Supplementary Materials: Exposure Assessment of Multiple Mycotoxins and Cumulative Health Risk Assessment: A Biomonitoring-Based Study in the Yangtze River Delta, China

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S1. Detailed UHPLC-MS/MS Method

The method for accurate determination of 23 mycotoxins/metabolites in urine samples was performed via a Waters ACQUITY UHPLC system (Milford, MA, USA) coupled with an AB SCIEX QTRA® 5500 tandem mass spectrometer (AB SCIEX instruments, Foster City, Canada). Chromatographic separation was achieved on an Agilent Poroshell 120 EC-C_{18} column (3.0 mm × 100 mm, 2.7 μm) (Agilent, USA) with the mobile phase consisting of methanol (A) and 5 mmol/L ammonium acetate (B). A linear gradient elution program was designed as follows: initial 10% (A), 1 min 10% (A), 6 min 90% (A), 6.5 min 90% (A), 6.7 min 10% (A), and 8 min 10% (A) and held for a further 2 min for re-equilibration, yielding a total run time of 10 min. The flow rate was 0.4 mL min^{-1}, and the injection volume was 3 μL. The column temperature and the sample temperature were maintained at 40 °C and 5 °C, respectively.

For MS/MS analysis, the electrospray ionization source was operated in both positive (ESI^+) mode and negative (ESI^-) mode with the parameters set as follows: ion spray voltage, 5.5 kV (positive ion mode) and 4.5 kV (negative ion mode); desolvation temperature, 500 °C; source temperature, 150 °C. Nebulizing gas and desolvation gas flow rates were 7.0 bar and 1000 L/h, respectively. Multiple reaction monitoring (MRM) mode was developed for quantification of the targeted analytes. Data processing was performed using MultiQuant 3.0.2 (Analyst; AB SCIEX, Framingham, MA, USA). The parameters and the collision energies of precursor and product ions are listed in Supplementary Table S2.

Table S1. The demographic characteristics of participants in the three sampling areas.

	All	Jiangsu	Zhejiang	Shanghai
Total	227	76	76	75
Male	114	30	43	41
Female	113	46	33	34
Age (years)	45.4 ± 17.5	46.5 ± 15.1	51.9 ± 20.3	37.8 ± 13.4
BMI[1] (kg m^{-2})	23.1 ± 3.2	23.2 ± 3.1	23.1 ± 2.8	22.9 ± 3.6
Creatinine (mg dL^{-1})	131.5 ± 64.7	132.6 ± 59.5	127.7 ± 69.2	134.3 ±64.7

[1]BMI: body mass index.

Table S2. Co-occurrence of investigated mycotoxins and their metabolites in urine samples from Jiangsu, Zhejiang, and Shanghai.

Table 1.	Distribution across location			
	Total Number (prevalence)	Jiangsu Number (prevalence)	Zhejiang Number (prevalence)	Shanghai Number (prevalence)
2 Toxins	30 (13.22%)	11 (14.47%)	6 (7.89%)	13 (17.33%)
AFM1+DON-15-GlcA	2 (0.88%)	-[2]	-	2 (2.67%)
OTA+DON-15-GlcA	1 (0.44%)	-	-	-
OTA+ZEN	1 (0.44%)	-	1 (1.32%)	-
FB1+ZEN	2 (0.88%)	2 (2.63%)	-	-
FB1+DON-15-GlcA	1 (0.44%)	-	1 (1.32%)	-
DON+DON-15-GlcA	12 (5.29%)	2 (2.63%)	2 (2.63%)	8 (10.67%)
DON-3-GlcA+DON-15-GlcA	7 (3.08%)	3 (3.95%)	2 (2.63%)	2 (2.67%)
DON-15-GlcA+ZEN	4 (1.76%)	4 (5.26%)	-	-
3 Toxins	13 (5.73%)	5 (6.58%)	3 (3.95%)	5 (6.67%)
OTA+FB1+DON-15-GlcA	1 (0.44%)	-	-	1 (1.33%)
OTA+FB1+ZEN-14-GlcA	1 (0.44%)	-	-	1 (1.33%)
FB1+DON+DON-15-GlcA	1 (0.44%)	1 (1.32%)	-	-
DON-3-GlcA+DON-15-GlcA+ZEN	2 (0.88%)	2 (2.63%)	-	-
DON+DON-3-GlcA+DON-15-GlcA	5 (2.20%)	1 (1.32%)	1 (1.32%)	3 (4.00%)
DON-3-GlcA+DON-15-GlcA+ZEN-14-GlcA	1 (0.44%)	-	1 (1.32%)	-
DON+DON-15-GlcA+ZEN	2 (0.88%)	1 (1.32%)	1 (1.32%)	-
4 Toxins	8 (3.52%)	5 (6.58%)	2 (2.63%)	1 (1.33%)
AFM1+DON-3-GlcA+DON-15-GlcA+ZEN	1 (0.44%)	1 (1.32%)	-	-
AFM1+FB1+DON-3-GlcA+DON-15-GlcA	1 (0.44%)	-	1 (1.32%)	-
AFM1+FB1+DON+DON-15-GlcA	1 (0.44%)	-	-	1 (1.33%)
FB1+DON+DON-3-GlcA+DON-15-GlcA	2 (0.88%)	1 (1.32%)	1 (1.32%)	-
FB1+DON+DON-15-GlcA+ZEN	1 (0.44%)	1 (1.32%)	-	-
DON+DON-3-GlcA+DON-15-GlcA+ZEN	2 (0.88%)	2 (2.63%)	1 (1.32%)	-
Total	110 (48.46%)	43 (56.58%)	31 (40.79%)	36 (48.00%)

[1] Positive samples refer to urine samples containing mycotoxins with concentrations ≥ LOD.
[2] - , no sample.

Table S3. *P*-value of mycotoxin concentration distribution by age, sex, and BMI using the rank sum test (n = 227).

Mycotoxins	Age[1]	Sex	BMI[2]
AFM1	0.480	0.564	0.157
OTA	0.311	0.564	0.130
FB1	0.916	0.067	0.598
DON	0.712	0.162	0.375
DON-3-GlcA	0.221	0.370	0.678
DON-15-GlcA	0.508	0.137	0.506
ZEN	0.497	0.368	0.711
ZEN-14-GlcA	0.317	-[3]	0.317

[1]Including three age groups (20–45 years, 46–64 years and 65–88 years);
[2]Including three BMI categories (< 18.5, 18.5–23.9 and > 24 kg m^{-2}).
[3]*P*-value was not calculated since only two male urine samples were contaminated by ZEN-14-GlcA.

Table S4. Toxicities of FB1, OTA, DON, and ZEN from EFSA data.

Mycotoxin	Species	Duration	Critical effect	Dose (mg/kg bw/day)	TDI (μg/kg bw/day)	Reference
AFB1	Rat	Subchronic	Hepatocellular carcinomas	0.0004 ($BMDL_{10}$)	-	[1]
FB1	Mice	Chronic	Hepatotoxicity (increased incidence of megalocytic hepatocytes)	0.1 ($BMDL_{10}$)	1.0	[2]
OTA	Rat	Chronic	Neoplastic effect (kidney tumors)	0.0145 ($BMDL_{10}$)	-	[3]
	Mice	Chronic	Reduced body weight gain	0.11 ($BMDL_{05}$)	1.0	
DON	Rat	Subacute	Estrogenic activity (increase in germ cell degeneration, sperm retention and abnormal nuclear morphology; effects on serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH))	1 (NOAEL)	-	[4]
ZEN	Pig	Chronic	Estrogenic activity (disturbance of the estrous cycle, ovulation, conception and implantation)	0.01 (NOEL)	0.25	[5]

Abbreviation: NOEL: no observed effect level; $BMDL_{10}$: the 90th percentile benchmark dose lower confidence limit; NOAEL: no observed adverse effect level.

Table S5. Food consumption in the total population stratified by location.

Food	24 h consumption (g or mL) (Mean ± SD)				*P*[1]
	All	Jiangsu	Zhejiang	Shanghai	
Wheat	93.6 ± 103.0	80.6 ± 93.8	93.4 ± 107.1	107.0 ± 107.3	0.119
Maize	8.7 ± 33.1	7.9 ± 18.4	14.1 ± 52.2	4.0 ± 13.7	0.335
Rice	190.4 ± 119.4	176.3 ± 106.5	247.0 ± 126.4	147.3 ± 102.3	0.000*
Vegetables and fruit	179.9 ± 133.8	136.5 ± 79.5	206.6 ± 135.3	196.7 ± 163.6	0.015*
Meat	107.2 ± 92.9	109.2 ± 95.5	103.3 ± 101.3	109.0 ± 81.9	0.499
Nuts and seeds	24.4 ± 31.9	19.7 ± 24.6	29.6 ± 34.6	24.0 ± 35.0	0.189
Milk and dairy produce	47.1 ± 89.8	35.2 ± 80.0	49.3 ± 102.2	57.0 ± 85.6	0.022*
Beverages, coffee and tea	155.6 ± 213.6	249.0 ± 241.5	81.2 ± 166.5	136.3± 192.8	0.000*

[1]The *p*-value was calculated using the Kruskal–Wallis test, *$p < 0.05$.

Table S6. LC-MS/MS parameters for the detection of targeted mycotoxins.

Target	Molecular ion	Precursor ion (*m*/*z*)	Quantification ion (*m*/*z*)	CE[1] (Q) (eV)	Qualitative ion (*m*/*z*)	CE (q) (eV)	LOQ[2] (ng mL^{-1})	LOD[3] (ng mL^{-1})
AFB1	$[M+H]^+$	313	241	50	269	43	0.2	0.05
AFB2	$[M+H]^+$	315	259	38	287	34	0.5	0.2
AFG1	$[M+H]^+$	329	243	35	311	38	0.2	0.05
AFG2	$[M+H]^+$	331	245	40	285	37	0.5	0.2
AFM1	$[M+H]^+$	329	273	31	259	30	0.2	0.05
AFM2	$[M+H]^+$	331	189	54	216	48	0.2	0.05
OTA	$[M+H]^+$	404	239	34	221	22	0.1	0.05
OTα	$[M+H]^+$	257	102	40	221	22	0.1	0.05
FB1	$[M+H]^+$	722	334	50	352	50	0.2	0.1
DON	$[M+H]^+$	297	203	28	175	28	1	0.5
DON-3-GlcA	$[M-H]^-$	471	175	40	265	36	1	0.5
3-ADON	$[M+H]^+$	339	231	12	137	15	5	2
15-ADON	$[M+H]^+$	339	261	12	279	10	5	2
T-2	$[M+H]^+$	484	305	14	185	18	0.3	0.1
HT-2	$[M+H]^+$	442	263	10	215	10	1	0.5
FUS-X	$[M+H]^+$	355	229	15	247	13	10	4
ZEN	$[M-H]^-$	317	175	35	131	40	0.2	0.1
ZEN-14-GlcA	$[M-H]^-$	493	317	40	175	40	0.5	0.2
α-ZEL	$[M-H]^-$	319	174	38	130	45	0.5	0.2
β-ZEL	$[M-H]^-$	319	174	38	130	45	0.5	0.2
α-ZAL	$[M-H]^-$	321	277	31	161	40	0.5	0.2
β-ZAL	$[M-H]^-$	321	277	31	161	40	0.5	0.2

[1]Collision energies (CE) (eV) required for optimal signal strength.
[2]LOD (limit of detection).
[3] LOQ (limit of quantification).

References

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