Figure S1

30 adult male flies were collected, and transfered to vials containing control food (which contains 150 mM of sucrose), food with BPA, high sugar (450 mM of sucrose), DEHP, or the mixture (BPA + DEHP + 450 mM of sucrose).

After 48 hours the flies were flash frozen in liquid nitrogen.

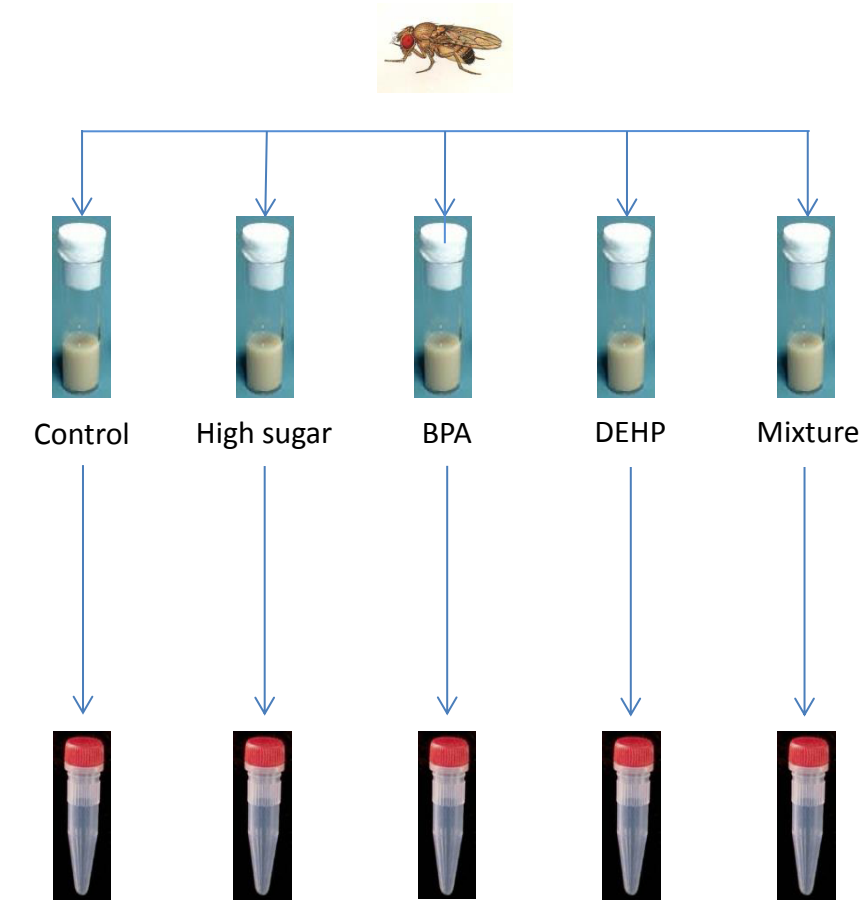


Figure S1. Cartoon with experimental design for acute exposure. We transferred adult male flies from the typical control laboratory diet to an identical diet in which BPA, DEHP, high sugar, or the mixture had been added. For the acute exposure flies were allowed to feed on the treatment diet for 48 hours. These flies were used to evaluate the effects of acute exposure on genome-wide gene expression. Four biological replicates were set up per treatment. Thirty males were exposed per biological replicate.

Figure S2

10 males and 15 female flies mated for 5 days in control food (which contains 150 mM of sucrose), food containing BPA, high sugar (450 mM of sucrose), or BPA and high sugar mixed together.

New male flies emerged from the food containing the compounds. They matured for 48hs in the same food they were raised.

After 48 hours the flies were flash frozen in liquid nitrogen.

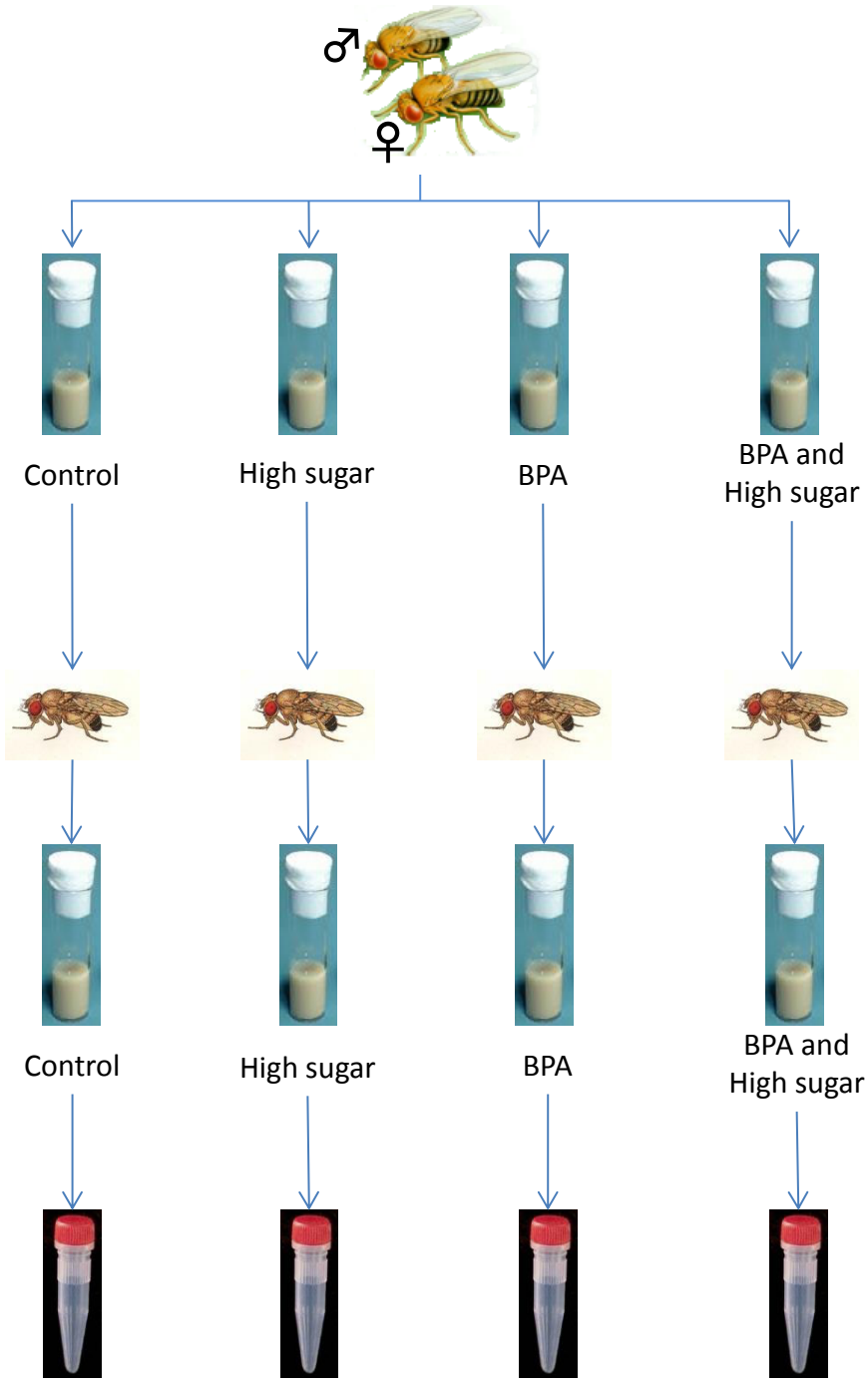


Figure S2. Cartoon with experimental design for chronic exposure. To study the chronic effect of BPA and high sugar, female flies laid eggs on the typical control laboratory diet or on an identical diet in which BPA, high sugar, or the mixture had been added. Flies were exposed to the treatment during development (from eggs to adults) and maintained in the treatment diet for 48hs upon emergence. These flies were used to evaluate genome-wide gene expression responses under chronic exposure. Four biological replicates were set up per treatment. Each treatment was further replicated in two genotypes.

Figure S3

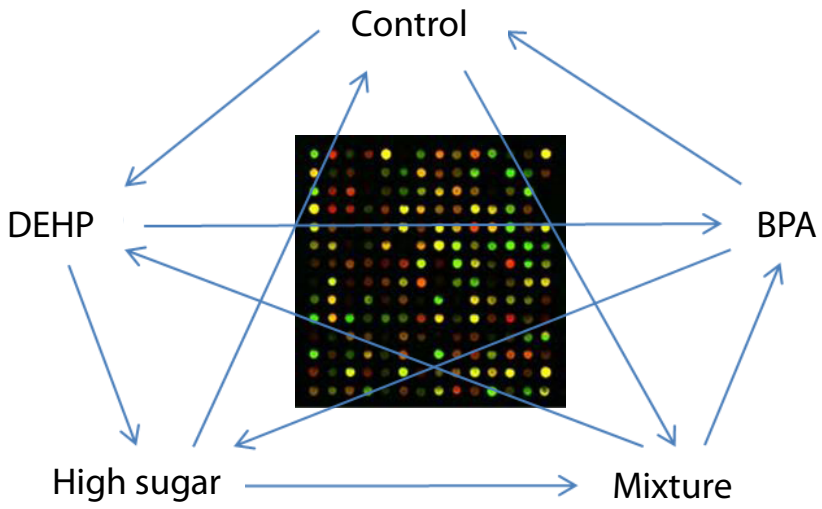


Figure S3. Microarray design for acute exposure. Dual-channel microarrays were used to study genome-wide gene expression. Two biological replicates were collected per treatment, with balanced dye swaps. Arrows represent the contrasts between 2 samples (1 microarray slide), and point from samples labeled with Cy5 to samples labeled with Cy3. In total, the expression signal of each treatment was collected from 4 distinct microarray slides.

Figure S4

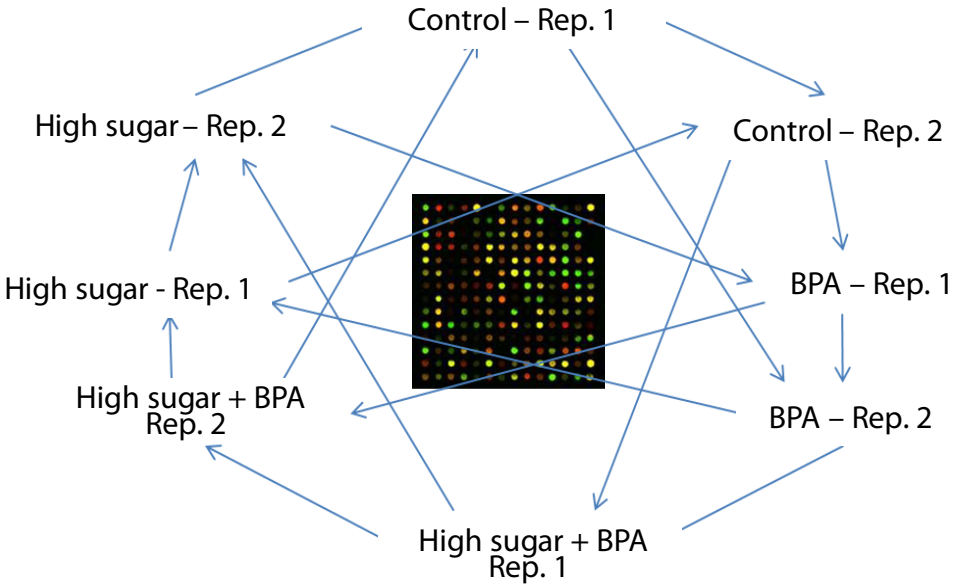


Figure S4. Microarray design for chronic exposure. Dual-channel microarrays were used for the genome-wide gene expression analysis. Each treatment was evaluated with two genotypes (rep 1 and rep 2). Each genotype replicate was evaluated twice (2 biological replicates per genotype and diet combination) with dye swaps. Arrows represent the contrasts between 2 samples (1 microarray slide), and point from samples labeled with Cy5 to samples labeled with Cy3. In total, the expression signal of each treatment was collected from 8 distinct microarray slides.

Figure S5

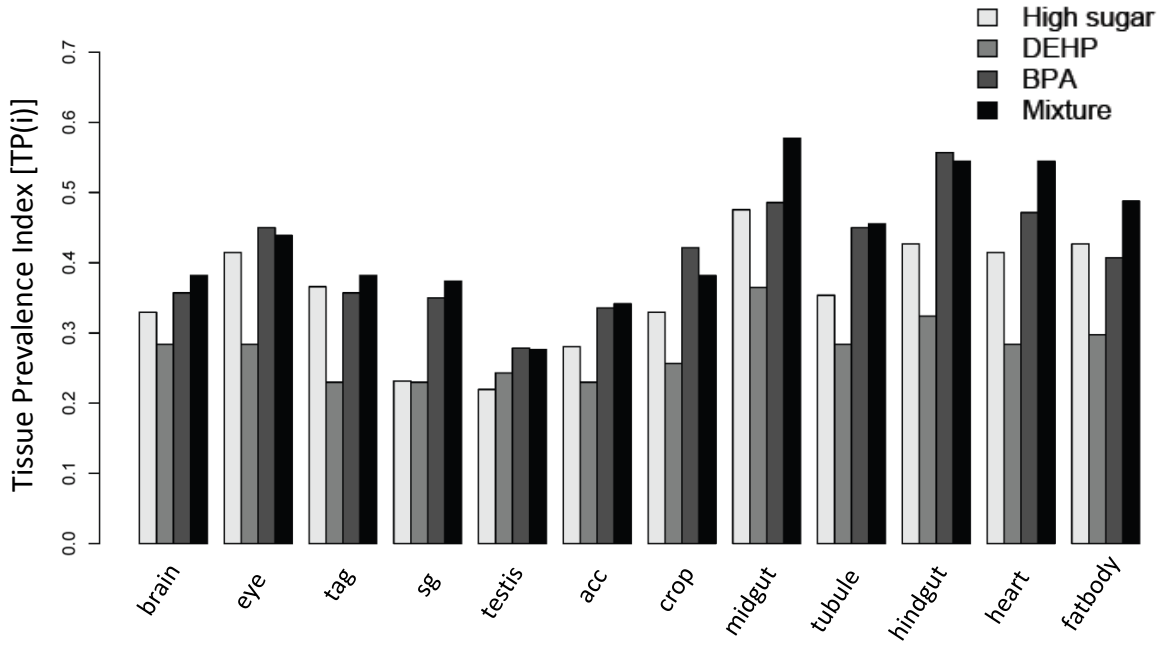


Figure S5. Tissue prevalence of genes up-regulated upon acute treatment with BPA, DEHP, and high sugar. Tissue prevalence indexes were calculated with the set of genes up-regulated in each treatment (BPP > 0.97). Tissue expression data are from the FlyAtlas database.

Figure S6

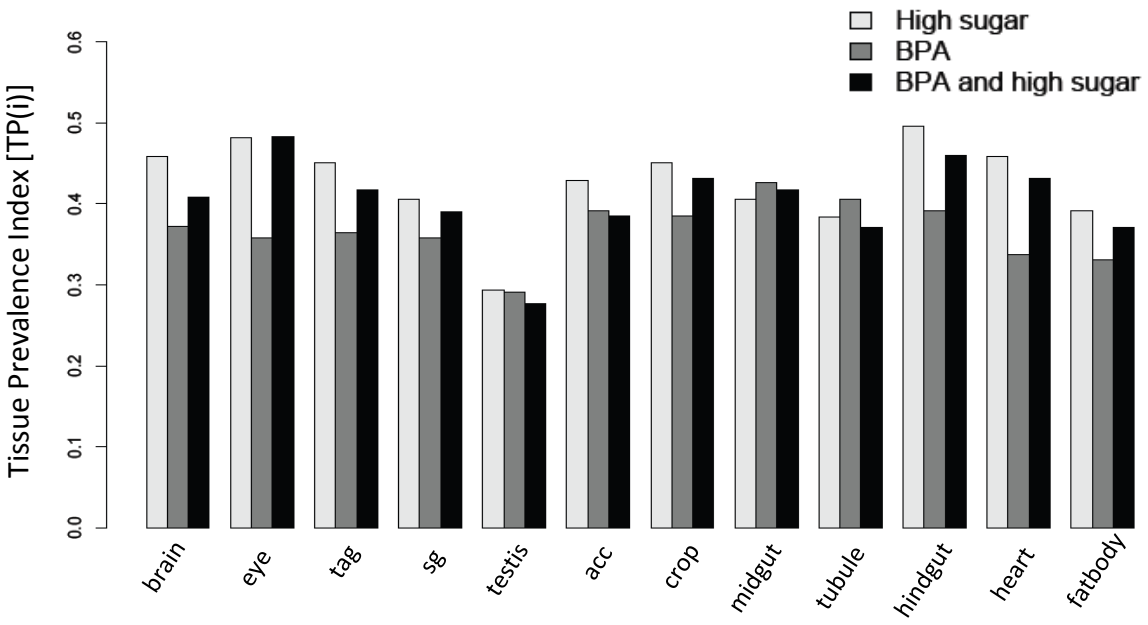
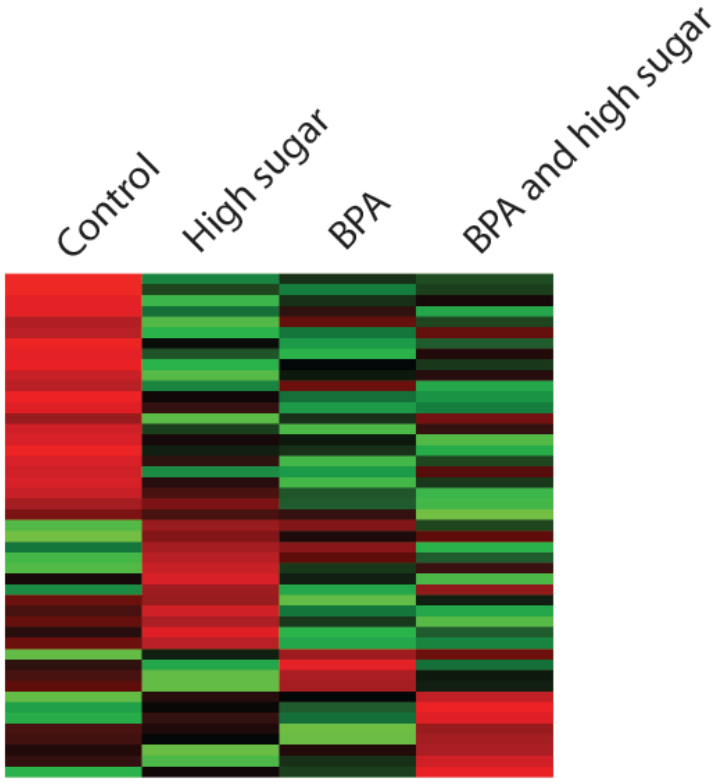


Figure S6. Tissue prevalence of genes up-regulated upon chronic exposure to BPA and high sugar. Tissue prevalence indexes were calculated with the set of genes up-regulated in each treatment (BPP > 0.97). Tissue expression data are from the FlyAtlas database.

Figure S7

A



B

Mitochondrial Ribosome	High sugar	BPA	BPA and High sugar
Up regulation	4	4	2
Down regulation	1	1	0

Figure S7. Expression profile of mitochondrial ribosome genes upon chronic exposure to BPA and high sugar. (A) Heat map shows the expression profile of 47 mitochondrial ribosome genes after chronic exposure to high sugar, BPA, and the mixture (BPA and high sugar). The scale color was normalized for each gene with green and red denoting high and low transcript abundance, respectively. (B) Number of mitochondrial ribosome genes significantly up-regulated (BPP > 0.95) in response to each treatment.

Table S1. Midgut salience ratios of genes differentially regulated in response to acute exposure. Data is shown for genes differentially expressed in each of 4 treatments (High sugar, DEHP, BPA, and the mixture). Background refers to the complete set of genes that passed the quality control steps and entered the analysis of differential expression. "Up" and "Down" identifies gene sets expressed in the midgut and at a level that is above or below the whole organism average, respectivelly.

Midgut	**Background**	**High sugar**	**DEHP**	**BPA**	**Mixture**
Dataset of **down-regulated** genes (BPP > 0.95)					
Up	1594	30	22	64	86
Down	3545	46	33	90	61
Ratio	0.45	0.65	0.66	0.71	1.41
P-value = 1.006e-11, Fisher's exact test					
Dataset of **down-regulated** genes (BPP > 0.98)					
Up	1594	11	8	39	56
Down	3545	12	10	23	9
Ratio	0.45	0.91	0.80	1.70	6.22
P-value < 2.2e-16, Fisher's exact test					
Dataset of **up-regulated** genes (BPP > 0.95)					
Up	1594	45	47	93	84
Down	3545	77	44	95	72
Ratio	0.45	0.58	1.07	0.98	1.17
P-value < 0.001, Fisher's exact test					
Dataset of **up-regulated** genes (BPP > 0.98)					
Up	1594	13	14	32	28
Down	3545	22	20	40	30
Ratio	0.45	0.59	0.70	0.80	0.93
P-value < 0.002, Fisher's exact test					

Table S2. Testis salience ratios of genes down-regulated in response to chronic exposure. (A) Dataset of down-regulated genes with BPP > 0.95. (B) Dataset of down-regulated genes with BPP > 0.97. Data is shown for genes differentially expressed in each of 3 treatments (BPA, high sugar, and the mixture). Background refers to the complete set of genes that passed the quality control steps and entered the analysis of differential expression. "Up" and "Down" identifies gene sets expressed in the testis and at a level that is above or below the whole organism average, respectivelly.

A

TESTIS	Background	BPA	High sugar	Mixture
Up	1733	82	60	125
Down	2722	66	73	70
Ratio	0.64	1.24	0.82	1.79

P-value = 1.05e-13, Fisher's exact test

B

TESTIS	Background	BPA	High sugar	Mixture
Up	1733	41	30	72
Down	2722	26	38	35
Ratio	0.64	1.58	0.79	2.06

P-value = 2.55e-10, Fisher's exact test

Table S3. Testis salience ratios of genes differentially regulated in response to acute exposure. Data is shown for genes differentially expressed in each of 4 treatments (High sugar, DEHP, BPA, and the mixture). Background refers to the complete set of genes that passed the quality control steps and entered the analysis of differential expression. "Up" and "Down" identifies gene sets expressed in the testis at a level that is above or below the whole organism average, respectivelly.

TESTIS	Background	High sugar	DEHP	BPA	Mixture
Dataset of **down-regulated** genes (BPP > 0.95)					
Up	1985	29	23	66	36
Down	3554	53	41	85	98
Ratio	0.55	0.55	0.56	0.78	0.37
P-value = 0.06, Fisher's exact test					
Dataset of **down-regulated** genes (BPP > 0.98)					
Up	1985	8	8	22	5
Down	3554	16	14	35	53
Ratio	0.55	0.50	0.57	0.63	0.09
P-value = 0.0001, Fisher's exact test					
Dataset of **up-regulated** genes (BPP > 0.95)					
Up	1985	20	19	25	12
Down	3554	106	72	167	146
Ratio	0.55	0.19	0.26	0.15	0.08
P-value = 2.20e-16, Fisher's exact test					
Dataset of **up-regulated** genes (BPP > 0.98)					
Up	1985	5	6	9	5
Down	3554	31	26	67	55
Ratio	0.55	0.16	0.23	0.13	0.09
P-value = 1.21e-11 Fisher's exact test					

Table S4. Tissue salience ratio of genes up-regulated in response to chronic exposures. (A) Dataset of up-regulated genes with BPP > 0.95. (B) Dataset of up-regulated genes with BPP > 0.99. CNS stands for central nervous system. Data is shown for genes differentially expressed in each of 3 treatments (BPA, high sugar, and the mixture). Background refers to the complete set of genes that passed the quality control steps and entered the analysis of differential expression. "Up" and "Down" identifies gene sets expressed in the tissue at a level that is above or below the whole organism average, respectivelly.

A

MIDGUT	Background	BPA	High sugar	Mixture
Up	1158	68	65	102
Down	2971	87	123	138
Ratio	0.39	0.78	0.52	0.74

P-value = 1.342e-08, Fisher's exact test

HINDGUT	Background	BPA	High sugar	Mixture
Up	1415	73	96	133
Down	2633	76	91	104
Ratio	0.54	0.96	1.05	1.28

P-value = 1.151e-14, Fisher's exact test

CNS	Background	BPA	High sugar	Mixture
Up	1704	62	75	88
Down	2481	101	115	148
Ratio	0.69	0.61	0.65	0.60

P-value = 0.6754, Fisher's exact test

TESTIS	Background	BPA	High sugar	Mixture
Up	1733	42	37	39
Down	2722	136	168	221
Ratio	0.64	0.31	0.22	0.18

P-value < 2.2e-16, Fisher's exact test

B

MIDGUT	Background	BPA	High sugar	Mixture
Up	1158	13	10	24
Down	2971	13	26	36
Ratio	0.39	1.0	0.38	0.67

P-value = 0.020, Fisher's exact test

HINDGUT	Background	BPA	High sugar	Mixture
Up	1415	20	18	33
Down	2633	7	20	27
Ratio	0.54	2.9	0.90	1.22

P-value = 1.80e-06, Fisher's exact test

CNS	Background	BPA	High sugar	Mixture
Up	1704	10	13	23
Down	2481	19	29	43
Ratio	0.69	0.53	0.45	0.53

P-value = 0.415, Fisher's exact test

TESTIS	Background	BPA	High sugar	Mixture
Up	1733	5	6	3
Down	2722	28	38	61
Ratio	0.64	0.19	0.16	0.05

P-value = 3.459e-13, Fisher's exact test