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Data in Brief

Interaction between bisphenol A and dietary sugar affects global gene transcription in *Drosophila melanogaster*

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ABSTRACT

Human exposure to environmental toxins is a public health issue. The microarray data available in the Gene Expression Omnibus database under accession number GSE55655 and GSE55670 show the isolated and combined effects of dietary sugar and two organic compounds present in a variety of plastics [bisphenol A (BPA) and Bis(2-ethylhexyl) phthalate (DEHP)] on global gene expression in *Drosophila melanogaster*. The study was carried out with samples collected from flies exposed to these compounds for a limited period of time (48 h) in the adult stage, or throughout the entire development of the insect. The arrays were normalized using the limma/Bioconductor package. Differential expression was inferred using linear models in limma and BAGEL. The data show that each compound had its unique consequences to gene expression, and that the individual effect of each organic compound is maximized with the joint ingestion of dietary sugar.

Specifications	
Organism/cell line/tissue	*Drosophila melanogaster*—whole body
Sex	Male
Sequencer or array type	22,000-feature custom cDNA array
Data format	Raw data: GPR file; normalized data: SOFT
Experimental factors	Acute and chronic exposures to BPA, DEHP, sugar
Experimental features	Global transcription analysis of fruit flies exposed to medium containing BPA, DEHP, sugar, or a mixture of these compounds. Exposure was done in adult flies for 48 h, or throughout the insect development
Consent	NA
Sample source location	Samples obtained from Bloomington Drosophila Stock Center at Indiana University and maintained in laboratory conditions at Harvard School of Public Health–Boston–USA

Direct link to deposited data.

Deposited data can be found here:http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE55655; http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE55670

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Experimental design, materials and methods

Bisphenol A

A remarkable increase in the industrial development of new chemical compounds is evident throughout the last few centuries. However, comprehensive studies on the effects of manufactured compounds on human health have lagged behind. This results in widespread human exposure to compounds with unknown consequences on biological pathways and physiology. Bisphenol A (BPA) is an organic compound used since the late 1950s as an ingredient to make polycarbonate and epoxy resins [1]. It is now one of the chemicals with the largest production worldwide [2]. It is used in the manufacture of a wide variety of products, such as plastic-based goods, industrial flooring, automotive primers, adhesives, and the lining of food cans. Thus, human populations are constantly and broadly exposed to BPA. Routes of exposure are varied, including dermal contact and ingestion, as is the case of canned food contaminated with leached BPA. The prevalence of BPA is such that it is present in the urine of more than 90% of all Americans [3,4], and chronic and persistent exposure to variable amounts of BPA has been associated with a broad spectrum of illness [5–7]. Biological effects have also been observed in the progeny of exposed adults [8,9], although causal pathways are often difficult to establish. Indeed, despite its abundance in the environment, the specifics of how BPA affects human health are a matter of debate. In addition, little is known about synergistic interactions between BPA and other common substances to which human populations are also exposed.

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Fruit fly exposures

To investigate the effects of BPA and its interaction with other common substances found in the human diet, BPA (3.7 g/L), sugar (102.7 g/L), Bis(2-ethylhexyl) phthalate (DEHP) (0.8% v/v), and mixtures containing these compounds were added to the reference diet of the flies (See details of the reference fly food at the website http://flystocks.bio.indiana.edu/Fly_Work/media-recipes/bloomfood.htm). Two approaches were used to expose the flies:

i) Acute exposure to evaluate short-term exposures. Four replicates of 30 adult flies (2-day-old *Yohio* males [10]) raised on regular control food were collected and transferred to vials containing fly food mixed with the toxins (BPA, DEHP, sugar, or a mix of the compounds). Flies were maintained for 48 h at 25 °C and 65% of relative humidity. After treatment, flies were flash-frozen in liquid nitrogen and stored at −80 °C.

ii) Chronic exposure to investigate the long-term effect of BPA and sugar. In this experiment, the genotypes *Yohio* and *Ycongo* [10] were exposed to the compounds throughout development (entire life-cycle, from egg to adult). Accordingly, four replicates of each genotype containing 15 virgin females and 10 males reared in reference food were combined in vials with medium containing BPA, sugar, or a mix of BPA and sugar. After 5 days laying eggs, adult flies were removed and the vials kept at 25 °C to collect the new emerged flies. Newly emerged adult males were aged for 48 h in the same rearing condition before they were flash-frozen in liquid nitrogen and stored at −80 °C [11].

Table 1
Experimental contrasts with corresponding files deposited in the GEO data bank.

File	Dye	Sample[a,b]	Treatment[c]	N° of detected spots[d]	N° of spots after QC[e]
24.gpr	Cy5	Control “B” (Rep. 1)	Chronic exposure	7916	4013
	Cy3	Control “A” (Rep. 1)			
25.gpr	Cy5	BPA “B” (Rep. 1)	Chronic exposure	7859	3856
	Cy3	Control “A” (Rep. 2)			
26.gpr	Cy5	Control “A” (Rep. 1)	Chronic exposure	8521	4618
	Cy3	High sugar “B” (Rep. 1)			
27.gpr	Cy5	Control “A” (Rep. 2)	Chronic exposure	7541	3646
	Cy3	BPA + high sugar “B” (Rep. 1)			
34.gpr	Cy5	BPA + high sugar “A” (Rep. 1)	Chronic exposure	9810	6552
	Cy3	Control “B” (Rep. 1)			
35.gpr	Cy5	BPA “A” (Rep. 1)	Chronic exposure	10,023	6226
	Cy3	Control “B” (Rep. 2)			
36.gpr	Cy5	BPA “B” (Rep. 2)	Chronic exposure	9837	6426
	Cy3	BPA “A” (Rep. 1)			
37.gpr	Cy5	BPA + high sugar “A” (Rep. 2)	Chronic exposure	9963	5933
	Cy3	BPA “B” (Rep. 2)			
38.gpr	Cy5	Control “B” (Rep. 2)	Chronic exposure	10,427	6300
	Cy3	High sugar “A” (Rep. 2)			
39.gpr	Cy5	High sugar “B” (Rep. 3)	Chronic exposure	9205	5811
	Cy3	High sugar “A” (Rep. 1)			
40.gpr	Cy5	High sugar “B” (Rep. 3)	Chronic exposure	9450	5625
	Cy3	BPA + high sugar “A” (Rep. 1)			
41.gpr	Cy5	BPA “A” (Rep. 1)	Chronic exposure	8937	5076
	Cy3	High sugar “B” (Rep. 2)			
42.gpr	Cy5	High sugar “A” (Rep. 1)	Chronic exposure	9507	5283
	Cy3	BPA + high sugar “B” (Rep. 2)			
43.gpr	Cy5	High sugar “A” (Rep. 2)	Chronic exposure	9338	5135
	Cy3	BPA “B” (Rep. 1)			
44.gpr	Cy5	BPA + high sugar “B” (Rep. 1)	Chronic exposure	9310	5185
	Cy3	BPA “A” (Rep. 2)			
45.gpr	Cy5	BPA + high sugar “B” (Rep. 2)	Chronic exposure	8671	4661
	Cy3	BPA + high sugar “A” (Rep. 2)			
61.gpr	Cy5	DEHP (Rep. 1)	Acute exposure	12,026	7505
	Cy3	Control (Rep. 1)			
62.gpr	Cy5	High sugar (Rep. 2)	Acute exposure	12,464	8403
	Cy3	DEHP (Rep. 2)			
63.gpr	Cy5	BPA + DEHP + High sugar (Rep. 2)	Acute exposure	12,492	7762
	Cy3	High sugar (Rep. 1)			
64.gpr	Cy5	BPA (Rep. 2)	Acute exposure	11,555	7056
	Cy3	BPA + DEHP + high sugar (Rep. 1)			
65.gpr	Cy5	Control (Rep. 2)	Acute exposure	11,642	6913
	Cy3	BPA (Rep. 1)			
66.gpr	Cy5	Control (Rep. 1)	Acute exposure	11,936	6894
	Cy3	High sugar (Rep. 2)			
67.gpr	Cy5	DEHP (Rep. 2)	Acute exposure	12,768	8254
	Cy3	BPA + DEHP + high sugar (Rep. 2)			
68.gpr	Cy5	High sugar (Rep. 1)	Acute exposure	11,367	1999
	Cy3	BPA (Rep. 2)			
69.gpr	Cy5	BPA (Rep. 1)	Acute exposure	11,963	7327
	Cy3	DEHP (Rep. 1)			
70.gpr	Cy5	BPA + DEHP + high sugar (Rep. 1)	Acute exposure	11,884	6485
	Cy3	Control (Rep. 2)			

[a] “Rep” stands for “Replicate”, and denotes the sample used in the hybridization contrast.
[b] “A” and “B” represent, respectively, the genotypes *Yohio* and *Ycongo*. Noteworthy, acute exposure was carried out only with the *Yohio* genotype.
[c] Expression data from chronic and acute exposures were deposited in the GEO data bank, respectively, as GSE55670 and GSE55655.
[d] Number of genes displaying fluorescence above background in each microarray slide.
[e] Number of spots after exclusion of bad quality spots according to the rule for quality control (QC) described in the topic “Microarray analysis”. These spots were used for further analysis of differential expression.

Microarray features

Slides were a ~22,000-feature cDNA array spotted with *D. melanogaster* PCR products from autosomal and X-linked single exons, Y-linked genes, *Drosophila* testis ESTs, and transposon elements on poly-L-lysine coated slides [10,12]. The sequence of the primers used to amplify the DNA fragments used to make the slides, as well as the association of the reference IDs of the microarrays with Fly Base numbers and their respective genes, are available in the Gene Expression Omnibus as platform GPL6056 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL6056).

cDNA hybridizations

RNA was isolated with Trizol from all 4 replicas at same time to reduce variation associated with "batch effects", and each experiment was carried out with at least 2 biological replicates (See Table 1 for details). cDNA probes were synthetized from 20 μg of total RNA following procedures described in the manual of the 3DNA kit (Genisphere). The probe was concentrated by centrifugation through Amicon Ultra-0.5 mL (30 K mesh) columns for 30 min at 13,500 ×*g*. The recovered volume was adjusted to 27 μL with ultrapure water, and the hybridization solution prepared with dT Blocker and 2× Enhanced Hybridization Buffer supplied with the kit. The cDNA probe mix was warmed up to 65 °C, and, immediately, the total volume was applied on a microarray slide placed in a metal chamber, also pre-warmed to 65 °C, and covered with 24 x 60 mm glass coverslip. The combination of samples used for the experiments are illustrated in the Fig. 1, and details regarding biological and technical replicates described in the Table 1. SSC (2×) was added to the chamber to avoid evaporation of the hybridization solution, sealed, and incubated for 15 h immersed in a water bath adjusted to 63 °C. Additional steps, which consisted in washes after first hybridization, second hybridization with the dyes Cy3 and Cy5, and final washes, were carried out according to the manufacturer of the kit.

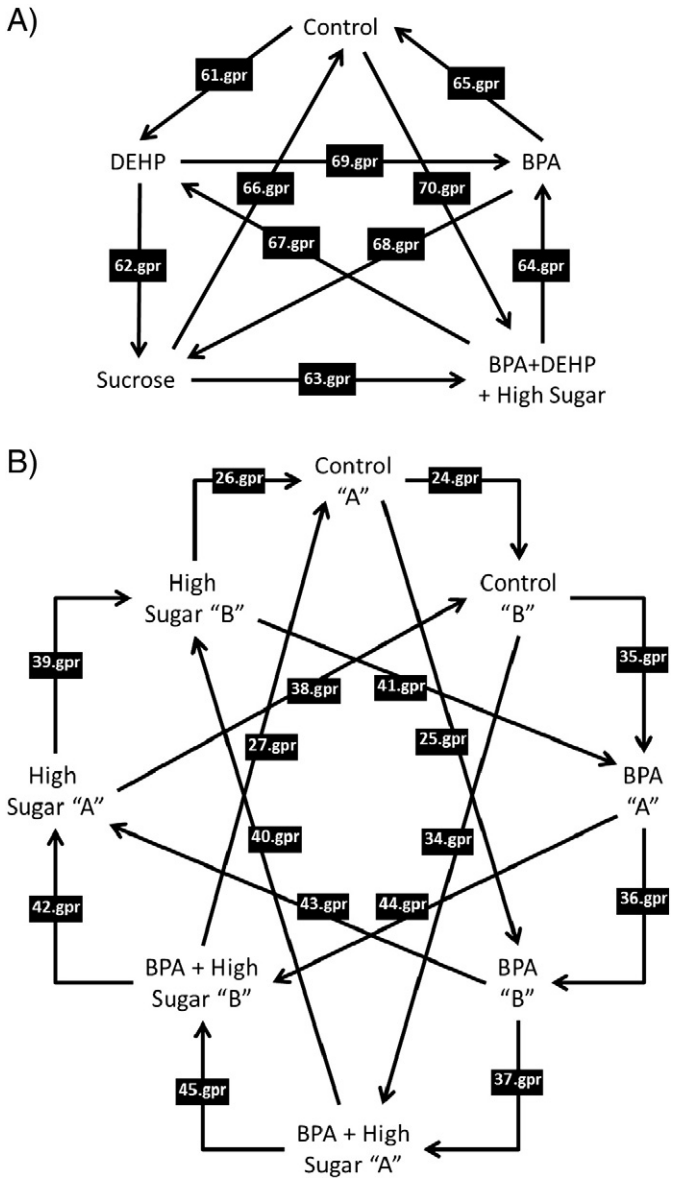

Fig. 1. Schematic representation of cDNA hybridizations. The figure shows the contrast of each microarray slide carried out for acute (A) and chronic (B) exposures. The arrows symbolize each hybridization, in which, the arrow's head indicates the sample probed with Cy5 and the base of it the sample labeled with Cy3 (See Table 1 for details). Black boxes indicate the .gpr files associated with the comparison and publicly available in the Gene Expression Omnibus database. Acute Exposure was carried out only with the *Yohio* genotype.

Microarray analysis

After hybridization, the fluorescence signal was collected with Axon 400B scanner (Axon Instruments). The data was extracted with the software GenePix Pro 6.0 using the rule: ([F635 Median − B635] > 4 *[B635 SD] Or [F532 Median − B532] > 4 *[B532 SD]) And ([% > B635 + 2 SD] > 70 Or [% > B532 + 2SD] > 70) And [F635 % sat.] < 45 And [F532 % sat.] < 45 And ([B532 Median] < 4 *[B635 Median] And [B635 Median] < 4 *[B532 Median]) And [Sum of Medians (635/532)] > 100 And [SNR 635] > 2 And [SNR 532] > 2 And [Rgn R2 (635/532)] > 0.5 And [Circularity] > 0.45. This quality control criterion ensures consistency of foreground intensity reads for both channels [10]. Normalization within arrays was done with the method of local linear regression Loess and an offset value = 50, as implemented in the package limma/Bioconductor [13,14]. Normalization between arrays was performed with the Aquantile method also using the limma package.

After quality control and normalization (Table 1), differential expression values were assessed with Bayesian Analysis of Gene Expression Levels (BAGEL) [15]. BAGEL is a reliable approach to identify differentially expressed genes with data from dual-channel microarrays. By using only ratio data, this method fixes common effects of dual-channel microarrays, such as dye bias and spot saturation. Yet, it has no requirements regarding balanced data [16]. Expression data generated by BAGEL analyses were further checked with linear models in limma, and false discovery rates were estimated by permutation of the dataset. The data reported here can be obtained at the Gene Expression Omnibus database under accession number GSE55655 and GSE55670.

Final data was organized in standard spreadsheets, and only values that had a Bayesian Posterior Probability larger than 95% were considered for further analyses. Analyses included investigation of Gene Ontology enrichment in sets of differentially expressed genes.

Discussion

Here we describe the dataset generated in the study published recently by Branco and Lemos (2014) [11]. The dataset shows that the effect of the organic compound BPA on genome-wide gene expression of *D. melanogaster* can be enhanced by the ingestion of sugar. This observation indicates that assessments of biological toxicity based exclusively on individual components are not satisfactory. Toxic effects need to be evaluated in conjunction with assessments of dosage responses and tissue-specific disruptions. The data highlight the potential for interactions between BPA and other substances, which include ingredients of the human diet.

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