Supporting Information for:

Impact of Hurricane Maria on Drinking Water Quality in Puerto Rico

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Part 1. Tap water sampling scheme and ethics compliance.

A total of 36 tap water samples were collected with collaboration among Mayaguez and Medical Sciences Campus, University of Puerto Rico (UPR) under the NIEHS PROTECT program (https://web.northeastern.edu/protect/) and Northeastern University (NU) in Boston. The research protocols were approved by the ethics committees at the University of Puerto Rico, Northeastern University, Cornell University, University of Michigan—School of Public Health, and University of Georgia. All participants were provided full details of this study and gave informed consent prior to enrollment.

The 36 tap water samples consist of 16 samples collected before Hurricane Maria ("B") and 20 after Hurricane Maria ("P"), either in northern PR ("N") or in southern PR ("S", other regions to the south of the PROTECT cohort). The sample sites include 15 municipalities in PR: Quebradillas (QU), Ciales (CI), Barceloneta (BA), Arecibo (AR), Camuy (CA), Manati (MN), Vega Alta (VA), San Juan (SJ), Carolina (CL), Hatillo (HA), Aguadilla (AG), Mayagüez (MY), Guayanilla (GU), Cayey (CY), and Humacao (HU).



Figure S1. Map distribution of tap water sampling locations in Puerto Rico (PR) based on GPS coordinates. The circle denoting each location was enlarged to protect participants' privacy under the agreement and policy of the Puerto Rico Test site for Exploring Contamination Threats (PROTECT) program. Eight sampling locations within the PROTECT cohort (BN7, BN8, BN9, BN10, BN11, BN12, BN13, and PN2) are not shown because of the absence of GPS information.

		Collection	Before	After	Within	Analysis performed ^b			
Sample ID	Municipality	time	Hurricane	Hurricane	PROTECT ^a	Trace	Organic	Yeast	RT-
			Maria	Marıa	cohort?	elements	micropollutants	assay	qPCR
BN1-QU	Quebradillas	03/23/2016	N		N		N	N	1
BN2-CI	Ciales	04/02/2016	N		N		N	N	N
BN3-BA	Barceloneta	05/04/2016	N		N		N	N	N
BN4-AR	Arecibo	05/28/2016	N		N		N	N	N
BN5-CA	Camuy	06/04/2016			N		N	N	\checkmark
BN6-CI	Ciales	10/14/2016	V		V			V	
BN7		04/04/2017	V		V	N		V	\checkmark
BN8	-	05/02/2017							
BN9	-	05/08/2017				\checkmark		\checkmark	
BN10	-	05/31/2017			\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
BN11	-	06/01/2017			\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
BN12	-	06/15/2017	\checkmark		\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
BN13	-	06/19/2017	\checkmark		\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
BN14-BA	Barceloneta	06/21/2017	\checkmark		\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
BN15-BA	Barceloneta	06/21/2017	\checkmark		\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
BN16-MN	Manati	06/21/2017	\checkmark		\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
PN1-VA	Vega Alta	02/06/2018			\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
PN2	-	02/19/2018		\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
PN1-SJ	San Juan	12/17/2017		\checkmark		\checkmark	\checkmark	\checkmark	\checkmark
PN1-CL	Carolina	12/18/2017		\checkmark		\checkmark	\checkmark	\checkmark	\checkmark
PN1-MN	Manati	12/18/2017			\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
PN1-HA	Hatillo	12/18/2017				\checkmark	\checkmark	\checkmark	\checkmark
PN1-AG	Aguadilla	12/19/2017				\checkmark	\checkmark	\checkmark	\checkmark
PS1-MY	Mayagüez	12/19/2017				\checkmark	\checkmark	\checkmark	\checkmark
PS1-GU	Guayanilla	12/19/2017				\checkmark	\checkmark	\checkmark	\checkmark
PS1-CY	Cavey	12/20/2017				\checkmark	\checkmark	\checkmark	\checkmark
PS1-HU	Humacao	12/20/2017				\checkmark		\checkmark	\checkmark
PN2-SJ	San Juan	02/21/2018					\checkmark	\checkmark	\checkmark
PN2-CL	Carolina	02/21/2018						\checkmark	\checkmark
PN2-MN	Manati	02/20/2018			\checkmark			\checkmark	\checkmark
PN2-HA	Hatillo	02/20/2018		\checkmark			\checkmark	\checkmark	\checkmark
PN2-AG	Aguadilla	02/20/2018		\checkmark			\checkmark	\checkmark	\checkmark
PS2-MY	Mavag üez	02/19/2018					\checkmark	\checkmark	
PS2-GU	Guavanilla	02/19/2018		\checkmark			\checkmark	\checkmark	\checkmark
PS2-CY	Cavey	02/19/2018		\checkmark			\checkmark	\checkmark	\checkmark
PS2-HU	Humacao	02/18/2018		\checkmark			\checkmark		\checkmark

Table S1. Detailed Information on Tap Water Samples Collected at Puerto Rico (PR).

^{*a*} PROTECT: The Puerto Rico Test site for Exploring Contamination Threats (PROTECT) program, an on-going collaborative project starting in 2011 aiming to investigate potential relationship(s) between environmental contamination exposure and risk of adverse birth outcomes such as preterm birth (less than 37 completed weeks of gestation) in Puerto Rico.

^{*b*} All 36 tap water samples were analyzed for organic micropollutants and yeast toxicogenomics assay, but trace element analysis and human cell RT-qPCR assay were not performed for certain samples because of the limited sample volume.

^{*c*} No municipality information because of the lack of GPS coordinates.



Figure S2. A workflow of effect-directed drinking water quality analysis before and after Hurricane Maria (HM) in Puerto Rico (PR).

Part 2. Method description on trace elements and targeted organic micropollutants analyses.

A total of 21 pre-acidified tap water samples were analyzed for 18 trace elements using ICP-MS method by Prof. Philip Larese-Casanova's team at the Northeastern University, including aluminium (Al), arsenic (As), barium (Ba), chromium (Cr), copper (Cu), iron (Fe), gallium (Ga), lanthanum (La), manganese (Mn), nickel (Ni), lead (Pb), rubidium (Rb), scandium (Sc), selenium (Se), strontium (Sr), thorium (Th), uranium (U), and zinc (Zn).

Organic extracts from the 36 tap water samples were first diluted with Milli-Q water to achieve the final enrichment factor of 1000 times, filtered with 0.22-µm polytetrafluoroethylene (PTFE) membrane (Fisher Scientific), and then subjected to target screening for 200 organic micropollutants (Table S2) by means of high-performance liquid chromatography (HPLC) coupled to high-resolution mass spectrometry (HRMS, quadrupole-orbitrap, Thermo Scientific).^{1,2} These targeted chemicals include 13 PROTECT-priority chemicals and other emerging micropollutants identified in surface waters around the world (Table S3). A mixture of all target micropollutants was first prepared in Milli-Q water at 5 mg/L. The mixture was diluted with Milli-Q water to create an eleven-point calibration curve ranging between 0 and 1000 ng/L. A mixture of 44 isotope-labeled internal standards (ILISs) ^{1,2} was likewise created in Milli-Q water at 5 mg/L and was spiked into each calibration standard and sample extract at a fixed mass of 100 ng prior to sample analysis. A previously reported HPLC-HRMS method was used to quantify the micropollutants in each sample extract.^{1,2} Briefly, the mobile phase consisted of LC-MS-grade water and LC-MS-grade methanol, each with 0.1% formic acid (98-100%, Thermo Scientific). The extracted samples were injected at 20 µL on an XBridge C-18 analytical column $(2.1 \times 50 \text{ mm}, \text{ particle size } 3.5 \text{ }\mu\text{m}, \text{Waters})$ at 25°C. Mobile phase was pumped at a flowrate of 0.200 mL/min following a linear gradient. The instrument method acquired full-scan MS data in

a mass-to-charge ratio (m/z) range of 100-800 with rapid polarity switching mode and heated electrospray ionization. Data dependent MS2 scans were acquired with an inclusion list consisting of the target micropollutants. The target micropollutants were quantified using the ILISs based on the ratio of the area responses of the target micropollutant to its assigned internal standard and by 1/x weighted linear least-squares regression. Limits of quantification (LOQs, Table S2) were determined by the lowest linear calibration point with five MS scans and the presence of a diagnostic fragment. Method blank, solvent blank, and calibration checks were included in the chemical analyses to account for laboratory sources of contamination, solvent carryover during sample extraction process, and to verify the precision and accuracy of the calibration. Concentrations of the organic micropollutants quantified in the 36 tap water sample extracts (relative enrichment factor, REF = 1000) were divided by 1000 to reflect the actual contamination levels in raw tap water samples (REF = 1), as shown in Table S8.

Table S2. Limits of Quantification (LOQs) and Calibration Quality of 200 OrganicMicropollutants Analyzed in This Study.

Micropollutant Name	Limits of quantification (LOQ, µg/L)	Calibration R ^{^2}
10,11-dihydrocarbamazepine	2.5	0.9981
2,4-Dichlorophenoxyacetic acid (2,4-D)	1	0.9988
2,6-dichlorobenzamide	10	0.9905
2,6-dimethoxyphenol	5	0.9803
2-aminobenzimidazole	0.5	0.9819
2-ethyl-2-phenyl-malonamide	5	0.9669
2-methylisothiazolin-3-one_(MI)	10	0.9970
6-benzylaminopurine	0.5	0.9969
Abacavir	0.5	0.9915
Abiscisic acid	25	0.9999
Acebutolol	1	0.9966
Acephate	5	0.9967

Acesulfame	0.5	0.9983
Acetaminophen	10	0.9952
Acetamiprid	2.5	0.9984
Acetochlor alachlor	25	0.9909
Adrenalone	25	0.9351
Adrenosterone	5	0.9956
Albuterol	2.5	0.9999
Aldicarb	2.5	0.9677
Amcinonide	25	0.8992
Amisulpride	1	0.9978
Amitriptyline	1	0.9906
Amphetamine	1	0.9987
Ampicillin	5	0.9945
Arecoline	2.5	0.9956
Atenolol	0.5	0.9990
Atenolol acid	1	0.9990
Atomoxetine	2.5	0.9917
Atorvastatin	250	NA
Atrazine-2-hydroxy	0.5	0.9973
Atrazine-desethyl	1	0.9977
Atrazine	1	0.9987
Atrazine-desethyl-desisopropyl	25	0.9934
Atropine	0.5	0.9914
Azoxystrobin	10	0.9998
Baclofen	1	0.9969
Bendiocarb	2.5	0.9975
Bentazon	0.5	0.9973
Benzisothiazolin-3-one_(BIT)	5	0.9997
Benzophenone	2.5	0.9909
Benzotriazole	1	0.9968
Benzotriazole-methyl-1H	0.5	0.9958
Benzoylecgonine	2.5	0.9991
Bromacil	5	0.9960
Bultalbital	25	0.9922
Bupropion	1	0.9914
Butocarboxim	>250	NA
Caffeine	5	0.9980
Candesartan	5	0.9896
Carbamazepine	2.5	0.9981
Carbaryl	5	0.9967
Carbazole	250	NA

Carbendazim	0.5	0.9993
Carbofuran	2.5	0.9991
Carisoprodol	10	0.9925
Celecoxib	10	0.9889
Chloridazon	10	0.9915
Chlorpheniramine	2.5	0.9968
Cimetidine	250	NA
Ciprofloxacin	100	1.0000
cis-Diltiazem	2.5	0.9976
Citalopram	0.5	0.9981
Clarithromycin	25	0.9972
Climbazol	2.5	0.9924
Clofibric acid	0.5	0.9994
Codeine	1	0.9989
Corticosterone	10	0.9954
Cotinine	2.5	0.9925
Coumarin	1	0.9898
Cyanazine	>250	NA
Cyflufenamid	25	0.9870
DEET	0.5	0.9978
Dehydroacetic acid	10	0.9938
Desmethylvenlafaxine	0.5	0.9932
Dexamethasone	10	0.9965
Dextromethorphan	1	0.9985
Diclofenac	10	0.9900
Diethyl phthalate	5	0.9946
Dikegulac	>250	NA
Dimethachlor	2.5	0.9948
Dimethoate	2.5	0.9987
Diphenhydramine	0.5	0.9935
Diuron	5	0.9980
Efavirenz	50	0.9781
Estriol	250	NA
Estrone	100	1
Ethofumesate	10	0.9933
Ethyl-butylacetylaminopropionate	1	0.9931
Famiciclovir	1	0.9952
Famotidine	2.5	0.9988
Fexofenadine	5	0.9993
Fluconazole	1	0.9996
Flucytosine	250	NA

Fluoxetine	2.5	0.9963
Folic acid	250	NA
Furosemid	2.5	0.9979
Gabapentin	0.5	0.9978
Gemfibrozil	50	0.9937
Gibberellic acid	2.5	0.9976
Hexazinone	1	0.9994
Hexomethylphosphoramide	0.5	0.9977
Hydrochlorothiazide	100	1.0000
Hydrocodone	1	0.9976
Hydrocortisone	10	0.9964
Ibuprofen	250	NA
Imidacloprid	5	0.9937
Iodocarb	10	0.9981
Iopromide	>250	NA
Ioxynil	0.5	0.9908
Irbesartan	2.5	0.9627
Isophorone diisocyanate	10	0.9917
Isoproturon	1	0.9988
Ketamin	0.5	0.9935
Ketoprofen	10	0.9971
Lamotrigine	1	0.9941
Levetiracetam	10	0.9787
Lidocaine	0.5	0.9956
Linuron	5	0.9863
Losartan	2.5	0.9848
Mabuterol	0.5	0.9930
Malaoxon	5	0.9974
MCPA	0.5	0.9995
Mecoprop	0.5	0.9993
Melamine	50	0.9814
Meprobamate	25	0.9886
Metalaxyl	2.5	0.9985
Metamitron	0.5	0.9963
Metaxalone	10	0.9961
Metformin	10	0.9983
Methadone	1	0.9937
Methocarbamol	10	0.9987
Methomyl	5	0.9969
Metolachlor	5	0.9924
Metolachlor-ESA	100	1.0000

Metoprolol	1	0.9981
Metribuzin	1	0.9983
Metsulfuron-methyl	25	0.9715
Molinate	2.5	0.9965
Morphine	2.5	0.9973
N,N-didesmethylvenlafaxine	1	0.9990
N4-acetylsulfamethoxazole	25	0.9999
Nadolol	1	0.9993
Naproxen	25	0.9879
Nicotine	10	0.9450
Norfloxacin	100	1.0000
Ofloxacin	50	0.9765
Oxazepam	5	0.9971
Oxcarbazepine	25	0.9940
Oxybenzone	5	0.9972
Paraxanthine-theophylline	2.5	0.9990
Penciclovir	5	0.9414
Pentoxyfylline	2.5	0.9930
Perfluorobutanoic acid	0.5	0.9994
Perfluorooctanoic acid	0.5	0.9765
Phenobarbital	10	0.9844
Phenytoin	25	0.9946
Pirimicarb	0.5	0.9993
Pirimiphos-ethyl	5	0.9838
Primidone	10	0.9980
Progesterone	10	0.9936
Prohexadione	0.5	0.9895
Prometon	0.5	0.9946
Propachlor	2.5	0.9987
Propachlor-ESA	0.5	0.9978
Propachlor-OXA	25	0.9979
Propazine	0.5	0.9973
Propoxur	5	0.9996
Propranolol	1	0.9988
Propyzamide	10	0.9863
Pseudoephredrine	0.5	0.9749
Pyrazophos	10	0.9846
Ranitidine	2.5	0.9987
Ritalinic acid	0.5	0.9978
Serotonin	2.5	0.9989
Sertraline	2.5	0.9987

Siduron	2.5	0.9907
Simazine	1	0.9956
Sitagliptin	2.5	0.9991
Sucralose	2.5	0.9965
Sulfadimethoxine	2.5	0.9963
Sulfamethazine	2.5	0.9950
Sulfamethoxazole	5	0.9987
Sulfathiazole	2.5	0.9856
TDCPP	250	NA
Temazepam	5	0.9978
Terbutylazine	2.5	0.9962
Testosterone	5	0.9969
Thiabendazole	0.5	0.9993
Tramadol	0.5	0.9935
Triamterene	1	0.9882
Tributyl phosphate	5	0.9942
Triclosan	2.5	0.9968
Trimethoprim	1	0.9973
Trinexapac-ethyl	5	0.9888
Tris(2-chloroethyl)phosphate	10	0.9946
Valsartan	25	0.9971
Venlafaxine	0.5	0.9985
Verapamil	5	0.9722
Warfarin	5	0.9873

Table S3. List of Puerto Rico (PR) Human Exposure-Relevant Chemicals Analyzed.

Туре	Chemical(s) ^a
Trace elements	As, Cr, Mn, Pb
	2,4-D, atrazine, atrazine-2-hydroxy, bromacil,
Pesticides and metabolites	DEET, diuron, 2-methyl-4-
	chlorophenoxyacetic acid (MCPA)
Organonhagnhata	tris(1,3-dichloroisopropyl)phosphate
Organophosphate	(TDCPP)
Phenols and parabens	benzophenone, triclosan
Par and polyflyoroalkyl substances	perfluorobutanoic acid (PFBA),
rei- and poryndoroarkyr substances	perfluorooctanoic acid (PFOA)
Phthalate	diethyl phthalate (DEP)

^a Chemicals were detected in urine, whole blood, and/or serum samples from pregnant women in

PR as previously reported.³⁻⁵

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Part 3. List of proteins in cellular stress response pathways ensemble yeast cell library.

Table S4. List of Proteins, Related Functions and Pathways Information in Five Known Stress

Stress	Function	Pathway		Protein involved		
	Matchaliam	Trehalose synthesis		TPS1, TPS2		
General	Wietabolisin	Osmotic stress		HOG1		
stress	Concerl function	Signal transduct	tion	TPK1, TPK2, CDC28		
	General function	Apoptosis		CDC48, NMA111, Tat-D, FIS1		
		Yap1p regulation	on	YAP1, YBP1		
	Sensor/regulator	Skn7p regulatio	n	SKN7, SLN1		
		Msn2p regulation		MSN2		
		Glutathione/	Glutathione	GSH1, GSH2		
Stress		Glutaredoxin	Glutaredoxin	GRX1, GRX2		
50055	Defense system	Thioredoxin	Thioredoxin	TRX1, TRX2, TRX3		
	Derense system	Enzymatic	SOD	SOD1, SOD2, CCS1		
		system	Catalase	CTT1		
		system	CYC related	CCP1		
	Membrane, cell wa	ll and cell structu	re	PUN1, HSP12		
Chemical	ATP-binding casset	te (ABC) transpo	PDR1, PDR3, YCF1, PDR5,			
stress			SNQ2, BPT1, ATM1			
54055	Major facilitator su	perfamily (MFS)	FLR1, QDR2, ATR1, TPO1,			
				AQR1, QDR3, TOP2		
	Cytosolic	Molecular chap	erones	HSP104, HSP42, HSP78, HSP26		
	unfolded protein	Ubiquitin-proteasome system		UBC5, UBC8		
	response (cytUPR)					
Protein		Sensor/regulato	r	IRE1, HAC1		
stress	Endoplasmic reticulum UPR (erUPR)	Molecular chap	erones	KAR2		
		ER-associated	protein	UBC1, UBC7		
		degradation (EF	RAD)			
	Mitochondrial	Molecular chap	erones	HSP60, HSP78		
	UPR (mtUPR)	Proteolytic syste	em	OXA1, PIM1		
	Autophagy			ATG1, UTH1		
		DNA damage si	ignaling (DDS)	CHK1, RAD9		
		Direct reversal	repair (DRR)	PHR1		
		Translesion syn	thesis (TLS)	RAD30		
		Base excision re	epair (BER)	OGG1, NTG1, NTG2, UNG1,		
				MAG1		
DNA		Nucleotide e	excision repair	RAD4		
stress	DNA repair	(NER)				
50055		Mismatch repair	r (MMR)	MSH2		
		Double strand b	oreak (DSB)	XRS2, MRE11		
		Homologous	recombination	RAD51		
		(HR)				
		Non-homologou (NHEJ)	us end joining	YKU70		

Response Pathways Ensemble Yeast Cell Library.

Part 4. Protein expression profiling data processing.

As previously described,¹⁻⁴ the quantitative toxicogenomics-based assay employs a library of in frame GFP fusion proteins of *Saccharomyces cerevisiae*, consisting of 74 reporter strains (key proteins) covering five key cellular stress response categories. It measures *in situ* and real-time protein expression changes in exposure to tap water samples, yielding sample-specific temporal response profiles (fingerprints) within 2 hours. Temporal raw data of optical density (OD) and green fluorescent protein (GFP) signal are first corrected by background OD and GFP signal of medium control with or without chemical, respectively. Protein expression P for a given protein open reading frame (ORF) *i* in treatment *x* at a given time point *t* is normalized by cell density as:

$$P_{i,x,t} = \frac{GFP_{corrected,i,x,t}}{OD_{corrected,i,x,t}}$$
(1)

where, $GFP_{corrected,i,x,t}$ is defined as the GFP reading of ORF *i* in treatment *x* at time *t*, corrected by the GFP reading of medium control at time *t*; and $OD_{corrected,i,x,t}$ is defined as the OD600 reading of ORF *i* in treatment *x* at time *t*, corrected by the OD600 reading of medium control at time *t*.

The *P* values of both treated experiments and untreated controls are normalized against internal control (housekeeping protein PGK1⁵), based on the average value of P_{PGK1} (vehicle control). The alteration in protein expression for a given protein ORF *i* in treatment *x* at time point *t* due to chemical exposure, also referred as induction factor, $I_{i,x,t}$, is then calculated as:

$$I_{i,x,t} = \frac{P_{treated,i,x,t-normalized}}{P_{untreated,i,t-normalized}} (2)$$
$$I_{i,x,t} = \frac{Q_{i,x,t}}{Q_{i,untreated,t}} (2)$$

where, $Q_{i,x,t}$ is the *P* value of ORF *i* in treatment *x* at time *t*, normalized against the internal control; and

 $Q_{i,control,t}$ is the *P* value of ORF *i* at time *t* in the control condition without chemical exposure, normalized against the internal control.

To quantify the chemical-induced protein expression level changes of a treatment, Protein Effect Level Index (PELI) was derived as a molecular quantifier.¹⁻⁴ The accumulative altered protein expression change over the 2 h exposure period for a given protein (ORF) i was calculated as:

$$PELI_{ORF,i} = \frac{\int_{t=0}^{t} I_{(up-regulated)}dt}{exposure\ time} (3)$$

where, *t* is the exposure time.

For up-regulated protein, $I_{(up-regulated)} = I$, when $I \ge 1$; and for proteins that showed downregulation, $I_{(up-regulated)} = 1$ when I < 1. This is based on the understanding that up-regulation of the biomarkers selected indicate potential activation of specific DNA damage repair, and the overall protein down regulations have been observed to be related to nonspecific cellular suppression effects.

The pathway activation response are calculated by integrating the protein expression changes for all the proteins (ORFs) in a pathway as:

$$PELI_{pathway j} = \frac{\sum_{i=1}^{n} w_i \times PELI_{ORF i}}{n}$$
(4)

where, *n* is the number of ORFs in one particular pathway, and

 w_i is the weight factor of *ORF* $_i$. For this study, we assigned value of 1 for all the weight factors.

Finally, the overall cellular stress response are quantified by integrating the protein expression changes for all pathways as:

$$PELI_{total} = \frac{\sum_{j=1}^{n} w_j \times \text{PELI}_{pathway j}}{n} (5)$$

where, *n* is the number of cellular stress response pathways in yeast library, and

 w_j is the weight factor of Pathway j. For this study, we assigned value of 1 for all

the weight factors.

Reference

(1) Lan, J.; Gou, N.; Gao, C.; He, M.; Gu, A. Comparative and Mechanistic Genotoxicity Assessment of Nanomaterials via A Quantitative Toxicogenomics Approach Across Multiple Species. *Environ. Sci. Technol.* **2014**, 48 (21), 12937-45.

(2) Lan, J.; Gou, N.; Rahman, S. M.; Gao, C.; He, M.; Gu, A. Z. A quantitative toxicogenomics assay for high-throughput and mechanistic genotoxicity assessment and screening of environmental pollutants. *Environ. Sci. Technol.* **2016**, 50 (6), 3202-3214.

(3) Lan, J.; Hu, M.; Gao, C.; Alshawabkeh, A.; Gu, A. Z. Toxicity Assessment of 4-Methyl-1-cyclohexanemethanol and Its Metabolites in Response to a Recent Chemical Spill in West Virginia, USA. *Environ. Sci. Technol.* **2015**, 49 (10), 6284-6293.

(4) O'Connor, S. T. F.; Lan, J.; North, M.; Loguinov, A.; Zhang, L.; Smith, M. T.; Gu, A. Z.; Vulpe, C. Genome-wide functional and stress response profiling reveals toxic mechanism and genes required for tolerance to benzo [a] pyrene in S. cerevisiae. *Front. Genet.* **2012**, 3, 316.

(5) Nakatani, Y.; Yamada, R.; Ogino, C.; Kondo, A. Synergetic effect of yeast cell-surface expression of cellulase and expansin-like protein on direct ethanol production from cellulose. *Microb. Cell Fact.* **2013**, 12 (1), 66.

Part 5. Maximum Cumulative Ratio (MCR) for Identification of Potential Cumulative Risk.¹

To untangle toxicant interactions of complex mixtures, we applied the recently introduced maximum cumulative ratio (MCR) concept to identify potentially high-risk mixtures that may require further investigation, and the major chemicals possibly driving the cumulative risk in tap water samples.¹⁻⁴ MCR is the cumulative exposure to multiple chemicals divided by the maximum chemical-specific exposure of a single chemical, when exposures are described using a common metric. In this study, the hazard quotient (HQ)/hazard index (HI) approach was used to normalize exposures across different chemicals for the MCR calculation. Application of HI and MCR for surface water mixture toxicity evaluation has been previously reported.¹⁻³ The HQ compares environmental concentration of a contaminant to its health based permitted dose as follows: ^{2.5}

$HQ = \frac{Chemical \ Concentration}{Permitted \ Dose}$

For the purposes of placing study findings in the context of human health, permitted dose were selected as regulatory USEPA Maximum Contaminant Levels (MCLs) for contaminants regulated in drinking water under the Safe Drinking Water Act (SDWA) or non-regulatory U.S. Geological Survey (USGS) Health-Based Screening Levels (HBSLs) for unregulated contaminants, when available. If neither of the two human-health benchmarks is available for certain chemicals, then other health-based advisory values were used for HQ calculation such as Drinking Water Health Advisory by USEPA, or World Health Organization (WHO) Guidelines for Drinking-water Quality, or the Guideline Value by the Minnesota Department of Health (MDH) as indicated in Table 1 and S5. HQs of the components are summed to provide a measure of cumulative exposure as the HI:

$$HI = \sum_{i=1}^{n} HQ_i$$

where *n* is the number of chemicals in a tap water sample.

Based on the assumption that dose additivity applies to all chemicals in the samples, MCR is calculated as:

$$MCR = \frac{HI}{HQ_{MAX}}$$

where HQ_{MAX} is the maximum of multiple HQ values calculated for each tap water sample. Larger MCR values (>2) indicate a greater need for cumulative risk assessment since the chemical-by-chemical approach would underestimate overall toxicity when the combined exposures to chemicals result in a cumulative toxicity level that exceeds the toxicity of the most toxic chemical. ^{2, 4} Contaminant mixtures in different samples can be categorized into four risk-associated groups based on the calculated values of the HI and the MCR (Table S5). ¹

Group	Combined risk	Individual chemicals risk	MCR	Implications
				The mixture presents a
Ι	HI > 1	$HQ_{MAX} > 1$		potential risk already based
				on individual components
П	Н І – 1	$HO_{M} = 1$		The assessment does not
11	$\Pi \leq 1$	$\Pi Q_{MAX} < 1$		identify a health risk concern
				The majority of the health risk
IIIA	HI > 1	$HQ_{MAX} < 1$ N	MCR < 2	caused by the mixture is
				driven by one contaminant
				The potential health risk is
IIIB	HI > 1	$HI > 1$ $HQ_{MAX} < 1$		driven by multiple contaminants
				in a specific sample

Table S5. Characteristics of Mixtures Based on the Values of HI and MCR.¹

Reference

(1) Vallotton, N.; Price, P. S. Use of the Maximum Cumulative Ratio as an Approach for Prioritizing Aquatic Coexposure to Plant Protection Products: A Case Study of a Large Surface Water Monitoring Database. *Environ. Sci. Technol.* **2016**, *50* (10), 5286-93.

(2) Han, X.; Price, P. S. Determining the maximum cumulative ratios for mixtures observed in ground water wells used as drinking water supplies in the United States. *Int. J. Environ. Res. Public Health* **2011**, *8* (12), 4729-4745.

(3) Price, P.; Zaleski, R.; Hollnagel, H.; Ketelslegers, H.; Han, X. Assessing the safety of coexposure to food packaging migrants in food and water using the maximum cumulative ratio and an established decision tree. *Food Additives and Contaminants: Part A* **2014**, *31* (3), 414-421.

(4) Price, P. S.; Han, X. Maximum cumulative ratio (MCR) as a tool for assessing the value of performing a cumulative risk assessment. *Int. J. Environ. Res. Public Health* **2011**, *8* (6), 2212-2225.

(5) Toccalino, P. L.; Norman, J. E.; Hitt, K. J. *Quality of source water from public-supply wells in the United States, 1993-2007*; U. S. Geological Survey: 2010.

Part 6. Disease enrichment analysis using Comparative Toxicogenomics Database (CTD).

The Comparative Toxicogenomics Database (CTD) is a publicly available database developed by North Carolina State University and the National Institute of Environmental Health Sciences (NIEHS), aiming to provide insights into mechanisms of chemical actions, disease susceptibility, toxicity, and therapeutic drug interactions by curating and integrating data describing relationships between chemicals, genes/proteins, and human diseases.¹ A disease is considered enriched if the proportion of genes annotated to it in a test set is significantly larger than the proportion of all genes annotated to it in the genome. A total of 27 organic micropollutants and 18 trace elements detected in tap water samples were queried in CTD for diseases enrichment following the basic steps below:

(1) Select target chemicals: in our case, we screened 45 exposure-relevant chemicals (27 organics and 18 trace elements) detected in the 36 tap water samples collected in Puerto Rico (Table S6 and Table S7);

(2) Search and confirm CAS numbers of target chemicals;

(3) Log on to the CTD website: http://ctdbase.org/;

(4) Use the "batch query" function in CTD to generate lists of genes associated with each of the 45 chemicals, using CAS numbers as the search input;

(5) Filter the resulting chemical genelists by organisms (human, mouse and rat genes only) in order to restrict our results to data obtained from the most commonly used mammalian animal models (mouse, rat) and most relevant species (human);

(6) Use the "Set Analyzer" tool on CTD website to test for enriched diseases for each chemical gene set (corrected *p*-value for enrichment < 0.05). "Set Analyzer" tool iterates over the

list of diseases annotated to the gene set to determine the significance of enrichment by the hypergeometric distribution and adjusted for multiple testing using the Bonferroni method;

(7) Assign top five enriched diseases associated with each chemical gene set (corrected *p*-value < 0.05) based on the ranking of the degree of disease enrichment as $-\log 10$ (corrected *p*-value).

Reference

(1) Mattingly, C. J.; Colby, G. T.; Forrest, J. N.; Boyer, J. L. The comparative toxicogenomics database (ctd). *Environ. Health Perspect.* **2003**, *111*(6), 793.

Part 7. Hurricane Maria impact on drinking water trace element concentration.

	Detection	Detection Minimum		Median Maximum		Average concentration ^a (µg/L)		Human-
Element	frequency (n=21)	concentration ^a (µg/L)	concentration ^a (µg/L)	concentration ^a (µg/L)	Before Hurricane Maria	After Hurricane Maria	benchmark (µg/L)	benchmark type ^c
Al	18	0.84	19.35	195.74	21.74	35.23	6000	HBSL
As*	21	1.68	3.20	9.91	2.68	4.67	10	MCL
Ba	21	0.49	28.18	88.98	26.26	34.76	2000	MCL
Cr	21	0.11	0.20	2.01	0.30	0.42	100	MCL
Cu	21	4.06	32.49	168.00	31.58	52.81	1300	MCL
Fe	21	10.96	23.36	61.60	26.32	32.63	4000	HBSL
Ga*	7	0.05	0.06	0.08	0.07	BDL b	-	-
La	17	0.01	0.02	0.04	0.01	0.02	-	-
Mn	16	0.06	0.16	5.77	0.09	1.28	300	HBSL
Ni	15	0.15	0.27	4.23	0.22	0.90	100	HBSL
Pb	21	0.04	0.21	0.54	0.23	0.31	15	MCL
Rb	21	0.23	1.02	1.84	0.97	1.05	-	-
Sc	7	0.18	2.32	5.27	1.00	2.87	-	-
Se	8	0.21	0.37	1.24	0.32	0.65	50	MCL
Sr	18	28.25	154.49	338.34	156.30	164.44	4000	HBSL
Th*	4	0.01	0.01	0.01	0.01	BDL	2	MCL
U	15	0.04	0.24	1.08	0.30	0.39	30	MCL
Zn	9	6.15	7.62	22.87	8.89	13.42	2000	HBSL

Table S6. Trace Element Concentration Statistics, Detection Frequency, and Human-Health Benchmark.

* A red asterisk (*) shows contaminant concentrations were significantly different in the tap

water samples collected before and after HM according to both unpaired t-test and Mann-

Whitney U test (p < 0.05);

^{*a*} Apply to concentrations detected above the detection limit;

^{*b*} BDL—below the detection limit;

^c Human-health benchmark values were current as of August 2019. MCL: Maximum

Contaminant Levels under the Safe Drinking Water Act (SDWA) by U.S. Environmental

Protection Agency; HBSL: Non-cancer Health-Based Screening Levels for unregulated

contaminants obtained from the HBSL website.

Part 8. The primary usage and physicochemical properties of the 27 organic micropollutants detected.

Table S7. The Primary Use Groups and Physicochemical Properties of the 27 OrganicMicropollutants Detected in at Least one of the 36 Tap Water Samples Collected at Puerto Rico.

Micropollutant	CAS number	Primary Use ^a	Structure ^b	pKa ^a	Log D ^{<i>a</i>} (pH=6)
2,4-D	94-75-7	Herbicide Agricultural-derived	HO HO CI	2.81	-0.52
Acesulfame	33665-90-6	Sweetener Wastewater-derived		2.00	-1.97
Atenolol acid	56392-14-4	Pharmaceutical degradation product (atenolol and metoprolol) Wastewater-derived		3.54	-1.24
Atrazine-2-hydroxy	2163-68-0	Herbicide degradation product (atrazine) Agricultural-derived		12.48	-2.84
Atrazine	1912-24-9	Herbicide Agricultural-derived		3.20	0.98
Benzophenone	119-61-9	Photosensitizer Wastewater-derived		-7.5	3.18
Benzotriazole	95-14-7	Ultraviolet stabilizer Wastewater-derived	Z,Z ZE	0.22	1.30
Benzotriazole-methyl-1H	136-85-6	Corrosion inhibitor and antifreeze Wastewater-derived	N NH	0.45	1.81
Benzoylecgonine	519-09-5	Illicit drug metabolite (cocaine) Wastewater-derived		3.15	-0.59
Caffeine	58-08-2	Stimulant Wastewater-derived	H ₃ C O	-1.16	-0.55
Coumarin	91-64-5	Anticoagulant Wastewater-derived	°	n/a	1.78

Micropollutant	CAS number	Primary use ^a	Structure ^b	pKa ^a	Log D ^{<i>a</i>} (pH=6)
DEET	134-62-3	Insect repellent Wastewater-derived		-0.95	2.50
Dehydroacetic acid	520-45-6	Fungicide Agricultural-derived	Fungicide Agricultural-derived		0.42
Diethyl phthalate (DEP)	84-66-2	Plasticizer Wastewater-derived	Plasticizer Wastewater-derived		2.69
Fluconazole	86386-73-4	Antifungal Wastewater-derived	Antifungal Wastewater-derived $\begin{pmatrix} & & & & \\ & & & & \\ & & & & \\ & & & & $		0.56
Hexazinone	51235-04-2	Herbicide Agricultural-derived	Herbicide Agricultural-derived		1.37
Imidacloprid	138261-41-3	Insecticide Agricultural-deried	cticide icultural-deried		1.02
МСРА	94-74-6	Herbicide, fungicide Agricultural-derived	Herbicide, fungicide Agricultural-derived		-0.17
Mecoprop	93-65-2	Herbicide Agricultural-derived		3.47	0.49
Metoprolol	51384-51-1	Beta-blocker Wastewater-derived		9.67	-1.34
Perfluorooctanoic acid (PFOA)	335-67-1	Perfluorochemical Wastewater-derived	P F F F O	-4.20	1.58
Prometon	1610-18-0	Herbicide Agricultural-derived	$\mathcal{A}_{\mathrm{H}}^{\mathrm{H}}\mathcal{A}_{\mathrm{H}$	-1.66	-0.70
Simazine	122-34-9	Herbicide Agricultural-derived	$\mathbf{A}_{\mathbf{H}}^{\mathbf{H}} \mathbf{A}_{\mathbf{H}}^{\mathbf{H}} \mathbf{A}_{\mathbf{H}}^{\mathbf{H}}^{\mathbf{H}} \mathbf{A}_{\mathbf{H}}^{\mathbf{H}} \mathbf{A}$	3.23	0.58
Sucralose	56038-13-2	Sweetener Wastewater-derived		11.91	-0.47

Micropollutant	CAS number	Primary Use ^a	Structure ^b	pKa ^a	Log D ^{<i>a</i>} (pH=6)
Temazepam	846-50-4	Hypnotic Wastewater-derived		-1.40	2.79
Tributyl-phosphate	126-73-8	Plasticizer Wastewater-derived		n/a	4.09
Tris(2-chloroethyl) phosphate	115-96-8	Flame retardant, plasticizer Wastewater-derived		n/a	2.11

^a Information on chemical primary use and properties were obtained from the supporting

information of a previous study by Carpenter and Helbling.¹

^b Structures were obtained from ChemSpider (<u>http://www.chemspider.com</u>).

Reference

(1) Carpenter, C. M.; Helbling, D. E. Widespread micropollutant monitoring in the Hudson River Estuary reveals spatiotemporal micropollutant clusters and their sources. *Environ. Sci. Technol.* **2018**, *52* (11), 6187-6196.

Part 9. Hurricane Maria impact on drinking water organic micropollutant level.

Table S8. Organic Micropollutant Concentration Statistics and Detection Frequency Before and After

 Hurricane Maria (HM).

Compound	Detection	Minimum	Median	Maximum	Average con (ng/	centration ^a L)
(detection frequency from high to low)	frequency (n=36)	concentration ^a (ng/L)	concentration ^a (ng/L)	concentration ^a (ng/L)	Before Hurricane Maria	After Hurricane Maria
DEET	36	1.3	5.0	59.6	8.7	9.4
Diethyl phthalate (DEP)	34	6.6	20.4	850.5	111.3	19.5
Benzophenone	32	3.0	6.2	16.6	5.6	8.0
Sucralose*	29	2.9	18.3	859.4	17.8	133.0
Perfluorooctanoic acid (PFOA)*	22	0.5	1.0	4.0	0.6	2.0
2,4-D	17	1.0	4.0	16.4	3.7	7.6
Fluconazole	17	1.1	3.9	8.4	4.5	4.2
Atrazine-2-hydroxy*	10	0.7	2.3	5.6	0.7	2.7
Benzotriazole*	8	1.1	2.3	18.6	<loq<sup>b</loq<sup>	4.3
Coumarin	8	1.3	1.4	3.6	1.7	2.1
Acesulfame*	7	0.6	0.9	1.7	<loq< td=""><td>1.0</td></loq<>	1.0
Prometon*	5	0.6	0.6	1.8	<loq< td=""><td>1.0</td></loq<>	1.0
Benzoylecgonine	4	4.0	6.1	6.8	<loq< td=""><td>5.7</td></loq<>	5.7
Caffeine	4	8.2	9.5	13.0	10.4	9.0
Atrazine	3	1.2	1.8	2.0	1.8	1.6
Benzotriazole- methyl-1H	3	0.9	1.7	10.1	<loq< td=""><td>4.2</td></loq<>	4.2
Hexazinone	3	1.0	1.3	11.4	11.4	1.2
Mecoprop	3	0.5	0.9	1.0	<loq< td=""><td>0.8</td></loq<>	0.8
Temazepam	3	14.7	16.5	24.8	<loq< td=""><td>18.6</td></loq<>	18.6
Atenolol acid	2	2.3	6.1	9.9	<loq< td=""><td>6.1</td></loq<>	6.1
Dehydroacetic acid	2	106.3	169.3	232.3	<loq< td=""><td>169.3</td></loq<>	169.3
MCPA	2	1.1	1.1	1.2	<loq< td=""><td>1.1</td></loq<>	1.1
Simazine	2	1.0	1.2	1.4	1.4	1.0
Imidacloprid	1	6.5	6.5	6.5	6.5	<loq< td=""></loq<>
Metoprolol	1	2.1	2.1	2.1	<loq< td=""><td>2.1</td></loq<>	2.1
Tributyl-phosphate	1	9.5	9.5	9.5	<loq< td=""><td>9.5</td></loq<>	9.5
Tris(2-chloroethyl) phosphate	1	10.4	10.4	10.4	<loq< td=""><td>10.4</td></loq<>	10.4

* A red asterisk (*) shows contaminant concentrations were significantly different in the tap

water samples collected before and after HM according to both unpaired t-test and Mann-

Whitney U test (p < 0.05);

^{*a*} Apply to concentrations detected above the detection limit;

^{*b*} Below limit of quantification.

Part 10. Concentration statistics of top 10 frequently detected chemicals in the tap water samples collected at Northern Puerto Rico region before and after Hurricane Maria (HM).



Figure S3. Box plots showing concentration statistics of top 10 frequently detected (a) trace elements, (b) organic micropollutants and those showed significant (p < 0.05) changes after Hurricane Maria (HM) in the tap water samples collected at Northern Puerto Rico region. The black line within each box is the median with box top and bottom as 75th percentile and 25th percentile, respectively. The maximum observation (after removal of outliers) and minimum value are also shown. Outliers are defined based on the interquartile range (IQR) rule.

Part 11. Hurricane Maria impact on drinking water molecular toxicity levels/profiles as revealed by the quantitative toxicogenomics-based yeast assay and human cell RT-qPCR assay.

Table S9. Statistics of Pathway PELI Values in Exposure to Tap Water Samples Collected atPuerto Rico Before and After Hurricane Maria (HM) Based on the QuantitativeToxicogenomics-Based Assay in Yeast Strains.

			No. of	Before Huri	ricane Maria ^a	^{<i>a</i>} After Hurricane Maria ^{<i>a</i>}		
Stress categories	Functional pathways	No. of biomarkers	biomarkers with PELI > 1.5	PELI- Median	PELI- Maximum	PELI- Median	PELI- Maximum	
	Trehalose synthesis	2	2	1.71	3.76	1.44	5.39	
General -	Osmotic stress	1	1	1.00	4.35	1.00	5.48	
	Signal transduction	3	3	1.51	3.89	1.46	2.47	
	Apoptosis	4	4	1.19	1.94	1.06	4.63	
	Cell wall damage, membrane desiccation*	2	2	1.27	2.63	1.00	2.08	
Chemical	ATP-binding cassette (ABC) transporters	7	6	1.31	2.23	1.10	2.10	
	Major facilitator superfamily (MFS) transporters	7	7	1.20	2.52	1.13	1.56	
	DNA damage signaling (DDS)	2	2	1.00	2.91	1.00	2.98	
	Direct reversal repair (DRR)	1	1	1.00	6.51	1.00	5.16	
	Translesion synthesis (TLS)	1	1	1.00	4.68	1.00	2.13	
	Base excision repair (BER)	5	5	1.32	2.93	1.31	3.55	
DNA	Nucleotide excision repair (NER)	1	1	1.00	6.19	1.00	4.43	
	Mismatch repair (MMR)	1	1	1.40	7.63	1.00	4.50	
	Double strand break (DSB)	2	2	1.29	3.49	1.00	2.90	
	Homologous recombination (HR)	1	1	1.00	8.33	1.00	2.11	
	Non-homologous end joining (NHEJ)	1	1	1.00	6.68	1.00	1.92	
	Yap1p regulation	2	2	1.00	4.77	1.00	2.62	
	Skn7p regulation*	2	2	1.63	3.76	1.00	3.34	
	Msn2p regulation	1	1	1.32	4.26	1.00	3.06	
	Glutathione*	2	2	2.01	4.60	1.00	3.25	
Oxidative	Glutaredoxin	2	2	1.00	3.24	1.00	2.05	
	Thioredoxin	3	3	1.30	3.26	1.08	2.95	
	SOD	3	2	1.00	2.20	1.00	2.42	
	Catalase	1	1	1.00	3.56	1.00	1.93	
	CYC related	1	1	1.00	5.95	1.00	3.74	

	Cytosolic unfolded protein response (cytUPR)	6	6	1.35	3.75	1.05	2.16
Protein	Endoplasmic reticulum UPR (erUPR)*	5	5	1.61	3.46	1.00	2.02
	Mitochondrial UPR (mtUPR)*	4	4	1.34	3.06	1.00	1.78
	Autophagy	2	2	1.00	1.95	1.00	1.90

* A red asterisk (*) shows the expression level of a stress-response pathway in yeast cells that was significantly different in the samples before and after the HM according to both unpaired t-test and Mann-Whitney U test (p < 0.05);

^{*a*} Minimum PELI values for all functional pathways were equal to 1.00 (no expression change relative to the untreated control).



Figure S4. Frequency of differential biomarker expression (PELI > 1.5) in exposure to tap water samples collected before and after Hurricane Maria (REF = 200). X-axis: biomarkers grouped by different stress response categories which are color-coded.

Table S10. Statistics of Molecular Toxicity Quantifiers in Exposure to Tap Water Samples Collected at Puerto Rico Before and After Hurricane Maria (HM) as Fold Difference (*I*) of 12 Biomarkers in Human Epithelial A549 Lung Cells ^{*a*} Based on The RT-qPCR Assay.

		No. of samples with fold			samples fold	Fold difference, <i>I</i>					
Stress	Biomarker	differe	nce > 2	differ 0	ence < .5	Before HM After			After HM	ter HM	
Category		Before	After	Before	After						
		HM	HM	HM	HM	Min.	Median	Max.	Min.	Median	Max.
		(n=14)	(n=20)	(n=14)	(n=20)						
Oxidative	HO1	0	0	0	0	0.74	1.07	1.83	0.56	1.05	1.75
DNA	<i>Rad51</i> *	4	1	0	1	1.01	1.88	3.93	0.47	1.25	2.08
damage	KU70	0	0	0	0	0.82	0.92	1.29	0.64	0.92	1.25
Inflammation	$TNF-\alpha^*$	0	5	8	4	0.08	0.39	1.13	0.18	1.01	3.73
Anontosis	Casp3	0	0	0	1	0.51	0.96	1.25	0.47	0.88	1.35
Apoptosis	p53	0	0	0	0	0.90	1.08	1.71	0.72	0.99	1.66
Chemical	MTF-1	0	2	0	0	0.66	1.03	1.38	0.61	0.89	2.13
Endocrine	ESR2*	0	0	3	1	0.28	0.61	0.85	0.47	1.12	1.75
disruption (ED)	AR	0	0	1	0	0.32	0.77	1.10	0.52	0.74	0.93
Aryl	AhR	0	0	0	0	0.71	0.87	1.47	0.67	0.90	1.59
hydrocarbon	CYP1A1	14	20	0	0	3.14	7.09	17.64	2.55	8.44	43.33
receptor (AHR)	CYP1B1	13	20	0	0	1.95	4.00	6.62	2.04	4.24	7.87

^{*a*} Human epithelial A549 lung cells were selected in this study because they have been widely used to study cytotoxic, genotoxic, oxidative and inflammatory responses, etc., of heavy metals, nano-structured compounds, pharmaceuticals, bisphenols, and other emerging organic pollutants. ¹⁻⁴ Although the A549 cells might not be the primary cells that come in direct contact with ingested drinking water, still certain detected chemicals of trace levels in PR tap water samples such as arsenic, perfluorooctanoic acid (PFOA), some pesticides, phthalates, etc., were previously reported to induce toxic effects on lung function. ⁵⁻⁸

* A red asterisk (*) shows the expression level of a specific biomarker in human A549 cells that was significantly different in exposure to the samples before and after the HM according to both unpaired t-test and Mann-Whitney U test (p < 0.05).

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Figure S5. (a) Molecular toxicity changes, as PELI values, in exposure to the 18 tap water samples collected on December 2017 and February 2018 after the Hurricane Maria (REF = 200) as determined by toxicogenomics assay in yeast strains. Y-axis: altered protein expression changes relative to the untreated control as expressed by the PELI for the five stress response categories which are color-coded and indicated in the legends. (b) Gene expression analysis in human A549 cells after 6 h exposure to tap water extracts at REF=200, as shown in fold changes (*l*) of eight selected biomarkers based on the comparative CT method. The eight biomarkers selected showed differential expression (fold difference > 2 or < 0.5) relative to untreated control in at least 1 sample tested. Error bar represent standard error of the mean.





Figure S6. Spatial distribution of overall molecular toxicity levels in exposure to tap water extracts (relative enrichment factor, REF = 200) across Puerto Rico (PR) determined by (**a**) yeast toxicogenomics-based assay as Protein Effect Level Index (PELI) (**b**) human cell RT-qPCR assay as average fold change (*I*) in relative to untreated control.

Part 12. Comparisons of contaminant concentrations to human-health benchmarks for tap water samples collected across Puerto Rico (PR) during 2016 to 2018.

Table S11. Summary of Hazard Quotients (HQ) of the 29 Detected Contaminants with AvailableHuman-Health Benchmarks in Tap Water Samples Collected Across PR.

Compound	Detection Frequency (n=21)	Percentage of detections with HQ > 1	Percentage of detections with 0.1 < HQ ≤ 1	Human-health benchmark (µg/L)	Human-health benchmark type ^a
Al	18	0	0	6000	HBSL
As	21	0	100%	10	MCL
Ba	21	0	0	2000	MCL
Cr	21	0	0	100	MCL
Cu	21	0	4.76%	1300	MCL
Fe	21	0	33.33%	4000	HBSL
Mn	16	0	0	300	HBSL
Ni	15	0	0	100	HBSL
Pb	21	0	0	15	MCL
Se	8	0	0	50	MCL
Sr	18	0	0	4000	HBSL
Th	4	0	0	2	MCL
U	15	0	0	30	MCL
Zn	9	0	0	2000	HBSL
2,4-D	14	0	0	70	MCL
Atrazine-2-hydroxy	5	0	0	200	WHO
Atrazine	2	0	0	3	MCL
DEET	21	0	0	200	MDH
Diethyl phthalate (DEP)	19	0	0	5000	HBSL
Hexazinone	2	0	0	300	HBSL
Imidacloprid	1	0	0	360	HHBP
MCPA	1	0	0	30	HBSL
Mecoprop	1	0	0	10	WHO
Metoprolol	0	0	0	3	MDH
Perfluorooctanoic acid (PFOA)	15	0	0	0.07	НА
Prometon	3	0	0	300	HBSL
Simazine	1	0	0	4	MCL
Tributyl-phosphate	1	0	0	4	HBSL low
Tris(2-chloroethyl) phosphate	1	0	0	2	HBSL low

^{*a*} Human-health benchmark values were current as of August 2019. MCL: Maximum Contaminant Levels under the Safe Drinking Water Act (SDWA) by USEPA;¹ HBSL: Non-cancer Health-Based Screening Levels for unregulated contaminants obtained from the HBSL

website; HBSL low: low end of HBSL range corresponding to a 10⁻⁶ (1 in 1 million) cancer risk, the HBSL range corresponds to a 10⁻⁶ to 10⁻⁴ cancer risk range; WHO: a health-based guideline value for drinking-water quality established by World Health Organization; MDH: a health-based guidance value for drinking water quality developed by the Minnesota Department of Health; HHBP: chronic non-cancer Human Health Benchmarks for Pesticides established by USEPA; HA: the lifetime health advisory level in drinking water established by USEPA.²

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