens to estrogens in males same as in females

**Feminization:**

- feminization
- relationship of androgens to estrogens in males same as in females

**Eggs/oocytes:**

- vg/vn immunoreactivity reduced in males

MISCELLANEOUS OUTCOMES							
	body weight	maturation	growth	mortality	proteins	DNA/ gene expression	hormones & receptor binding affinity
Change	0	3	1	0	1	3	1
No change	2	2	1	2	0	0	0
Total	2	5	2	2	1	3	1
% Change	0%	60%	50%	0%	100%	100%	100%

**Body weight:**

- no change

**Maturation:**

- arrest of differentiation at gastrula stage
- suppressed thyroid hormone induced maturation as shown by tail shortening and hind limb lengthening

- inhibited T3 induced metamorphosis
- interfered with normal development of intestines
- inhibited T3 induced metamorphosis by blocking most of the T3 signaling pathways

**Growth:**

- growth reduction at early life stages
- increase in larval malformations as skeletal deformities at pluteus stage

**Mortality:**

- no changes

**Proteins:**

- altered some globin proteins but not total protein content

**DNA/gene expression:**

- suppressed EGFP activity
- upregulated hemoglobin genes HbA, HbB, HbC, and downregulated HbE
- inhibited T3-induced transcription
- inhibited T3-induced gene expression for TRβ, stromelysin-3, TH/bZIP
- 33% of T3 upregulated genes were downregulated
- 36% of T3 downregulated genes were upregulated
- majority of genes with the most up or down regulation showed interference in action by BPA
- inhibits metamorphosis by blocking most of the T3 signaling pathways

**Hormones & Binding affinity:**

- unregulated ER mRNA activity in whole organism

EARLY OFFSPRING OUTCOMES			ORGAN & SYSTEM OUTCOMES				
	embryos	sex ratio	liver	thyroid	kidney	blood	other
Change	1	1	2	2	0	4	2
No change	0	0	2	0	1	0	0
Total	1	1	4	2	1	4	2
% Change	100%	100%	50%	100%	0%	100%	100%

**Embryos:**

- growth reduction at early life stages
- increase in larval malformations as skeletal deformities at pluteus stage
- reduced normal development

**Sex ratio:**

- decreased number of males

**Liver:**

- increased VTG levels
- constant or decreasing VTG levels at lower dose groups
- increased esterase levels in bile of males

**Thyroid:**

- interference with spontaneous and thyroid induced metamorphosis
- inhibited T3-induced transcription and metamorphosis
- inhibited T3-induced gene expression for TRβ, stromelysin-3, TH/bZIP
- inhibits T3 induced metamorphosis by blocking most of the T3 signaling pathways

**Kidney:**

- no changes

**Blood:**

- increased VTG levels
- increased ZRP levels cod and turbot
- more ZRP in plasma of males vs females
- changes in plasma calcium concentrations.
- upregulated hemoglobin genes HbA, HbB, HbC, and downregulated HbE
- altered some globin proteins

**Other:**

- exposed sperm resulted in decreased offspring quality
- interfered with normal development of intestines

**Adult Non-Mammal (n=55)**

MALE REPRODUCTIVE SYSTEM OUTCOMES							FEMALE REPRODUCTIVE SYSTEM OUTCOMES				
	testes	sperm	prostate	penis	testosterone	feminization	ovaries	vagina	uterus	♀ organs	eggs/ oocytes
Change	8	7	1	2	1	4	5	1	1	2	8
No change	4	0	0	0	0	0	0	0	0	0	3
Total	12	7	1	2	1	4	5	1	1	2	11
% Change	67%	100%	100%	100%	100%	100%	100%	100%	100%	100%	73%

**Testes:**

- elevated *rm-cyp19a* mRNA expression in gonads
- decreased portion of testes occupied by sperm.
- altered structured and intersexual gonads with previtellogenic oocytes
- gonad reabsorption
- abnormalities
- downregulated p53 mRNA expression
- induced expression of *GnRHR* in gonads of secondary males
- upregulated expression of N-ras mRNA in gonads

**Sperm:**

- reduction in semen mass, sperm motility, average velocity
- reduced sperm counts
- reduction in ripe/mature spermatozoa
- almost empty spermatid cysts with few spermatozoa showing early spawning
- attenuated 20 $\beta$ -S upregulation of sperm motility
- fertilization success decreased
- exposed sperm resulted in decreased offspring quality

**Prostate:**

- decreased length

**Penis:**

- decreased length

**Testosterone:**

- decreased testosterone levels and steroid ratio decreased

**Feminization:**

- induction of choriogenin in males and female specific proteins
- induction female specific proteins in males

**Ovaries:**

- decrease in ovary growth
- superfeminization of female organs
- malformation of the oviduct
- morphological changes in ovaries
- gonad resorption
- downregulated p53 mRNA expression

**Vagina:**

- superfeminization of female organs

**Uterus:**

- superfeminization of female organs

**Eggs/oocytes:**

- increase in spawning mass
- increased egg production
- changed time of ovulation
- increasing atresia of PVG oocytes
- 50% had reduced ovarian follicles empty of oocytes, other 50% had ovarian follicles filled with atretic oocytes
- increase in embryos in brood pouch
- decreased number of atretic early vitellogenic oocytes
- faster maturation of oocytes
- shortened reproductive cycle

MISCELLANEOUS OUTCOMES								EARLY OFFSPRING OUTCOMES		
	body weight	maturation	growth	mortality	enzymes	proteins	DNA/ gene expression	embryos	birth rate	sex ratio
Change	1	2	2	3	3	4	13	5	1	0
No change	1	0	1	3	1	0	0	0	3	1
Total	2	2	3	6	4	4	13	5	4	1
% Change	50%	100%	67%	50%	75%	100%	100%	100%	25%	0%

**Body weight:**

- decreased body weight

**Maturation:**

- longer time to maturation
- arrest of differentiation at gastrula stage

**Growth:**

- decreased growth in males
- growth reduction at early life stages
- increase in larval malformations as skeleton deformities at the pluteus stage

**Mortality:**

- increased mortality
- increased female mortality
- increased second generation mortality

**Enzymes:**

- increase in CYP content
- increases in phosphotyrosine levels in gills and mantle
- increased GSR & GST
- increased glutathione
- decreased catalase at 3&15 ng/g
- increased catalase at 60 ng/g
- decreased lysosomal latency
- increased neutral lipid intensity at all doses

**Protein:**

- females - down regulated 26 protein peaks, up regulated 13; males - down regulated 19, up regulated 12
- decrease in Hsp 90
- Hsp 70 overexpressed and upregulated

**DNA/gene expression:**

- induction of micronuclei
- increased ER, CYP19 and p53 activation
- increase in ER mRNA in females, males less sensitive but showed increased responses
- induced expression of *GnRHR* in intestines of secondary males
- downregulated p53 mRNA expression
- dose dependent upregulation of both *omChgH* & *omChgL* in both sexes
- upregulated 29 Ets and 18 TCs, gene coding for homobox proteins, all coding for VTGs
- regulated genes that code for proteins involved in metabolism
- DNA damage in erythrocytes
- choriogenin L and H cDNA induced
- reduced transcriptional levels of CYP2C33-like, CYP2Y3, CYP3C1-like
- upregulated Hsp 70 mRNA

**Embryos:**

- increase in embryo production in a inverted u response pattern
- decreased hatching success
- increase in unshelled embryos
- increase in total numbers of embryos
- increase in larval malformations as skeleton deformities at the pluteus stage

**Birth rate:**

- decrease

**Sex ratio:**

- no changes

	BRAIN & BEHAVIOR OUTCOMES		ORGAN & SYSTEM OUTCOMES					other
	brain	behavior	liver	pancreas	bones	immune system	blood	
Change	4	1	8	1	1	1	7	5
No change	2	0	3	0	0	0	1	0
Total	6	1	12	1	1	1	8	5
% Change	67%	100%	73%	100%	100%	100%	88%	100%

**Brain:**

- mRNA overexpressed
- upregulated gene transcription as shown by ERE-TK-LUC activity
- induced expression of *GnRHR* in brain and pituitary of secondary males
- downregulated p53 mRNA expression

**Behavior**

- increased embryo sheltering behavior

**Liver:**

- Re-cyp1a mRNA & p53 mRNA downregulated in liver
- induced expression of *GnRHR* in secondary males
- dose dependent upregulation of both *omChgH* & *omChgL* in both sexes
- males reacted at lower doses and displayed higher-fold changes
- in hepatopancreas: decreased MeER1 expression, increased MeER2 expression
- choriogenin L and H cDNA induced
- reduced transcriptional levels of CYP2C33-like, CYP2Y3, CYP3C1-like

**Pancreas:**

- decreased MeER1 expression and increased MeER2 expression in hepatopancreas

**Bones:**

- decreased the activity of TRAP and ALP in scales

**Immune system:**

- significant lysosomal membrane destabilization
- dramatic decrease in phosphorylation of p38 MAPK, and CREB-like transcription factor

**Blood:**

- increase in plasma VTG levels in males
- decreased E2 in both sexes
- changes in VTG in both males and females
- induction of micronuclei
- DNA damage in erythrocytes

**Other:**

- exposed sperm resulted in decreased offspring quality
- induced expression of *GnRHR* in intestines of secondary males
- downregulated p53 mRNA expression in intestines
- upregulated expression of *N-ras* mRNA in intestines
- decreased acetylcholine esterase activity in claw muscles

**Multiple Life Stages Exposure (n=11)**

MALE REPRODUCTIVE SYSTEM OUTCOMES						FEMALE REPRODUCTIVE SYSTEM OUTCOMES		
	testes	sperm	prostate	penis	feminization	ovaries	♀ organs	eggs/ oocytes
Change	3	1	1	1	0	0	1	2
No change	2	0	0	0	1	1	0	1
Total	5	1	1	1	1	1	1	3
% Change	60%	100%	100%	100%	0%	0%	100%	67%

**Testes:**

- levels of ntERα and ntERβ2 mRNA changed dependent on time in development
- upregulated *GnRHR* gene expression in testes of secondary males
- upregulated *R-ras1* mRNA in gonads of hermaphrodites and secondary males

**Sperm:**

- decrease in stored sperm

**Prostate:**

- decreased length

**Penis:**

- decreased length

**Feminization:**

- no change

**Ovaries:**

- no change

**♀ organs:**

- enlarged albumen and capsule glands
- rupture of the pallial oviduct and abortive capsule masses

**Eggs/oocytes:**

- enhancement of spawning mass and egg production
- decreased egg production past 7 generations

MISCELLANEOUS OUTCOMES							EARLY OFFSPRING OUTCOMES	
	body weight	maturation	growth	mortality	DNA/ gene expression	hormones & receptor binding affinity	birth rate	sex ratio
Change	2	1	1	1	3	1	0	1
No change	0	1	0	0	0	0	1	0
Total	2	2	1	2	3	1	1	1
% Change	100%	50%	100%	100%	100%	100%	0%	100%

**Body weight:**

- decrease in larval wet weight
- increased body weight in generations 4,5,& 7

**Growth:**

- suppressed juvenile growth my half of control

**Mortality:**

- increase

**DNA/ gene expression**

- suppressed MT mRNA expression in intestine and liver in hermaphrodites & secondary males

- suppressed MT mRNA expression in brain in secondary males
- upregulated *GnRHR* gene expression in brain, pituitary, & gonads of secondary males
- upregulated *GnRHR* gene expression in intestine and liver of hermaphrodites & secondary males
- upregulated R-*ras1* mRNA expression in brain, liver and gonads of hermaphrodites & secondary males
- upregulated R-*ras1* mRNA expression in intestine of hermaphrodites
- upregulated R-*ras2* mRNA expression in brain, intestine and liver of hermaphrodites & secondary males
- upregulated R-*ras2* mRNA expression in gonads, of secondary males
- upregulated R-*ras3* mRNA expression in intestine of hermaphrodites
- upregulated R-*ras3* mRNA expression in liver of hermaphrodites & secondary males

**Hormones & receptor binding affinity**

- increased levels of juvenile hormone (II & III) at all days

**Birth rate:**

- no changes

**Sex ratio:**

- reduced number of females in all generations at 0.1 ppm

BRAIN & BEHAVIOR OUTCOMES			ORGAN & SYSTEM OUTCOMES		
	brain	pituitary	liver	blood	other
Change	3	1	3	2	4
No change	0	0	0	0	0
Total	3	1	3	2	4
% Change	100%	100%	100%	100%	100%

**Brain:**

- suppressed MT mRNA expression, upregulated *GnRHR* gene expression & R-*ras1*&2 mRNA expression in brain in secondary males
- upregulated R-*ras1*&2 mRNA expression in brain of hermaphrodites

**Pituitary:**

- upregulated *GnRHR* gene expression in pituitary of secondary males

**Liver:**

- suppressed MT mRNA expression, upregulated R-*ras1*, 2 & 3 & *GnRHR* gene expression in liver in secondary males & hermaphrodites

**Blood:**

- increase in VTG

**Other:**

- deformation of mouthparts
- suppressed MT mRNA expression & upregulated *GnRHR* gene expression in intestine in hermaphrodites & secondary males
- upregulated R-*ras1*, 2 & 3 mRNA expression in intestine of hermaphrodites
- upregulated R-*ras2* mRNA expression in intestine of secondary males