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What emerging contaminants are in the urine of college students and what are their associated risks? analysis method development and applications

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Emerging contaminants (ECs) are widespread in the environment and pose notable health risks, yet their exposure levels among specific groups, such as college students, are underexplored. This study investigated the occurrence of ECs in human urine through suspect screening (537 ECs) and target analysis (50 prioritized ECs), alongside a human health risk assessment. An optimized solid-phase extraction method was compared with liquid–liquid extraction and supported-liquid extraction and was coupled with LC-TQMS analysis. This method demonstrated high reliability ($r = 0.997$), precision (0.05–14.7%), recoveries (52.6–113%) and sensitivity (LOD: 0.05–5.00 ng mL^{-1}). Urine samples were collected twice from 43 freshmen and once from 33 seniors (students from other grades), with accompanying questionnaires assessing their living environments and lifestyle habits. Eleven ECs were detected, with atrazine exhibiting a 100% detection frequency. Significant variations were observed in the urinary concentrations of 2,4-dinitrophenol, ethylparaben, metformin, and mycophenolic acid between freshmen and seniors, suggesting differences in exposure patterns influenced by living environments and personal habits. Statistical analyses identified correlations between EC exposure and personal care product use, with monobenzyl phthalate being a notable example. Health risk assessments indicated low overall risks but revealed higher hazard quotient (HQ) values for atrazine, 2,4-dinitrophenol, and mycophenolic acid, warranting further investigation. This study successfully developed a high-throughput and sensitive LC/MS method by integrating suspect screening with target analysis. It also provided a preliminary evaluation of EC exposure in a young student population through urine analysis, offering valuable insights for future research on environmental exposure and associated health risks.

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Environmental significance

This study highlights significant exposure to emerging contaminants (ECs) among college students, revealing compounds like atrazine, 2,4-dinitrophenol and mycophenolic acid as potential health risks. Advanced detection methods provide a robust framework for ongoing biomonitoring and environmental health assessments. Findings indicate the pervasive presence of ECs linked to lifestyle and the environment, emphasizing the need for stricter controls and public health measures. This research not only deepens understanding of interactions between humans and ECs, but also informs policy-making for enhanced environmental protection.

1 Introduction

Emerging contaminants (ECs), a diverse group of synthetic and naturally occurring chemicals, have raised increasing concern due to their widespread presence in the environment and potential risks to human health.[1–7] These compounds, which include pharmaceuticals, personal care products (PCPs), pesticides, and industrial chemicals, enter the environment through human activities, such as industrial discharge, agricultural runoff, and the improper disposal of consumer products.[8]

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Unlike traditional pollutants, ECs are not fully regulated in many regions, and their long-term health effects remain poorly understood. This has prompted growing interest in developing advanced analytical methodologies to assess human exposure to ECs and their associated health risks.[9–12]

Biomonitoring, particularly through urinary analysis, has proven to be a powerful tool for evaluating internal exposure to ECs.[13–15] Urine is a non-invasive and easily accessible biological matrix that reflects the metabolic processing of contaminants, thereby providing critical insights into both short-term and cumulative exposure levels.[16–20] However, traditional biomonitoring methods often fall short in capturing the complexity of EC exposure due to their limited sensitivity and scope, which are insufficient for identifying a broad range of contaminants, particularly those previously uncharacterized.[21–25] To address these challenges, innovative analytical approaches that integrate high throughput screening with precise quantification have emerged as essential tools for comprehensive exposure assessment.[26]

Liquid chromatography high-resolution mass spectrometry (LC-HRMS) has revolutionized biomonitoring by enabling suspect screening of thousands of potential contaminants in a single analysis.[27–29] Its exceptional sensitivity and resolution make it highly effective for detecting trace levels of ECs, even in complex biological matrices such as urine.[30] Complementing LC-HRMS, liquid chromatography coupled with triple quadrupole tandem mass spectrometry (LC-TQMS) offers unparalleled capabilities for the targeted quantification of specific analytes, ensuring high accuracy, sensitivity and reproducibility.[21,31,32] The integration of these advanced technologies provides a balanced approach that combines the extensive coverage of LC-HRMS with the precision and reliability of LC-TQMS, allowing for a comprehensive assessment of EC exposure.

To contextualize the biomonitoring findings, a Hazard Quotient (HQ) analysis was conducted to evaluate potential health risks. By comparing exposure levels to established safety thresholds, contaminants with elevated HQ values were identified as priorities for further investigation. This streamlined approach aligns quantitative exposure data with actionable health risk insights, directly supporting the study's goal of identifying high-priority contaminants.[25,33]

This study leverages the strengths of LC-HRMS and LC-TQMS to develop and apply a robust methodology for the biomonitoring of ECs in human urine. By screening 537 suspect ECs and quantifying 50 prioritized ECs, this research characterizes exposure levels and dynamic fluctuations of ECs within a group of college students in North China. The use of Solid-Phase Extraction (SPE) for sample preparation further enhances the sensitivity and reliability of the analytical process. Additionally, a health risk assessment based on Hazard Quotient (HQ) calculations is conducted to evaluate the potential health implications of the detected contaminants. This study investigates whether urinary contaminants form distinctive regional fingerprints or reflect ubiquitous compounds by comparing newly enrolled students (freshmen) from diverse areas and students from other grades already residing on the university campus (seniors), while also assessing how these regional differences evolve—attenuating or enhancing—after 100 days in a standardized campus environment through repeat sampling. Through this integrated approach, this study provides valuable insights into the prevalence, variability, and risk profiles of EC exposure in young adults, offering a foundation for future environmental management and public health strategies.

2 Materials and methods

2.1 Chemicals and reagents

Chemical standards of the 50 ECs and internal standards including metformin-D6 HCl, metoprolol-D7 HCl, telmisartan-D3, and ranitidine-D6 HCl were acquired from Beijing Jindizehao Technology Co., Ltd and Beijing Putian Tongchuang Biotechnology Co., Ltd Target Chemicals: 4-hydroxytamoxifen (4-OHT), 7-amino-4-methylcoumarin (7-AMC), 7-hydroxycoumarin (7-HC), acephate (ACP), acetamiprid (ATP), albendazole (ABZ), amiodarone (AMD), atrazine (ATZ), benzocaine (BNC), bezafibrate (BZB), bicalutamide (BCT), fluticasone propionate (FP), candesartan (CDT), captopril (CAP), celecoxib (CLC), chloridazon (CLZ), diflubenzuron (DFZ), dimethenamid (DMA), exemestane (EXM), fluoxastrobin (FLX), glyburide (GLY), ketoprofen (KP), lidocaine (LDC), linuron (LNR), marbofloxacin (MBF), mebeverine (MBE), metconazole (MTC), metformin (MET), metoprolol (MTP), nitrendipine (NTD), mirtazapine (MRZ), oryzalin (OYZ), oxybutynin (OXY), monobenzyl phthalate (MBzP), promethazine (PMT), raloxifene (RLX), sotalol (STL), sulfadimethoxine (SDM), sulfathiazole (STZ), telmisartan (TLS), pendimethalin (PDM), terbuthylazine (TBZ), thiabendazole (THZ), triclocarban (TCC), mycophenolic acid (MPA), perfluorohexanoic acid (PFHxA), ethylparaben (EP), 2,4-dinitrophenol (DNP), methylparaben (MP) and ranitidine (RNT). The molecular formulae and CAS numbers of ECs are provided in Table 1. Standard stock solutions were prepared by weighing the chemical standards and dissolving them in the corresponding amount of methanol. These stock solutions were stored at −20 °C to ensure stability.

Methanol (HPLC grade, Sigma-Aldrich, USA), formic acid (HPLC grade, Aladdin Biochemical Technology Co., Ltd, China), ammonium formate (HPLC grade, Macklin Biochemical Technology Co., Ltd, China), phosphate buffer (Tianjin Chemical Reagent Third Co., China), diethyl ether (Xilong Scientific Co., China), and Milli-Q water. Oasis® HLB (200 mg/6 mL, Waters, USA), Oasis® HLB (60 mg/3 mL, Waters, USA), Poly-Sery HLB Pro SPE (60 mg/3 mL, CNW, Germany), Cleanert® SLE Diatomaceous Earth (Neutral) (6 mL/1 mL Agela Technologies, China), PTFE-Q membrane syringe filters (0.2 μm, Agilent, USA), and centrifuge tubes (50 mL, Beijing Jindizehao Technology Co., Ltd, China) were used. For chromatographic separation, a Kinetex-C18 column (3.0 mm × 100 mm, 2.6 μm, Phenomenex, USA) was employed.

2.2 Instruments

The primary analytical instruments used in this study were an Agilent 1290 Infinity II-6550 ultra-high-performance liquid

Table 1 Name, molecular formula, CAS number, LC-TQMS precursor ions, product ions, and retention time information for the 50 ECs

EC name	Formula	CAS no.	ESI mode	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	Fragmentor (V)	CE (eV)	Retention time (min)
4-Hydroxytamoxifen	$C_{26}H_{29}NO_2$	680 47-06-3	Positive	388	58/72	182	25/29	16.4
7-Amino-4-methylcoumarin	$C_{10}H_9NO_2$	260 93-31-2	Positive	176	77/120	116	50/25	10.2
7-Hydroxycoumarin	$C_9H_6O_3$	93-35-6	Positive	163	91/107	116	25/25	9.42
Acephate	$C_4H_{10}NO_3PS$	305 60-19-1	Positive	184	94.9/143	60	28/4	2.67
Acetamiprid	$C_{10}H_{11}ClN_4$	135 410-20-7	Positive	223	56/125.9	116	13/21	9.84
Albendazole	$C_{12}H_{15}N_3O_2S$	549 65-21-8	Positive	266	191/234	116	37/21	16.5
Amiodarone	$C_{25}H_{29}I_2NO_3$	1951-25-3	Positive	646	100/58	182	33/45	19.7
Atrazine	$C_8H_{14}ClN_5$	1912-24-9	Positive	216	131.8/174	118	25/15	15.3
Benzocaine	$C_9H_{11}NO_2$	1994/9/7	Positive	166	94/138	50	17/9	17.4
Bezafibrate	$C_{19}H_{20}ClNO_4$	418 59-67-0	Positive	362	121/138.9	121	36/28	12.0
Bicalutamide	$C_{18}H_{14}F_4N_2O_4S$	903 57-06-5	Positive	431	94.9/216.9	182	49/17	16.5
Fluticasone propionate	$C_{25}H_{31}F_3O_5S$	804 74-14-2	Positive	501	275.1/293.1	116	29/13	19.2
Candesartan	$C_{24}H_{20}N_6O_3$	139 481-59-7	Positive	441	192/263	116	29/9	16.8
Captopril	$C_9H_{15}NO_3S$	625 71-86-2	Positive	218	75/116	116	20/12	8.17
Celecoxib	$C_{17}H_{14}F_3N_3O_2S$	169 590-42-5	Positive	382	281.5/362	150	45/29	18.6
Chloridazon	$C_{10}H_8ClN_3O$	1698-60-8	Positive	222	103.9/77	116	25/45	9.72
Diflubenzuron	$C_{14}H_9ClF_2N_2O_2$	353 67-38-5	Positive	311	141/158	101	40/12	18.8
Dimethenamid	$C_{12}H_{18}ClNO_2S$	876 74-68-8	Positive	276	168/244	116	25/13	17.0
Exemestane	$C_{20}H_{24}O_2$	107 868-30-4	Positive	297	93/121	116	29/21	17.1
Fluoxastrobin	$C_{21}H_{16}ClFN_4O_5$	361 377-29-9	Positive	459	110.9/427	116	50/17	18.5
Glyburide	$C_{23}H_{28}ClN_3O_5S$	102 38-21-8	Positive	494	168.9/369	116	49/13	18.8
Ketoprofen	$C_{16}H_{14}O_3$	220 71-15-4	Positive	255	105/209	130	24/12	16.4
Lidocaine	$C_{14}H_{22}N_2O$	137-58-6	Positive	235	58/86	108	40/15	6.26
Linuron	$C_9H_{10}Cl_2N_2O_2$	330-55-2	Positive	249	62/160	116	13/21	16.8
Marbofloxacin	$C_{17}H_{19}FN_4O_4$	115 550-35-1	Positive	363	320/72	132	15/25	7.03
Mebeverine	$C_{25}H_{35}NO_5$	3625/6/7	Positive	430	121/149	116	45/25	13.3
Metconazole	$C_{17}H_{22}ClN_3O$	125 116-23-6	Positive	320	125/70	110	50/29	19.7
Metformin	$C_4H_{11}N_5$	657-24-9	Positive	130	71/60	116	25/13	1.36
Metoprolol	$C_{15}H_{25}NO_3$	513 84-51-1	Positive	268	116/56	116	17/33	8.67
Nitrendipine	$C_{18}H_{20}N_2O_6$	395 62-70-4	Positive	361	283/315	116	25/5	18.0
Mirtazapine	$C_{17}H_{19}N_3$	613 37-67-5	Positive	266	72/195	116	21/29	9.51
Oryzalin	$C_{12}H_{18}N_4O_6S$	190 44-88-3	Positive	347	217/288	116	28/16	18.5
Oxybutynin	$C_{22}H_{31}NO_3$	5633-20-5	Positive	358	72/124	182	45/21	14.9
Monobenzyl phthalate	$C_{15}H_{12}O_4$	2528-16-7	Positive	257	65/91	70	50/16	15.8
Promethazine	$C_{17}H_{20}N_2S$	60-87-7	Positive	285	71/86	94	50/16	13.4
Raloxifene	$C_{28}H_{27}NO_4S$	844 49-90-1	Positive	474	84/112	182	50/33	13.3
Sotalol	$C_{12}H_{20}N_2O_3S$	3930-20-9	Positive	273	176/133	101	20/32	2.36
Sulfadimethoxine	$C_{12}H_{14}N_4O_4S$	122-11-2	Positive	311	65/165	116	50/21	11.4
Sulfathiazole	$C_9H_9N_3O_2S_2$	72-14-0	Positive	256	92/156	116	29/13	5.56
Telmisartan	$C_{33}H_{30}N_4O_2$	144 701-48-4	Positive	515	497/276	248	41/50	18.3
Pendimethalin	$C_{13}H_{19}N_3O_4$	404 87-42-1	Positive	282	194/212	90	17/9	22.0
Terbuthylazine	$C_9H_{16}ClN_5$	5915-41-3	Positive	230	68/174	116	45/17	17.3
Thiabendazole	$C_{10}H_7N_3S$	148-79-8	Positive	202	65/175	182	50/29	8.87
Triclocarban	$C_{13}H_9Cl_3N_2O$	101-20-2	Positive	315	93/127	116	49/50	20.7
Mycophenolic acid	$C_{17}H_{20}O_6$	242 80-93-1	Negative	319	199/275	116	24/16	6.77
Perfluorohexanoic acid	$C_6HF_{11}O_2$	307-24-4	Negative	313	119/269	50	25/5	7.09
Ethylparaben	$C_9H_{10}O_3$	120-47-8	Negative	189	153/109	110	16/28	5.99
2,4-Dinitrophenol	$C_6H_4N_2O_5$	51-28-5	Negative	165	137/92	116	13/25	6.31
Methylparaben	$C_8H_8O_3$	99-76-3	Negative	151	136/92	90	12/24	5.89
Ranitidine	$C_{13}H_{22}N_4O_3S$	663 57-35-5	Negative	313	142/170	116	8/12	1.45

chromatograph coupled with a quadrupole-time-of-flight mass spectrometer (UPLC-QTOF/MS) (Agilent Technologies, USA), and a 1260–6470 liquid chromatography-triple quadrupole tandem mass spectrometer (LC-TQMS) (Agilent Technologies, USA). Additional equipment included a Solid-Phase Extraction (SPE) apparatus (Waters, USA), a nitrogen evaporator (Oulutec, Shanghai, China), a Milli-Q ultrapure water system (Millipore, USA), a vortex mixer (Dragon Lab, China), and micropipettes (Eppendorf, Germany).

2.3 Method development

2.3.1 Sample collection

2.3.1.1 Pooled urine sample for method development. University student populations represent an ideal cohort for investigating long-term cumulative exposure to emerging contaminants and associated health impacts,[34] owing to their enclosed lifestyles, concentrated exposure pathways, and well-defined temporal gradients. To establish and refine the

parameters for the LC/MS analytical methodology, morning urine samples (approximately 50 mL) were obtained from 10 college student volunteers (aged 22–28 years), each of whom had abstained from water intake prior to collection. A 100 mL pooled sample was prepared by taking and mixing 10 mL from each volunteer. A portion of this pooled sample was immediately subjected to LC-HRMS analysis, while the remainder was preserved at −20 °C for subsequent use.

2.3.1.2 Urine sample collection. In this study, 76 college students residing in campus dormitories were enlisted to provide urine samples, including 43 first-year students who had just enrolled during sampling and 33 senior students (37 males and 39 females; male-to-female ratio: 1 : 1.05). To determine whether individual variations in the living environment, diet, and water source influence human environmental exposure, sampling was performed twice for these first-year students. The 1st sampling was performed upon their first enrollment at the university (in September 2024) from their hometowns in different regions of China (sample numbers: 1–1 to 1–43). The 2nd sampling was performed after 100 days of dormitory residence for all 76 enlisted students (sample numbers: 2–1 to 2–76). This study was approved by the Ethics Committee of Shanxi Medical University (Serial Number: 2024SJL7), with proof available upon request. It adhered to ethical principles, respecting participants' privacy rights, and informed consent was obtained.

Morning urine samples (the first urine pass of the day, approximately 50 mL) were collected from the volunteers. These volunteers had fasted and refrained from drinking water prior to collection. Questionnaires gathering information on their pre-university residence, surrounding environment, smoking habits, and frequency of personal care product use were completed by volunteers. All different options for questionnaire factors were assigned a score from 0 to 5 (see the content in Table S3 of the SI). All participants voluntarily provided both their urine samples and personal information. The samples were divided into two aliquots and stored at −20 °C. On the morning of urine collection, Milli-Q water was used as procedural blanks and methanol was used as a solvent blank.

2.3.2 Suspect screening. Pooled urine samples from ten volunteers were pre-treated by SPE according to the method described in the literature[35] and UPLC-QTOFMS suspect screening was executed using a 537 ECs suspect screening list based on our previous studies[36] and the literature. The suspect list includes pesticides and some metabolites, pharmaceuticals (*e.g.* antibiotics, antidepressants, antifungals, antihypertensives, analgesics, *etc.*), industrial chemicals (plasticizers, solvents, flame retardants, organic chemical intermediates, *etc.*), personal care products (cosmetics, sunscreens, antimicrobials, and fragrances), and others such as PFAS (per- and polyfluoroalkyl substances) and UV stabilizers. The detailed database information and references can be found in SI Table S1.

Instrumental analysis was performed on the UPLC-QTOFMS. The LC and QTOFMS conditions were as follows: a Kinetex-C18 column (100 mm × 3.0 mm, 2.6 μm) was used and the mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (methanol). The column temperature was maintained at 22 °C. The gradient elution was programmed as follows: 10% solvent B was held constant from 0 to 3.2 min, followed by a linear increase to 95% B between 3.2 and 21 min. The 95% B composition was maintained from 21 to 41 min, after which it was reduced back to 10% B between 41 and 50 min. The flow rate was 0.2 mL min^{-1} and the injection volume was 20 μL. Mass spectrometry analysis utilized an electrospray ionization (ESI) source. Nitrogen was employed as both the drying and nebulizing gas, with the drying gas temperature set at 300 °C at a flow rate of 14 L min^{-1}. The nebulizer operated at a pressure of 3 psi, while the capillary voltage was set at 3.5 kV, and the fragmentor voltage was maintained at 380 V. The sheath gas temperature was 350 °C, with a flow rate of 11 L min^{-1}. For detection, a full-scan mode was employed, covering a mass-to-charge ratio (m/z) range of 100–1000 with a scan rate of 1.4 spectra per second and a cycle time of 714.3 milliseconds per spectrum. Additionally, target MS/MS mode (data independent analysis, DIA) was utilized for fragmentation analysis, with collision energies set at 10 eV, 30 eV, and 50 eV respectively to detect daughter ions within the 50–1000 m/z range.

Peaks were selected based on the following criteria: signal intensity greater than 2,000, a signal-to-noise ratio (S/N) exceeding 3, and peak values at least five times higher than the blank samples. The matching parameters for compound identification were established as follows: mass tolerance was 15 ppm, retention time window was 0.5 min, and ion forms in both positive ion mode ($[M + H]^+$ and $[M + Na]^+$) and negative ion mode ($[M - H]^-$).

Compounds with high match scores were subjected to further analysis using target MS/MS mode. These results were compared against publicly available mass spectrometry databases, such as MassBank (**https://massbank.eu/MassBank/**) and DrugBank (**https://go.drugbank.com/**). As a result, 50 ECs were selected as priority ECs, for establishing the quantification analytical approach in later steps. The details of these 50 ECs are provided in Table 1.

2.3.3 Target analysis

2.3.3.1 LC-TQMS. Due to the extremely low concentrations of ECs in biological samples, a more sensitive quantification method was developed using LC-TQMS in multiple reaction monitoring (MRM) mode for the 50 selected ECs. Detailed information can be found in Table 1. The instrumental parameter settings were as follows: a Kinetex-C18 column (3.0 mm × 100 mm, 2.6 μm) was used and the mobile phase consisted of solvent A (methanol) and solvent B (0.1% formic acid + 10 mmol L^{-1} ammonium formate in water). The gradient elution in positive ion mode was as follows: from 0 to 1 min, 10% solvent A was maintained, followed by an increase from 10% to 90% A from 1 to 21 min, then the percentage of A decreased from 90% to 10% from 21 to 21.5 min, and finally 10% A was maintained from 21.5 to 25 min. The gradient elution in negative ion mode was as follows: from 0 to 0.5 min, 10% A was maintained, followed by an increase from 10% to 90% A from 0.5 to 4.5 min, then the percentage of A decreased from 90% to 10% from 4.5 to 8 min, and finally 10% A was

maintained from 8 to 10 min. The column temperature was operated at a temperature of 40 °C, with a flow rate of 0.3 mL min^{-1}, and the injection volume was 5 μL. Mass spectrometric detection was carried out using an Agilent Jet Stream (AJS) Electrospray ionization (ESI) source. The capillary voltage was set to 3500 V, with a nebulizer gas pressure of 45 psi. The temperature of the nebulizing gas was set at 300 °C, with a flow rate of 5 L min^{-1}.

2.3.4 Sample extraction protocol

2.3.4.1 Selection of extraction methods. In this study, three extraction techniques—Liquid–Liquid Extraction (LLE), Supported-Liquid Extraction (SLE), and Solid-Phase Extraction (SPE)—were evaluated for their efficiency in extracting targeted ECs from urine samples. Diazepam-D5 and a mixed standard solution of 50 ECs were spiked into urine samples, with three parallel extractions performed before and after spiking. Recovery rates were used as the primary evaluation metric. The details of extraction procedures of SLE, LLE and SPE are listed in SI Text S1.

2.3.4.2 Selection of SPE cartridges. Three cartridges were tested to optimize extraction: Oasis® HLB (200 mg/6 mL), Oasis® HLB (60 mg/3 mL), and CNW Poly-Sery HLB Pro SPE (60 mg/3 mL). The adsorption efficiency of SPE sorbents is influenced by both the pK_a of the compounds and the pH of the sample. To optimize recovery, different sample pH levels (3.0, 7.0, and 9.0) and elution solvents (5% formic acid in methanol; methanol; 5% ammonia in methanol) were tested.

2.3.4.3 The optimal extraction protocol. 1 mL of the pooled urine sample was pipetted into a tube. Subsequently, 20 μL of a 10 μg mL^{-1} mixed internal standard solution was added. Following this, 2 mL of phosphate buffer (pH = 7) was introduced, and the mixture was vortexed thoroughly before being applied to the CNW Poly-Sery HLB Pro SPE (60 mg/3 mL) column. The SPE column was preconditioned with 3 mL of methanol, followed by 3 mL of deionized water. The urine sample was allowed to pass through the column under gravity, after which the column was rinsed with 3 mL of deionized water. The SPE column was subsequently dried under negative pressure for 20 min. Elution was performed with 1.5 mL of methanol followed by 1.5 mL of 5% ammoniated methanol. The eluted solution was evaporated at 45 °C under a nitrogen stream, reconstituted in 0.2 mL of the initial mobile phase, vortexed, filtered through a 0.2 μm membrane, and transferred to an amber injection vial for LC-TQMS analysis.

2.3.4.4 Method validation. Linearity, Limits of Detection (LODs), and Limits of Quantification (LOQs): a mixed standard solution containing the 50 ECs was spiked into 1 mL of pooled urine sample to prepare calibration standards at concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0, 50.0, 100.0, 200.0, and 500.0 ng mL^{-1}, respectively. The extraction and analysis were performed as described in the optimal extraction protocol section.

The precision and accuracy were evaluated by spiking pooled urine samples with the mixed standard solution of the 50 ECs at low, medium, and high concentration levels, along with four internal standards with a constant concentration of 200 ng mL^{-1}. Six replicates of each sample were analyzed. Intra-day precision was assessed by same-day repeated analyses, while inter-day precision was assessed over three consecutive days. Precision was expressed as relative standard deviation (RSD) and accuracy as analyte recovery.

2.3.4.5 Target analysis of 50 priority emerging contaminants (ECs). The optimized method was applied to analyze the urine samples collected from 76 college students, following the sampling procedure outlined in Section 2.3.1. Data analysis for qualification and quantification was executed using MassHunter software (Agilent, USA). Statistical evaluations were conducted *via* R (version 4.4.0; R Core Team, 2024) and SPSS Statistics software (IBM, USA). To compare urine results among three distinct groups—freshmen 1st sampling (F1), freshmen 2nd sampling (F2), and senior students (S)—the Kruskal–Wallis test was utilized, followed by Dunn's test as a post-hoc method, with a significance threshold of $p < 0.05$. Additionally, Spearman correlation analysis was applied to examine the relationship between questionnaire factors and the results, with significant correlations determined at $p < 0.05$.

2.4 Health risk assessment

The Hazard Quotient (HQ) is a widely used metric for evaluating whether the chemical concentrations detected in urine samples could pose potential health risks. A HQ value of less than 1 typically signifies low risk, indicating that the exposure concentration is below the toxicological reference level and is considered acceptable. Conversely, a HQ greater than 1 suggests elevated risk, meaning that the exposure exceeds the reference level and may potentially lead to adverse health effects, necessitating the implementation of risk mitigation strategies.[37] The HQ is calculated based on the following formula:

$$\text{HQ} = \text{estimated daily intake (EDI)/reference dose (RfD)} \quad (1)$$

where EDI is the estimated daily intake, expressed in mg per kg per day. RfD is the reference dose, which is a toxicologically derived safe exposure level, often determined by regulatory agencies like the U.S. Environmental Protection Agency (EPA). It represents the daily exposure level that is not expected to cause adverse health effects over a lifetime. Alternatively, if no explicit RfD is available for prescription drugs, therapeutic doses may serve as reference values, and safety doses derived from toxicological studies including NOAEL (No Observed Adverse Effect Level) or LOAEL (Lowest Observed Adverse Effect Level), can be used, adjusted with uncertainty factors.

$$\text{HQ} = \text{estimated daily intake (EDI)/NOAEL or LOAEL} \quad (2)$$

The formula for estimating daily intake (EDI) is as follows:

$$\text{EDI} = C_u \times V_u / (F \times \text{BW}) \quad (3)$$

where C_u is the concentration of the EC in urine, typically in mg L^{-1}. V_u is the daily urine excretion volume, assumed to be 1.5 L per day for an average adult in this study. BW is the body weight, in kg (with the average adult body weight in China being 60 kg, according to the 2020 Report on Nutrition and Chronic Diseases of Chinese Residents). F is the absorption coefficient,

representing the fraction of the chemical excreted in urine relative to total intake. For certain chemicals, this coefficient can be obtained from the literature. If no absorption coefficient is available, the bioavailability score can be used as a substitute. The bioavailability score predicts the likelihood of a compound being absorbed systemically following oral ingestion, based on its physicochemical properties. This score was estimated using the online tool SWISSADME (**http://www.swissadme.ch/**) in this study, which predicts bioavailability based on the SMILES of ECs.

This method assumes that the concentration of an EC in urine reflects the daily intake, and critical factors such as excretion volume and body weight are essential for estimating the daily exposure dose. In this study, the median and the 90th percentile concentration were used respectively to simultaneously assess typical risks and high-exposure risks. Additionally, Table S4 in the SI provides detailed data on the body weight (BW), urine volume, *F*/BS ratio, and reference dose (RfD) of the 10 quantified ECs.

3 Results and discussion

3.1 Suspect screening of pooled urine samples by LC-HRMS

Using LC-HRMS, a total of 537 ECs were screened in pooled urine samples. In positive electrospray ionization mode (ESI^+), 408 features were identified by matching precursor ions against the suspect list, while 75 features were detected in negative electrospray ionization mode (ESI^-). After further refinement, 68 ECs in ESI^+ mode and 12 ECs in ESI^- mode with well-defined peak shapes were confirmed by matching product ions with online mass spectral databases, such as MassBank.

Subsequently, a detailed literature review was conducted to evaluate the toxicity of these compounds and their occurrence in the environment. This assessment aimed to prioritize substances that posed significant potential risks to human health and environmental safety. Finally, the availability of chemical standards was considered as a practical criterion for further analysis. Based on these factors—toxicity, environmental presence, and standard availability—a total of 50 ECs were selected as prioritized compounds for targeted analysis.

3.2 Establishment and validation of the LC-TQMS method

3.2.1 Optimization of instrumental parameters. For the organic mobile phase, methanol and acetonitrile were tested. It was observed that the peak area with methanol was higher than that with acetonitrile, leading to the selection of methanol as the organic mobile phase. When Milli-Q water was used as the aqueous mobile phase, some compounds did not elute. The addition of 0.1% formic acid to the aqueous mobile phase resulted in the appearance of two peaks for certain compounds. Consequently, different concentrations of ammonium formate (5, 10, and 20 mmol L^{-1}) were added to 0.1% formic acid water solution. At 10 mmol per L ammonium formate, most compounds showed the best performance. Therefore, the final aqueous mobile phase selected was 0.1% formic acid and 10 mmol per L ammonium formate in Milli-Q water.

To achieve optimal chromatographic separation, we further optimized the parameters for column temperature, flow rate and injection volume. Column temperatures of 20 °C, 30 °C, and 40 °C were tested while keeping other conditions constant. The column temperature of 40 °C provided the highest peak area. For the flow rate, 0.2 mL min^{-1}, 0.3 mL min^{-1} and 0.4 mL min^{-1} were tested. At 0.2 mL min^{-1}, elution was slow with a low response, while at 0.4 mL min^{-1}, the response and peak shape were inferior to those at 0.3 mL min^{-1}. Thus, 0.3 mL min^{-1} was selected as the optimal flow rate. Regarding injection volume, 5 μL, 10 μL and 15 μL were evaluated. At 15 μL, some compounds exhibited tailing (*e.g.*, raloxifene), while at 10 μL and 5 μL, the peaks were symmetrical, and the peak area reached a maximum of 10^9 orders of magnitude. Consequently, 5 μL was chosen as the injection volume to conserve the sample and prevent contamination of the instrument. This thorough optimization of LC-TQMS conditions ensures the precise and reliable detection of ECs in urine samples and supports high-throughput analysis with robust reproducibility.

3.2.2 Optimization of pretreatment conditions

3.2.2.1 Selection of extraction methods. The comparison of recoveries across LLE, SLE, and SPE extraction methods is shown in Fig. S1 in the SI. The results revealed that SPE provided remarkably higher recoveries, with 39 ECs showing recoveries between 70% and 110%, compared to 31 and 27 ECs for LLE and SLE, respectively. Consequently, SPE was chosen as the extraction technique for further parameter optimization.

3.2.2.2 Selection of SPE cartridge specifications. As displayed by Fig. S2 in the SI, the HLB (200 mg/6 mL) cartridge showed lower recoveries (around 60%) due to its larger sorbent mass, which may have retained analytes more strongly, making elution difficult. Recoveries for most ECs were similar between the Oasis® HLB (60 mg/3 mL) and CNW Poly-Sery HLB Pro SPE (60 mg/3 mL) cartridges. However, considering cost-effectiveness, the latter was selected for subsequent experiments.

Three sample pH values (3.0, 7.0 and 9.0) and three elution solvents (5% formic acid in methanol, methanol, and 5% ammonia in methanol) were tested. As displayed in Fig. 1, the analysis showed that the sample at pH 7 with 5% (v/v) ammonia in methanol was most effective. Thus, a two-step elution process was adopted: first with methanol, followed by 5% (v/v) ammonia in methanol, to maximize recoveries.

3.2.3 Method validation

3.2.3.1 Standard curve linear range, LODs and LOQs. The linear range and the LODs for the 50 ECs were determined following the procedures outlined in Section 2.3.3. All ECs exhibited excellent linearity across their respective ranges, with an average determination coefficient (r) of 0.997. The LODs for the ECs ranged from 0.05 to 5.00 ng mL^{-1}, while the LOQs spanned from 0.10 ng mL^{-1} to 10.0 ng mL^{-1} (as shown in Table 2).

3.2.3.2 Precision and accuracy. The precision and accuracy of the method were evaluated as outlined in Section 2.3.3. The intra-day RSD for the 50 ECs ranged from 0.05% to 13.0%, while

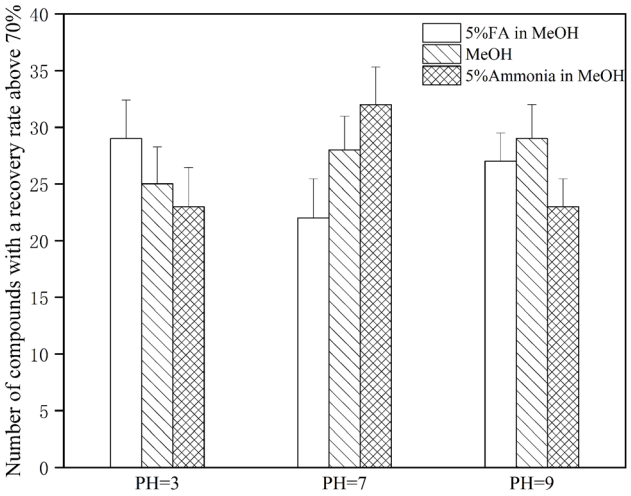

Fig. 1 Comparison of recoveries at three sample pH values using three elution solutions of SPE.

the inter-day RSD varied from 0.45% to 14.7%. The EC recoveries in spike samples were observed to range between 52.6% and 113%. Detailed results are provided in SI Table S2.

The method validation clearly demonstrates that this approach is robust, ensuring reliable and accurate quantification of the 50 selected ECs in urine samples. This validation process confirmed that the method meets the necessary requirements for precision, accuracy, and sensitivity, making it a reliable tool for biomonitoring and environmental studies involving trace-level contaminant analysis in biological fluids.

3.3 ECs in urine samples of college students

Urine samples from 76 college student volunteers, including 43 freshmen and 33 senior students, were analyzed using a multi-target detection LC-TQMS approach for 50 ECs. The analysis detected a total of 11 ECs, categorized as 4 pesticides, 3 pharmaceuticals, 3 personal care products, and 1 industrial chemical. Among them, as shown in Table 3, atrazine (ATR) exhibited 100% detection frequency across all students. Captopril (CAP) was present in 18.6% to 27.3% of samples from the F1 (freshmen 1st sampling), F2 (freshmen 2nd sampling), and S (senior student) groups, respectively. Monobenzyl phthalate (MBzP) and methylparaben (MP) had relatively high detection frequencies of 11.6% in the F1 group. Additionally, 2,4-dinitrophenol (DNP) was detected in 55.8% of samples from the F2 group, while ethylparaben (EP) showed a detection frequency exceeding forty percent in the S group.

Quantitative analysis was performed for 10 ECs, excluding pendimethalin, which had concentrations below the LOQ. The concentration ranges for the detected ECs were as follows: ATR (0.110–55.7 ng mL^{-1}), CAP (10.2–28.6 ng mL^{-1}), CLZ (1.08–1.72 ng mL^{-1}), DMA (29.6 ng mL^{-1}), MET (0.170–4833 ng mL^{-1}), MBzP (0.190–2.02 ng mL^{-1}), DNP (1.29–7.65 ng mL^{-1}), EP (5.94–37.9 ng mL^{-1}), MP (0.340–92.9 ng mL^{-1}), and MPA (0.0700–23.1 ng mL^{-1}).

Fig. 2 shows significant differences in the concentrations of four compounds—DNP, EP, MET, and MPA—among the three groups (F1, F2, and S). In particular, DNP and MET exhibited notable deviations between F1 and F2, while the concentration distributions of DNP, EP, MET, and MPA showed significant differences between the freshmen and senior groups. Such findings highlighted variations in EC exposure levels potentially influenced by lifestyle or environmental factors associated with different stages of college life.

3.3.1 Pesticides. As presented in Table 3, the mean concentrations of ATR, a widely used triazine herbicide, were comparable among the groups: 6.09 ng mL^{-1} and 8.19 ng mL^{-1} for freshmen in the first and second samplings, and 6.40 ng mL^{-1} for seniors, respectively. Statistical analysis using the Kruskal–Wallis test revealed no significant differences in ATR levels among the three groups ($p > 0.05$). Previous research on ATR exposure *via* urine has largely focused on agricultural workers and vulnerable populations such as pregnant women and children. These studies, though dated, reported ATR concentrations in urine ranging from 15 ng mL^{-1} to as high as 16 000 ng mL^{-1}, which significantly exceeds the levels observed in this study. ATR exposure has been associated with adverse effects on reproductive health and hormone regulation, highlighting its potential health risks.[38] It has been shown to induce oxidative stress in animal models, including rats and mice, as reported in previous studies.[39,40] Furthermore, our prior investigations identified tap water as a potential source of ATR exposure for humans. Tap water samples collected on the university campus between 2022 and 2023 demonstrated 100% detection frequency of ATR, with mean concentrations of 4.32 ng L^{-1} in autumn and 9.26 ng L^{-1} in winter, respectively. Similarly, tap water samples from various regions across China consistently showed ATR occurrence (100%) with concentrations ranging from 0.25 to 96.03 ng L^{-1}.[41] The findings underscore ATR's persistent presence in the environment and its potential for human exposure. Other research groups have also reported ATR contamination in food sources, including agricultural crops, fruits, tea, and raw bovine milk.[41–43]

CLZ, a selective herbicide historically regarded as relatively low in toxicity due to its widespread use over decades, was detected exclusively in the urine samples of freshmen during the second sampling in this study. The mean concentration observed was 1.43 ng mL^{-1}, and the detection frequency was 7.00%. Similarly, a study by Ahrens *et al.* in southern Sweden identified CLZ concentration at 1.60 ng L^{-1} in a passive water sample, which exceeds the EU's standard detection limit and aligns with the exposure levels observed in the present study.[44]

DMA, a frequently used glyphosate derivative and the second most widely employed herbicide globally after organophosphates, was detected in only one sample, with a concentration of 29.6 ng mL^{-1}. Despite its extensive application, DMA has been associated with potential skin sensitization, as highlighted by EFSA.[21] The limited detection in this study underscores the need for further research to evaluate the long-term health risks associated with its sporadic environmental presence and human exposure.

PDM, a dinitroaniline herbicide, was detected at a level above the LOD but below the LOQ. It is known to infiltrate the human body through dermal contact, inhalation, or ingestion.

Table 2 Calibration curves, linear ranges, LODs and LOQs of the 50 preferred ECs using the LC-TQMS method

ECs	Calibration curve	Correlation coefficient	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
4-OHT	$y = 0.0760x - 0.00250$	0.995	35.0–500	1.00	5.00
7-AMC	$y = 1.87x + 14.73$	0.995	5.00–500	0.05	0.10
7-HC	$y = 3.29x + 0.00510$	0.998	15.0–500	0.05	0.10
Acephate	$y = 11.2x + 0.654$	0.997	15.0–500	0.05	0.10
Acetamiprid	$y = 12.6x + 0.363$	0.998	15.0–500	0.05	0.10
Albendazole	$y = 2.05x - 0.00740$	0.997	15.0–500	0.05	0.10
Amiodarone	$y = 0.0543x - 0.00130$	0.996	30.0–500	0.10	5.00
Atrazine	$y = 1.29x - 0.0284$	0.997	15.0–500	0.01	0.10
Benzocaine	$y = 1.90x - 0.0386$	0.996	10.0–500	5.00	10.0
Bezafibrate	$y = 0.257x - 0.00820$	0.997	20.0–500	1.00	5.00
Bicalutamide	$y = 0.0930x + 0.00320$	0.996	20.0–500	0.50	1.00
FP	$y = 0.0437x + 9.78$	0.995	20.0–500	0.50	1.00
Candesartan	$y = 0.263x - 0.00340$	0.996	20.0–500	0.10	0.50
Captopril	$y = 0.0895x - 0.00310$	0.996	30.0–500	5.00	10.0
Celecoxib	$y = 0.197x - 9.090$	1.000	30.0–500	0.05	0.10
Chloridazon	$y = 5.01x + 0.114$	0.998	15.0–500	0.50	1.00
DFZ	$y = 0.279x - 0.00120$	0.998	20.0–500	0.50	1.00
Dimethenamid	$y = 3.24x - 0.0851$	0.997	15.0–500	0.05	0.10
Exemestane	$y = 0.483x + 0.0112$	0.995	20.0–500	0.05	0.10
Fluoxastrobin	$y = 1.64x + 0.0285$	0.995	20.0–500	0.05	0.10
Glyburide	$y = 0.270x + 0.0147$	0.994	20.0–500	0.05	0.10
Ketoprofen	$y = 1.14x + 0.0467$	0.995	20.0–500	0.05	0.10
Lidocaine	$y = 70.8x - 0.346$	0.999	20.0–500	0.05	0.10
Linuron	$y = 0.628x - 0.00320$	0.999	20.0–500	0.05	0.10
Marbofloxacin	$y = 11.7x - 0.302$	0.999	20.0–500	0.05	0.10
Metformin	$y = 1.01x + 2.75$	0.996	15.0–500	0.05	0.10
Sotalol	$y = 8.90x + 0.123$	0.999	15.0–500	0.05	0.10
Triclocarban	$y = 235x - 4.55$	0.992	40.0–500	5.0	10.0
MPA	$y = 2.81x + 0.0999$	0.996	5.00–500	0.05	0.10
PFHxA	$y = 70.8x + 3.41$	0.997	5.00–500	0.05	0.10
DNP	$y = 4.49x + 0.0838$	0.995	5.00–500	0.05	0.10
EP	$y = 1.78x + 0.0593$	0.995	5.00–500	0.05	0.10
MP	$y = 5.11x + 0.577$	0.996	5.00–500	0.05	0.10
Ranitidine	$y = 0.504x - 0.00480$	1.000	5.00–500	1.00	10.0
Metoprolol	$y = 6.81x$	0.997	20.0–500	0.05	0.10
Mebeverine	$y = 20.0x$	0.995	20.0–500	0.05	0.10
Metconazole	$y = 0.0938x$	0.996	20.0–500	1.00	5.00
Mirtazapine	$y = 4.27x$	0.997	30.0–500	0.05	0.10
MBzP	$y = 0.631x$	0.995	20.0–500	0.05	0.10
Nitrendipine	$y = 1.22x$	0.995	20.0–500	0.05	0.10
Oryzalin	$y = 0.0889x$	0.996	20.0–500	1.00	5.00
Oxybutynin	$y = 0.623x$	0.997	20.0–500	0.05	0.10
Promethazine	$y = 10.9x$	0.998	20.0–500	0.05	0.50
Raloxifene	$y = 1.22x$	0.998	25.0–500	0.05	0.10
SDM	$y = 8.26x$	0.999	20.0–500	0.05	0.10
Sulfathiazole	$y = 3.68x$	0.999	20.0–500	0.05	0.10
Pendimethalin	$y = 0.00370x$	0.999	40.0–500	5.00	10.0
Telmisartan	$y = 0.784x$	0.996	20.0–500	0.05	0.10
Terbuthylazine	$y = 1.42x$	0.996	15.0–500	0.05	0.10
Thiabendazole	$y = 14.3x$	0.998	35.0–500	0.05	0.10

Previous studies using HPLC-MS/MS have reported urinary concentrations of PDM ranging between 0.0386 ng mL^{-1} and 0.0396 ng mL^{-1}, suggesting relatively low exposure levels under experimental conditions.[21] Though the immediate risks appear minimal, the potential long-term health implications of PDM exposure warrant further investigation.

3.3.2 Pharmaceuticals. As outlined in Table 3, the detection frequencies of CAP among college students were 18.6%, 25.6%, and 27.3% for the F1, F2, and S groups, respectively. CAP, an angiotensin-converting enzyme (ACE) inhibitor commonly prescribed for hypertension management,[45] was detected at mean concentrations ranging from 7.94 ng mL^{-1} to 12.8 ng mL^{-1}. Statistical analysis using the Kruskal–Wallis test indicated no significant differences in CAP levels among the groups ($p > 0.05$), suggesting that environmental factors linked to living conditions may not significantly influence urinary exposure to CAP. While CAP exposure during pregnancy has

Table 3 Detected frequency, mean concentration (ng mL^{-1}) and HQs of 11 ECs detected in urine samples from 76 college students

	Freshmen 1st sampling (F1)				Freshmen 2nd sampling (F2)				Senior students (S)			
ECs	DF	Mean conc. (ng mL^{-1})	HQ_{Med}	HQ_{90}	DF	Mean conc. (ng mL^{-1})	HQ_{Med}	HQ_{90}	DF	Mean conc. (ng mL^{-1})	HQ_{Med}	HQ_{90}
ATR	100%	6.09	0.0053	0.0157	100%	8.19	0.0082	0.0179	100%	6.40	0.0073	0.0179
CAP	18.60%	12.8	0.0032	0.0046	25.60%	7.94	0.0026	0.0025	27.30%	11.5	0.0043	0.0046
CLZ	—	—	—	—	7.00%	1.43	0.0007	0.0008	—	—	—	—
DMA	—	—	—	—	—	—	—	—	3.00%	29.6[a]	0.0007	0.0007
MET	2.30%	4833[a]	0.403	0.403	18.60%	7.75	0.0004	0.0017	—	—	—	—
MBzP	11.60%	0.96	0.0002	0.0003	7.00%	0.987	0.0001	0.0004	21.20%	0.753	0.0001	0.0003
DNP	4.70%	1.53	0.0238	0.0246	55.80%	1.47	0.0223	0.025	9.10%	4.75	0.0802	0.112
EP	7.00%	9.32	0.0002	0.0003	16.30%	11.3	0.0002	0.0005	42.40%	7.81	0.0001	0.0003
MP	11.60%	48.1	0.0009	0.002	2.30%	6.16[a]	0.0001	0.0001	6.10%	2.59	0.0001	0.0001
MPA	7.00%	2.44	0.0145	0.0212	—	—	—	—	15.20%	8.03	0.0407	0.0903
PDM	4.70%	<LOQ	—	—	—	—	—	—	—	—	—	—

[a] The ECs were detected in only one sample; "DF" indicates detection frequency; "Mean Conc." indicates mean concentration; "HQ_{Med}" and "HQ_{90}" indicate HQs calculated based on median concentration and 90th percentile concentration; "—" indicates that the ECs were not detected.

been linked to congenital anomalies,[46] reports on its environmental prevalence remain scarce.

MET, an oral hypoglycemic agent frequently prescribed for the treatment of type 2 diabetes, was detected in freshmen urine with the detection frequencies of 2.30% (only one person) and 18.6% for the 1st and 2nd sampling, respectively, while it was not found in the urine of seniors. Notably, an exceptionally high MET concentration of 4833 ng mL^{-1} was detected in a single freshman's urine sample during the first sampling, though it was absent in the second sampling from the same individual. This finding was statistically significant, as Kruskal–Wallis and Dunn's post-hoc tests revealed differences in MET levels between the first and second samplings for freshmen ($p < 0.05$) and between freshmen and seniors ($p < 0.01$). The extreme value may represent an outlier since this student did not report a history of drug use in the corresponding questionnaire;

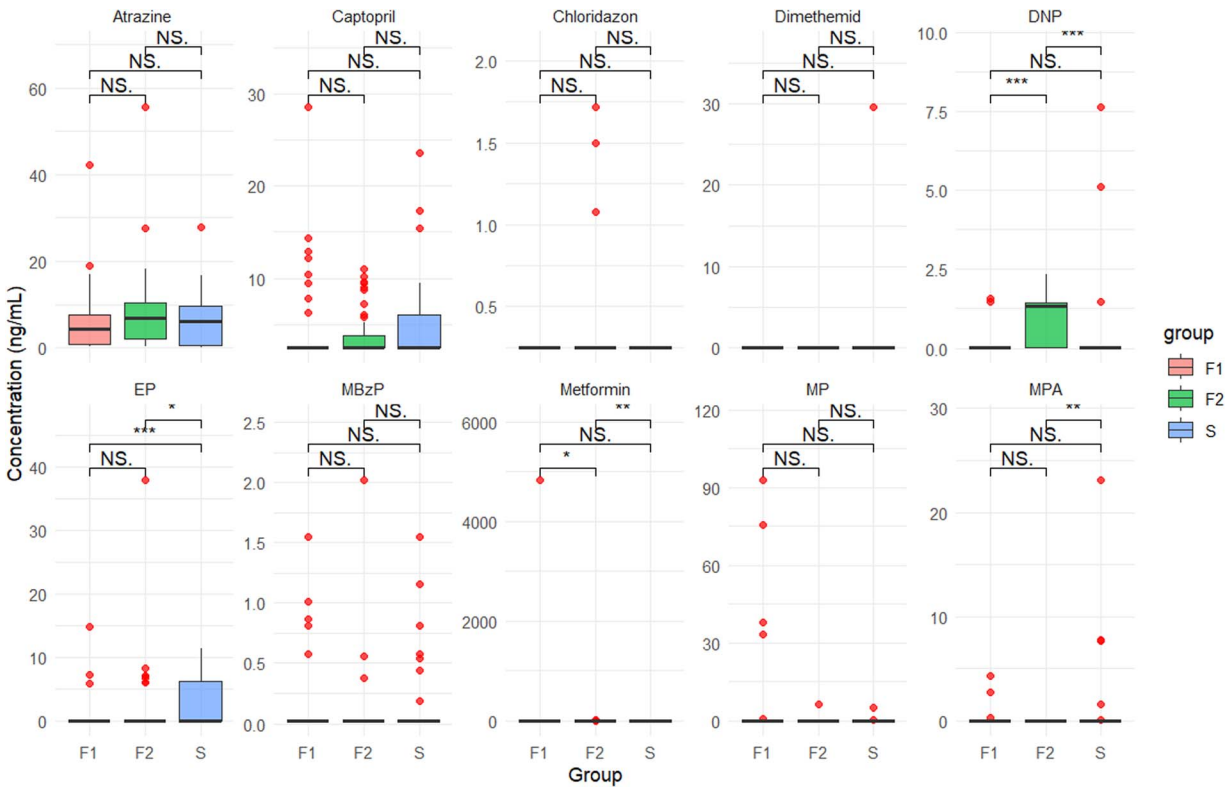

Fig. 2 Box plot of group-wise concentration distributions with Dunn's test pairwise comparisons of ten detected ECs from urine samples of 76 college students. "F1", "F2" and "S" represent three experimental groups (freshmen in the 1st sampling, freshmen in the 2nd sampling, and senior students). Pairwise comparisons were conducted using Dunn's test with Bonferroni correction, and the significance levels are represented as follows: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and NS (not significant).

despite this, the detected concentration remains significantly lower than that of counterfeit slimming formulations containing MET by mistake.[47,48] Herbal weight loss supplements are very popular and commonly used for weight loss in different countries. Sibutramine, methamphetamine, metformin, and bupropion are the most common illicit substances found in herbal weight loss products.[49] On average, MET concentrations in the second freshman sampling were 7.75 ng mL^{-1}. Most studies on MET residues have focused on aquatic ecosystems,[19] with limited research on its presence in human urine, particularly in healthy individuals. Given MET's poor metabolism in humans, it is largely excreted unchanged, contributing to its frequent detection in aquatic environments. Research indicates that MET can react with sodium hypochlorite during water treatment, forming chlorinated by-products that may inhibit stem cell proliferation and cause chronic damage in animal models,[50] though long-term human health implications remain uncertain.

MPA, a natural compound synthesized by molds of the *Penicillium* genus and developed into an immunosuppressive drug,[51,52] was detected at average concentrations of 2.44 ng mL^{-1} and 8.03 ng mL^{-1} among students. Statistical analysis revealed a significant difference in MPA levels between freshmen and seniors ($p < 0.01$). Prolonged exposure to MPA has been associated with immune system dysregulation, though its chronic health risks for humans are not well understood. MPA has been detected in household dust[53] and food items, such as fruit juices and cheese,[54,55] suggesting multiple potential exposure pathways, including inhalation and ingestion.

3.3.3 Personal care products. DNP was detected in both freshmen and senior students, with mean concentrations of 1.53 ng mL^{-1} (F1), 1.47 ng mL^{-1} (F2), and 4.75 ng mL^{-1} (S group). Statistical analysis *via* the Kruskal–Wallis test and Dunn's test revealed a highly significant difference in DNP levels between freshmen and senior students ($p < 0.001$). DNP, commonly employed as a preservative and bactericide in PCPs, industrial goods, and aquatic environments, has also been illicitly used as a weight-loss drug, despite its ability to inhibit ATP activity and induce fatal toxicity.[56] Numerous poisoning and fatality cases have been attributed to DNP exposure.[57–59]

EP and MP, widely used parabens in cosmetics, pharmaceuticals, and food products, were also analyzed. In this study, the mean concentrations of EP were 9.32 ng mL^{-1} and 11.3 ng mL^{-1} for freshmen (F1 and F2) and 7.81 ng mL^{-1} for senior students, respectively. The mean concentrations of MP were 48.1 ng mL^{-1}, 6.16 ng mL^{-1} and 2.59 ng mL^{-1} for freshmen (F1 and F2) and senior students, respectively. It is hypothesized that the higher exposure among freshmen during the first sampling may be attributed to increased use of sunscreen during outdoor military training at the start of university enrollment. Seasonal differences, with the second sampling conducted during autumn and winter, likely contributed to reduced PCP usage. However, a Kruskal–Wallis test revealed no statistically significant differences in EP concentrations between the two freshman samplings, while MP concentrations showed no significant differences among all student groups. Prolonged exposure to parabens has been linked to adverse effects such as skin irritation and endocrine disruption.[60] A study by Tkalec *et al.* identified median specific-gravity corrected EP and MP concentrations of 7.2 ng mL^{-1} and 6.0 ng mL^{-1} in Slovenian adolescents,[61] while Hajizadeh *et al.* reported median EP and MP concentrations of 9.64 ng mL^{-1} and 87.0 ng mL^{-1} in Iranian pregnant women.[62] The exposure levels observed in this study are consistent with those reported in the literature.

3.3.4 Industrial chemicals. MBzP, a metabolite of butyl benzyl phthalate, was identified in 7.00–11.6% of urine samples from freshmen and 21.2% from senior students. The mean concentrations were 0.96–0.987 ng mL^{-1} for freshmen and 0.753 ng mL^{-1} for senior students. MBzP, a plasticizer commonly utilized in packaging, toys, medical devices, and inks, has been associated with endocrine disruption, hepatotoxicity, nephrotoxicity, and reproductive toxicity. A study by Park *et al.* reported significantly higher MBzP concentrations in firefighters involved in fire suppression activities (geometric mean = 149.9 ng mL^{-1}) compared to those performing other tasks (geometric mean = 70.8 ng mL^{-1}).[63] Similarly, in young Danish men, MBzP concentrations ranged between 2.54 and 9.45 ng mL^{-1} (median) and 44.3 and 77.7 ng mL^{-1} (maximum) in urine collected from 2009–2017.[64] The relatively low MBzP levels observed in this study may reflect limited exposure among healthy individuals, consistent with its minor representation as a metabolite.

3.4 Correlation of questionnaire factors and exposure of ECs

To explore the relationship between the exposure factors derived from the questionnaire and the concentrations of the seven ECs detected in both the 1st and 2nd urine samples of freshmen, a Spearman correlation analysis was conducted as shown in Fig. 3. For a comprehensive overview of the questionnaire content, please refer to Table S3 in the SI.

3.4.1 Correlation analysis among ECs. Fig. 3 indicates a significant positive correlation between MET and MBzP ($r = 0.56$, $p < 0.05$). MET is commonly formulated as tablets, and MBzP, as a phthalate plasticizer, is often utilized in the production of tablet film coatings to enhance flexibility and durability. This manufacturing practice could potentially explain the observed positive correlation between the two compounds. However, other exposure pathways, such as food packaging materials, PCPs and indoor dust, might also contribute to urinary MBzP levels. Additionally, in this study, MET was detected in only one urine sample for the 1st sampling, which limits the representativeness and statistical reliability of the observed correlation. Given these limitations, the relationship between MET and MBzP warrants further investigation.

3.4.2 Correlation analysis between questionnaire factors and ECs. As illustrated in Fig. 3, gender exhibited a significant negative correlation with MBzP exposure levels among freshmen with a ratio of male and female students of 1.05 ($r = -0.21$, $p < 0.05$). Males are assigned a score of 1, and females are assigned a score of 0, as shown in Table S3 in the SI. This likely explains the higher MBzP exposure levels observed in female

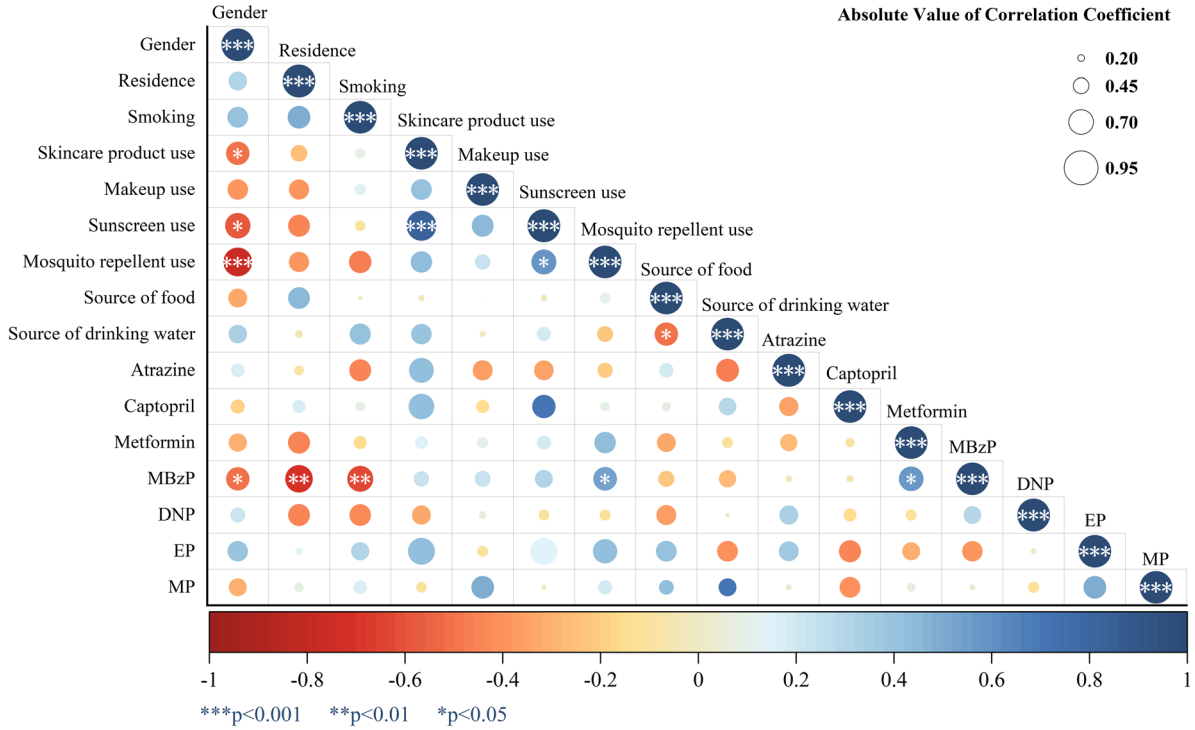

Fig. 3 Spearman correlation analysis of questionnaire factors with levels of detected ECs in freshmen urine samples.

participants. Phthalates commonly utilized as plasticizers and fragrance stabilizers in PCPs—such as soaps, shampoos, and makeup—are more frequently used by female students, as discussed in Section 3.4.1.

Furthermore, residential areas demonstrated a strong negative correlation with MBzP exposure levels ($r = -0.70$, $p < 0.01$), suggesting that even in regions without major commercial centers, such as hospitals or shopping malls, MBzP concentrations in urine remained elevated. This finding indicates that the complexity of an individual's residential environment may not be directly linked to MBzP exposure. To further clarify this relationship, future studies should consider collecting urine samples from family members of volunteers as controls, enabling a more comprehensive analysis of the connection between residential environments and personal exposure.

A significant negative correlation was also found between smoking status and MBzP exposure levels ($r = -0.63$, $p < 0.01$). Smokers, students who were exposed to secondhand smoking and non-smokers were assigned a score of 0, 1and 2, respectively, as shown in Table S3 in the SI. This likely explains the higher MBzP exposure levels observed in smoking participants. Previous studies have shown that numerous additives, including colorants, binders and plasticizers, are incorporated into cigarette production to enhance flavor, retain moisture and improve combustion properties. These additives can account for 10% to 25% of the overall weight of a cigarette, contributing to elevated MBzP exposure in smokers.[65]

Lastly, the frequency of mosquito repellent use was positively correlated with MBzP exposure ($r = 0.53$, $p < 0.05$). Phthalates are frequently employed as solvents in mosquito repellents, and MBzP is a known metabolite of these compounds. Therefore, individuals who regularly use mosquito repellents are more likely to experience heightened MBzP exposure.

3.5 Human health risk assessment

As illustrated in Table 3, the Hazard Quotients (HQs) of ECs were computed using the mean concentrations and the 90th percentile concentrations, following the equations outlined in Section 2.4. The calculated HQ values ranged from 0.0001 to 0.403. Notably, none of the HQs exceeded the threshold of 1, signifying a minimal exposure risk associated with the detected compounds. Nevertheless, the HQs for DNP (0.0223–0.112), MPA (0.0145–0.0903), and ATR (0.0053–0.0179) were comparatively higher than those of other ECs, necessitating greater vigilance. While these values remain within the acceptable risk range, they underscore the importance of ongoing monitoring, particularly among populations with elevated exposure potential.

The highest HQ value of 0.403 was observed in the first urine sample collected from a freshman, which detected MET at a concentration of 4833 ng mL^{-1}. However, this compound was not detected in the second urine sample collected from the same individual after a 100-day interval. Despite this exceptional case, the HQ range for MET (0.0004–0.0017) remains low and does not pose significant concern.

4 Conclusions

This study provides a comprehensive analysis of EC exposure among college students by integrating suspect screening and

quantitative analysis. Suspect screening effectively identified a wide range of ECs, while quantitative methods demonstrated robust analytical performance, ensuring the reliability of the findings. Method validation confirmed that the quantification approach exhibited excellent linearity, with LODs ranging from 0.05 to 5.00 ng mL^{-1} and LOQs spanning from 0.10 to 10.0 ng mL^{-1}, as well as strong precision and accuracy, making it a reliable tool for trace-level analysis in biological samples. Among the detected ECs, ATR showed a 100% detection frequency, underlining its significant role as an exposure source. Significant variations were observed for DNP, EP, MET and MPA—among samples from freshmen and senior students. Additionally, the presence of pharmaceuticals and pesticides revealed diverse exposure pathways, including environmental contamination, dietary intake, and lifestyle factors. Notably, MET and MBzP may share exposure routes, necessitating further investigation.

Health risk assessment indicated low overall risks for detected ECs, though relatively higher HQ values were observed for DNP, ATR and MPA, emphasizing the need for continued monitoring, particularly in high-risk populations. These findings underscore the importance of targeted public health strategies, such as raising awareness about EC-containing products, enhancing environmental monitoring, and encouraging safer lifestyle practices. This study highlights the value of advanced analytical techniques in biomonitoring and lays the groundwork for future research to explore longitudinal exposure trends, larger population studies, and detailed source assessment.

Author contributions

Conceptualization: Meng Hu and Chang-Er Chen. Data curation: Hongmei Zhang and Chao Zhang. Formal analysis: Jingjing Song. Funding acquisition: Meng Hu, Chang-Er Chen, and Haiyan Cui. Investigation: Xiangru Yi. Methodology: Jingjing Song, Xiangru Yi, Ziwei Yuan, and Chang Wang. Project administration: Chang-Er Chen and Meng Hu. Resources: Keming Yun and Tao Wang. Software: Rui Gao and Chenshan Lv. Supervision: Chang-Er Chen and Meng Hu. Validation: Hongyan Zou. Writing – original draft: Meng Hu. Writing – review & editing: Chang-Er Chen and Zhiwen Wei.

Conflicts of interest

There are no conflicts to declare. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Supplementary information: The urine suspect database of 537 ECs, precision and recovery of 50 preferred ECs, questionnaire content and body weight (BW), urine volume, *F*/BS and RfD of 10 quantified ECs. See DOI: https://doi.org/10.1039/d5sc05477j.

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References

1 S. D. Richardson and T. Manasfi, Water Analysis: Emerging Contaminants and Current Issues, *Anal. Chem.*, 2024, **96**(20), 8184–8219.
2 K. A. Murrell, P. D. Teehan and F. L. Dorman, Determination of contaminants of emerging concern and their transformation products in treated-wastewater irrigated soil and corn, *Chemosphere*, 2021, **281**, 130735.
3 R. Kumar, A. K. Vuppaladadiyam, E. Antunes, *et al.*, Emerging contaminants in biosolids: Presence, fate and analytical techniques, *Emerging Contam.*, 2022, **8**, 162–194.
4 A. F. Gilca, C. Teodosiu, S. Fiore, *et al.*, Emerging disinfection byproducts: A review on their occurrence and control in drinking water treatment processes, *Chemosphere*, 2020, **259**, 127476.
5 S. Berger, I. Oesterle, K. I. Ayeni, C. N. Ezekiel, A. Rompel and B. Warth, Polyphenol exposure of mothers and infants assessed by LC-MS/MS based biomonitoring in breast milk, *Anal. Bioanal. Chem.*, 2024, **416**(7), 1759–1774.
6 J. Robin, S. Lefeuvre, J. Guihenneuc, G. Cambien, A. Dupuis and N. Venisse, Analytical methods and biomonitoring results in hair for the assessment of exposure to endocrine-disrupting chemicals: A literature review, *Chemosphere*, 2024, **353**, 141523.
7 S. S. Shetty, D. Deepthi, S. Harshitha, *et al.*, Environmental pollutants and their effects on human health, *Heliyon*, 2023, **9**(9), e19496.
8 S. Jia, T. Xu, T. Huan, *et al.*, Chemical Isotope Labeling Exposome (CIL-EXPOSOME): One High-Throughput Platform for Human Urinary Global Exposome Characterization, *Environ. Sci. Technol.*, 2019, **53**(9), 5445–5453.
9 G. Ding, Y. Gao, H. Kan, *et al.*, Environmental exposure and child health in China, *Environ. Int.*, 2024, **187**, 108722.
10 C. Jiang, X. Wang, X. Li, *et al.*, Dynamic Human Environmental Exposome Revealed by Longitudinal Personal Monitoring, *Cell*, 2018, **175**(1), 277–291e31.
11 E. Park, J. Lee, J. Lee, *et al.*, Method for the simultaneous analysis of 300 pesticide residues in hair by LC-MS/MS and GC-MS/MS, and its application to biomonitoring of agricultural workers, *Chemosphere*, 2021, **277**, 130215.
12 M. Z. Hossain, M. L. Feuerstein, Y. Gu and B. Warth, Scaling up a targeted exposome LC-MS/MS biomonitoring method

by incorporating veterinary drugs and pesticides, *Anal. Bioanal. Chem.*, 2024, **416**(19), 4369–4382.
13 G. Sabbioni, A. Castaño, M. Esteban López, *et al.*, Literature review and evaluation of biomarkers, matrices and analytical methods for chemicals selected in the research program Human Biomonitoring for the European Union (HBM4EU), *Environ. Int.*, 2022, **169**, 107458.
14 K. Vorkamp, A. Castaño, J. P. Antignac, *et al.*, Biomarkers, matrices and analytical methods targeting human exposure to chemicals selected for a European human biomonitoring initiative, *Environ. Int.*, 2021, **146**, 106082.
15 B. Ringbeck, D. Bury, H. Hayen, T. Weiss, T. Brüning and H. M. Koch, Determination of specific urinary nonylphenol metabolites by online-SPE-LC-MS/MS as novel human exposure biomarkers, *J. Chromatogr. B:Anal. Technol. Biomed. Life Sci.*, 2021, **1177**, 122794.
16 S. F. Fernández, O. Pardo, I. Adam-Cervera, *et al.*, Biomonitoring of non-persistent pesticides in urine from lactating mothers: Exposure and risk assessment, *Sci. Total Environ.*, 2020, **699**, 134385.
17 S. Faure, N. Noisel, K. Werry, S. Karthikeyan, L. L. Aylward and A. St-Amand, Evaluation of human biomonitoring data in a health risk based context: An updated analysis of population level data from the Canadian Health Measures Survey, *Int. J. Hyg. Environ. Health*, 2020, **223**(1), 267–280.
18 N. Suwannarin, Y. Nishihama, T. Isobe, S. F. Nakayama, Japan Environment and Children's Study Group, Urinary concentrations of environmental phenol among pregnant women in the Japan Environment and Children's Study, *Environ. Int.*, 2024, **183**, 108373.
19 R. Zhang, Y. He, L. Yao, *et al.*, Metformin chlorination byproducts in drinking water exhibit marked toxicities of a potential health concern, *Environ. Int.*, 2021, **146**, 106244.
20 Y. Hu, Q. Zhu, Y. Wang, C. Liao and G. Jiang, A short review of human exposure to antibiotics based on urinary biomonitoring, *Sci. Total Environ.*, 2022, **830**, 154775.
21 EFSA Scientific Committee, S. J. More, V. Bampidis, *et al.*, Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals, *EFSA J.*, 2019, **17**(3), e05634.
22 J. Ao, T. Yuan, H. Xia, *et al.*, Characteristic and human exposure risk assessment of per- and polyfluoroalkyl substances: A study based on indoor dust and drinking water in China, *Environ. Pollut.*, 2019, **254**(Pt A), 112873.
23 J. Li, C. Wu, H. Zhao, *et al.*, Exposure Assessment of Bisphenols in Chinese Women during Pregnancy: A Longitudinal Study, *Environ. Sci. Technol.*, 2019, **53**(13), 7812–7820.
24 L. Šulc, T. Janoš, D. Figueiredo, *et al.*, Pesticide exposure among Czech adults and children from the CELSPAC-SPECIMEn cohort: Urinary biomarker levels and associated health risks, *Environ. Res.*, 2022, **214**(Pt 3), 114002.
25 T. Husøy, I. H. Caspersen, E. Thépaut, *et al.*, Comparison of aggregated exposure to perfluorooctanoic acid (PFOA) from diet and personal care products with concentrations in blood using a PBPK model - Results from the Norwegian biomonitoring study in EuroMix, *Environ. Res.*, 2023, **239**(Pt 2), 117341.
26 M. N. Newmeyer, L. Quirós-Alcalá, L. K. Kavi, L. M. Louis and C. Prasse, Implementing a suspect screening method to assess occupational chemical exposures among US-based hairdressers serving an ethnically diverse clientele: a pilot study, *J. Exposure Sci. Environ. Epidemiol.*, 2023, **33**(4), 566–574.
27 J. Chaker, D. M. Kristensen, T. I. Halldorsson, *et al.*, Comprehensive Evaluation of Blood Plasma and Serum Sample Preparations for HRMS-Based Chemical Exposomics: Overlaps and Specificities, *Anal. Chem.*, 2022, **94**(2), 866–874.
28 N. Caballero-Casero, G. Castro, M. Bastiaensen, *et al.*, Identification of chemicals of emerging concern in urine of Flemish adolescents using a new suspect screening workflow for LC-QTOF-MS, *Chemosphere*, 2021, **280**, 130683.
29 B. O. Clarke, The Role of Mass Spectrometry in Protecting Public Health and the Environment from Synthetic Chemicals, *J. Am. Soc. Mass Spectrom.*, 2024, **35**(9), 2248–2255.
30 H. Wang, D. Yang, H. Fang, *et al.*, Predictors, sources, and health risk of exposure to neonicotinoids in Chinese school children: A biomonitoring-based study, *Environ. Int.*, 2020, **143**, 105918.
31 M. Flasch, G. Koellensperger and B. Warth, Comparing the sensitivity of a low- and a high-resolution mass spectrometry approach for xenobiotic trace analysis: An exposome-type case study, *Anal. Chim. Acta*, 2023, **1279**, 341740.
32 J. Ueyama, A. Aoi, Y. Ueda, *et al.*, Biomonitoring method for neonicotinoid insecticides in urine of non-toilet-trained children using LC-MS/MS, *Food Addit. Contam., Part A: Chem., Anal., Control, Exposure Risk Assess.*, 2020, **37**(2), 304–315.
33 X. Cheng, Y. Zhang and Y. Gao, Progress in cumulative risk assessment of human health from combined exposure to environmental pollutants, *J. Shanghai Jiaotong Univ.*, 2024, **44**(8), 1037–1043.
34 X. M. Zhang, X. Y. Lou, L. H. Wu, C. Huang, D. Chen and Y. Guo, Urinary phthalate metabolites and environmental phenols in university students in South China, *Environ. Res.*, 2018, **165**, 32–39.
35 L. X. Han, X. Zhang and X. J. Hu, Determination of 12 typical personal care products in human urine samples by ultra performance liquid chromatography-tandem mass spectrometry, *Chin. J. Chromatogr.*, 2023, **41**(4), 312–322.
36 C. Lv, Y. Cao, X. Mu, H. Cui, T. Wang, Z. Wei, *et al.*, Rapid screening of 34 emerging contaminants in surface water by UHPLC-QTOF-MS, *J. Forensic Med.*, 2024, **40**(1), 30–36.
37 D. Zhang and S. Lu, Human exposure to neonicotinoids and the associated health risks: A review, *Environ. Int.*, 2022, **163**, 107201.
38 G. Mendaš, M. Vuletić, N. Galić and V. Drevenkar, Urinary metabolites as biomarkers of human exposure to atrazine: atrazine mercapturate in agricultural workers, *Toxicol. Lett.*, 2012, **210**(2), 174–181.

39 S. O. Abarikwu, Protective effect of quercetin on atrazine-induced oxidative stress in the liver, kidney, brain, and heart of adult wistar rats, *Toxicol. Int.*, 2014, **21**(2), 148–155.
40 S. Arabi, M. Heidari-Beni, P. Poursafa, M. Roshanaei and R. Kelishadi, A review of the potential adverse health impacts of atrazine in humans, *Rev. Environ. Health*, 2024, **40**(2), 347–359.
41 Z. Yuan, J. Song, X. Yi, *et al.*, Non-targeted screening to explore the spatiotemporal distribution and health risk assessment of emerging pollutants in tap water, *Chin. J. Forensic Med.*, 2024, **39**(06), 697–704.
42 J. Liu, N. Li, L. Ye, *et al.*, Triple modal aptasensor arrays driven by CHA-mediated DNAzyme for signal-amplified atrazine pesticide accumulation monitoring in agricultural crops, *J. Hazard. Mater.*, 2024, **476**, 135172.
43 N. Urseler, R. Bachetti, F. Biolé, V. Morgante and C. Morgante, Atrazine pollution in groundwater and raw bovine milk: Water quality, bioaccumulation and human risk assessment, *Sci. Total Environ.*, 2022, **852**, 158498.
44 L. Ahrens, A. Daneshvar, A. E. Lau and J. Kreuger, Concentrations, fluxes and field calibration of passive water samplers for pesticides and hazard-based risk assessment, *Sci. Total Environ.*, 2018, **637–638**, 835–843.
45 L. Han, T. Liu, D. Cui, *et al.*, Quantitative detection of captopril in urine by smartphone-assisted ratiometric fluorescence sensing platform, *Spectrochim. Acta, Part A*, 2022, **280**, 121562.
46 P. G. Jamkhande, K. D. Chintawar and P. G. Chandak, Teratogenicity: a mechanism based short review on common teratogenic agents, *Asian Pac. J. Trop. Dis.*, 2014, **4**(6), 421–432.
47 X. Zhang, X. Wang, D. I. Vernikovskaya, *et al.*, Quantitative determination of metformin, glyburide and its metabolites in plasma and urine of pregnant patients by LC-MS/MS, *Biomed. Chromatogr.*, 2015, **29**(4), 560–569.
48 D. O. M. Bonsu, C. Afoakwah and M. P. Aguilar-Caballos, Counterfeit formulations: analytical perspective on anorectics, *Forensic Toxicol.*, 2021, **39**(1), 1–25.
49 F. Firozian, A. Nili-Ahmadabadi, S. Moradkhani, M. Moulaei, Z. Fasihi and D. Ahmadimoghaddam, Adulteration of the Herbal Weight Loss Products by the Illegal Addition of Synthetic Antiobesity Medications: A Pilot Study, *J. Obes.*, 2021, **2021**, 9968730.
50 Q. Zheng, P. Dewapriya, G. Eaglesham, *et al.*, Direct injection analysis of oxypurinol and metformin in wastewater by hydrophilic interaction liquid chromatography coupled to tandem mass spectrometry, *Drug Test. Anal.*, 2022, **14**(8), 1519–1524.
51 J. O. Straub, R. Oldenkamp, T. Pfister and A. Häner, Environmental Risk Assessment for the Active Pharmaceutical Ingredient Mycophenolic Acid in European Surface Waters, *Environ. Toxicol. Chem.*, 2019, **38**(10), 2259–2278.
52 T. van Gelder, How cyclosporine reduces mycophenolic acid exposure by 40% while other calcineurin inhibitors do not, *Kidney Int.*, 2021, **100**(6), 1185–1189.
53 C. Viegas, B. Gomes, R. Pimenta, *et al.*, Microbial contamination in firefighter Headquarters': A neglected occupational exposure scenario, *Build. Environ.*, 2022, **213**, 108862.
54 E. Usleber, M. Dade, E. Schneider, R. Dietrich, J. Bauer and E. Märtlbauer, Enzyme immunoassay for mycophenolic acid in milk and cheese, *J. Agric. Food Chem.*, 2008, **56**(16), 6857–6862.
55 I. B. Sedova, Z. A. Chalyy and V. A. Tutelyan, Mycotoxins Contamination Of Grape Wines Marketed In The Russian Federation, *Health*, 2024, (4), 64.
56 D. Sousa, H. Carmo, R. Roque Bravo, *et al.*, Diet aid or aid to die: an update on 2,4-dinitrophenol (2,4-DNP) use as a weight-loss product, *Arch. Toxicol.*, 2020, **94**(4), 1071–1083.
57 A. Patankar, N. D. Karnik, M. Trivedi, *et al.*, Slim to Kill: 2, 4-Dinitrophenol (DNP), The Naïve Assassin, *Clin. Case Rep. Int.*, 2020, **4**, 1–2.
58 M. D. Newport and T. Said, Fatal poisoning with 2, 4-Dinitrophenol: learning *via* case study, *J. Paramedic Pract.*, 2020, **12**(2), 1–6.
59 T. Gziut and S. H. L. Thomas, International trends in systemic human exposures to 2,4 dinitrophenol reported to poisons centres, *Clin. Toxicol.*, 2022, **60**(5), 628–631.
60 P. A. A. Alaba, E. D. Cañete, B. S. S. Pantalan, J. M. C. Taguba, L. D. I. Yu and E. M. Faller, Toxic effects of paraben and its relevance in cosmetics: A review, *Int. J. Res.*, 2022, **2582**, 7421.
61 Ž. Tkalec, T. Kosjek, J. Snoj Tratnik, *et al.*, Exposure of Slovenian children and adolescents to bisphenols, parabens and triclosan: Urinary levels, exposure patterns, determinants of exposure and susceptibility, *Environ. Int.*, 2021, **146**, 106172.
62 Y. Hajizadeh, G. Kiani Feizabadi, K. Ebrahimpour, *et al.*, Urinary paraben concentrations and their implications for human exposure in Iranian pregnant women, *Environ. Sci. Pollut. Res. Int.*, 2020, **27**(13), 14723–14734.
63 S. Park, H. S. Kim, H. J. Oh, I. Chung, Y. S. Ahn and K. S. Jeong, Assessment of phthalate exposure at a fire site in Korean firefighters, *Int. J. Environ. Health Res.*, 2024, **34**(3), 1800–1809.
64 H. Frederiksen, O. Nielsen, H. M. Koch, *et al.*, Changes in urinary excretion of phthalates, phthalate substitutes, bisphenols and other polychlorinated and phenolic substances in young Danish men; 2009-2017, *Int. J. Hyg. Environ. Health*, 2020, **223**(1), 93–105.
65 M. Pötschke-Langer, Ingredients and additives in tobacco products, *Tob. Prev. Cessation*, 2016, **2**, 55.