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Human dietary and internal exposure to zearalenone based on a 24-hour duplicate diet and following morning urine study

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ABSTRACT

Zearalenone is a widespread mycotoxin with high estrogenic activity. This study aimed to characterize the exposure of ZEN in a Chinese population during harvest season in 2016. Exposure to ZEN was measured using both duplicate diet method and human biomonitoring approaches. Duplicate diet samples from 199 individuals (4–80 years old) and their following morning urine samples were collected and analyzed using LC-MS/MS methods sensitive for ZEN, ZAN, α/β-ZEL and α/β-ZAL. ZEN was detected in 59.8% of the food samples at a mean level of 1.21 ± 2.15 μg/kg. The estimated daily intake (EDI) of ZEN was calculated from food contamination and consumption data at a mean level of 25.6 ± 38.6 ng/kg bw/day, representing 10.2% of the tolerable daily intake (TDI) set by EFSA and 5.1% of the provisional maximum tolerable daily intake (PMTDI) set by JECFA, respectively. Wheat appears to be the main diet source of ZEN exposure, contributing over 80% of the mean EDI. Children had the highest EDI at 37.5 ± 56.3 ng/kg bw/day ($p < 0.05$). Urine samples were analyzed both before and after enzymatic hydrolysis to determine the free and total amounts of ZEN biomarkers. The majority of ZEN was excreted as conjugates with the mean fZEN/tBM ratio of 25.4%. Adolescents had the highest excretion of ZEN biomarkers among all age groups ($p < 0.05$). Probable daily intake (PDI) was calculated from ZEN biomarkers and an excretion rate of 36.8%, giving a mean value of 41.6 ± 65.5 ng/kg bw/day. Significant correlation between internal and external exposure measurement was evidenced in this study ($r = 0.344$, $p < 0.01$). Although the mean PDI was approximately 1.6 times the mean EDI, these two approaches resulted in similar calculated degrees of ZEN exposure, both markedly below the health-based guidance value. This study is the first to compare ZEN exposure in a same population based on both diet study and human biomonitoring approaches. Significant differences of PDI/EDI ratios were found in different age groups ($p < 0.05$), possibly indicative of diversified excretion capabilities and metabolism patterns within the population.

1. Introduction

Zearalenone (ZEN) is a secondary metabolite biosynthesized mainly by *Fusaruim graminearum*, *Fusarium culmorum, Fusarium equiseti* and *Fusarium sacchari* (EFSA, 2011). It contaminates various key grains, such as wheat, maize, millet and rice, and also occurs in cereal products and animal-derived food attributed to the carry-over from contaminated raw materials and feed. In mammals, ZEN is partially metabolized into stereo-isomeric metabolites α-/β-zearalenol (ZEL), α-/β-zearalanol (ZAL), and to a lesser extent zearalanone (ZAN), which can subsequently conjugate with glucuronic acid or sulfuric acid and be excreted in urine (Warth et al., 2013). In host plants, during fungal infection, ZEN can also be metabolized into its modified forms via Phase I and Phase II reactions. Reductive Phase I metabolites α-/β-ZEL, α-/β-ZAL, ZAN, and their phase II products such as glucosides and sulfates can be produced by *Fusarium* and occur in crops, with the amounts varying from a few up to 100% of ZEN (EFSA, 2016; Lorenz et al., 2019).

Despite their relatively low acute toxicity, ZEN and its derivatives exhibit potent estrogenic activity and are suspected as triggers for hyperestrogenism in humans (Zinedine et al., 2007) and central precocious puberty development in girls (Massart et al., 2008). ZEN has also been evidenced to be hepatotoxic, immunotoxic and genotoxic (Marin et al., 2013), and it is categorized as a Group 3 carcinogen by

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IARC (IARC, 1993). Based on comprehensive hazard assessments for ZEN, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) set a provisional maximum tolerable daily intake (PMTDI) as 0.5 mg/kg bw/day for ZEN in 2000 (JECFA, 2000), while recently EFSA proposed a group TDI of 0.25 mg/kg bw/day for ZEN and its modified forms (EFSA, 2016). According to these health-based guidance values (HBGVs), many countries or regions have established regulatory limits for ZEN to protect public health. The maximum levels (MLs) of ZEN fixed by the European Union range from 20 μg/kg for processed cereal/maize-based foods and baby foods for infants and young children to 400 μg/kg for refined maize oil (European Commission, 2007). In China, the MLs of ZEN in cereal/maize-based foods are 60 μg/kg (National Health Commission of PRC, China Food and Drug Administration, 2017).

Humans are easily exposed to ZEN via ingestion of contaminated food. The assessment of exposure level, usually called estimated daily intake (EDI), has been conventionally determined by combining the occurrence of ZEN in food with consumption data (Aldana et al., 2014; Cano-Sancho et al., 2012; EFSA, 2011; Bol et al., 2016). Since 2011, EFSA has launched a series of dietary exposure assessments on ZEN (EFSA, 2011) and its modified forms (EFSA, 2014; EFSA, 2016) in Europe to evaluate the chronic dietary exposure to ZEN for different age and consumer groups. Based on a total of 22,952 analytical results on ZEN occurrence in food provided by 19 European countries and food consumption data recorded in the EFSA Comprehensive European Food Consumption Database, a low risk for all age groups has been concluded (EFSA, 2011). Besides, ZEN has also been included in several national and regional total diet studies (TDSs), such as the first and second French TDS (Sirot et al., 2013), Netherlands TDS (Sprong et al., 2016), Spanish TDS (Eduardo et al., 2013), Hong Kong TDS, and the fourth (Wu and Li, 2015) and fifth (Wu et al., 2018) China TDS. All the results have evidenced a low health risk from ZEN dietary exposure.

Considering the feature of heterogeneous distribution of mycotoxins in food (Malik et al., 2010) and the probable insufficient representation of consumption data, an alternative strategy of human biomonitoring that directly measures mycotoxin biomarkers in human biological samples and then calculates a probable daily intake (PDI) using the corresponding excretion rate has been proposed (Baldwin et al., 2011; Routledge and Gong, 2011; Turner et al., 2012). It enables a less biased estimation and has gained increased acceptance in recent years. Besides, biomarker-based approaches cover almost all the possible sources of exposure such as diet, pharmaceuticals, environment, and occupational route, but still hardly identify the principal sources. These approaches have been widely adopted for internal exposure assessment of aflatoxin (Polychronaki et al., 2008; Romero et al., 2010) and deoxynivalenol (Ali et al., 2016; Wallin et al., 2013; Deng et al., 2018), whose biomarkers have been well studied and validated showing reliable and stable excretion rates (Cheng et al., 1997; Zhu et al., 1987; Wild and Turner, 2002; Turner et al., 2010; Fromme et al., 2016). For ZEN exposure, however, the metabolism and excretion pattern have not been thoroughly investigated. It is commonly considered that the total amount of urinary ZEN and its metabolites (free + conjugated) might be an appropriate biomarker to evaluate ZEN exposure (Mally et al., 2016). Up to now, only a few internal exposure studies have been carried out, most of which indicate a low ZEN exposure (Solfrizzo et al., 2014; Wallin et al., 2015; Föllmann et al., 2016; Li et al., 2018; Ali and Degen, 2018; Ali and Degen, 2019), despite the results of some individuals from the US (Bandera et al., 2011) and South Africa (Shephard et al., 2013) exceeding the HBGVs. However, most of the PDI was estimated using a piglet excretion data (Gambacorta, et al., 2013), owing to the lack of representative and large-scale studies on ZEN excretion pattern in humans. Only two sporadic studies have been reported for single volunteer (Warth et al., 2013; Mirocha et al., 1981), giving the excretion rate varying from 9.4%~20%. As a consequence, the internal exposure assessment for ZEN might be considered a rough estimate.

Until now, dietary exposure and internal exposure to ZEN have still been performed separately, with no exploration of their relationships and differences. In this study, a comprehensive exposure assessment of ZEN was made for the first time based on both the diet study and human biomonitoring. The duplicate diet of staple food and the following morning urine were collected and analyzed using reliable and sensitive LC-MS/MS methods established previously that have been adopted in China TDS (Wu and Li, 2015; Wu et al., 2018) and human biomonitoring study (Li et al., 2018). The occurrence and main food sources of ZEN, biomarker species and levels in urine, detailed analysis by gender and age groups, dietary and internal exposure, and their comparison were all discussed.

2. Materials and methods

2.1. Chemicals and materials

The following certificated standard solutions of ZEN and its metabolites were purchased from Biopure (Tulln, Austria): ZEN (100 μg/mL), α-ZEL (10 μg/mL), β-ZEL (10 μg/mL), ZAN (10 μg/mL), α-ZAL (10 μg/mL), β-ZAL (10 μg/mL), and $^{13}C_{18}$-ZEN (3 μg/mL). LC/MS-grade acetonitrile and methanol were purchased from Fisher Scientific (Leicestershire, UK). LC/MS-grade ammonia acetate, formic acid, and acetic acid were purchased from Merck (Darmstadt, Germany). The Oasis PRiME HLB 96-well μElution plate (3 mg) and CORTECS™UPLC® C18 column (2.1 × 100 mm, 1.6 μm) were obtained from Waters (Milford, MA, USA). The β-glucuronidase from *E. coli* (6.9 × 10^5 U/g solid) was from Sigma-Aldrich (MO, USA) and dissolved in 75 mM phosphate buffer (pH 6.8), for enzymatic treatment of urine samples.

2.2. Study subjects

The study was conducted on 199 healthy volunteers (96 males and 103 females) from 74 families living in rural area of Anhui province in China during September 2016. The subjects agreed to provide basic physical information, 24-h duplicate food portions, and urine samples the following morning. The basic information (name, gender, and age) of participants were obtained from their ID cards. The study protocol was approved by the ethics committee of China National Center for Food Safety Risk Assessment (No. 2016030063). The adult volunteers or parents on behalf of their children were fully informed of the study details and provided written informed consent for participation.

2.3. Duplicate diet sample collection

Here, 199 participants from 74 families were recruited to collect duplicate diet samples over a 24 h period. They were informed about the sampling procedure and instructed how to collect and store diet samples. Their physical information (height and body weight) was measured and recorded by the investigators on the day of training just before the 24 h period. As cereal is the main source of ZEN, only cereal-based foods and beverages (such as beer) were collected in this study. For each food sample, the participants placed duplicate amounts of their consumed food items into polyethylene bottles. Food categories and consumer names were written on the provided label on the outside of the bottle. The food samples were stored in a cooler with ice packs until the investigators collected the coolers in the following morning and took them to a local CDC laboratory, where each individual food item was weighed and recorded by the investigators. Then the same food samples collected by members of the same household were pooled together and homogenized for further analysis, since family members shared the same food. A total of 244 cereal samples were finally obtained, and an aliquot of each sample was transported to the NHC Key Laboratory of Food Safety Risk Assessment in Beijing and stored at −70 °C until further analysis. In this sampling strategy, family members shared the same data on ZEN concentration in food consumed and had individual respective consumption data.

2.4. Urine sample collection

Urine samples were collected from each participant the following morning after the food sampling. Urine samples were kept frozen at −70 °C until analysis.

2.5. Experimental analysis of food and urine samples

ZEN and its five derivatives of ZAN, β-ZEL, α-ZEL, β-ZAL and α-ZAL in food were analyzed using an isotope dilution method adapted from China Total Diet Study (Wu et al., 2017). Briefly, food samples were ultrasonized and centrifuged following the addition of isotope internal standard ^{13}C-ZEN and 10 mL extract solvent (acetonitrile/water mixture, 86/14) into 2 g of the samples. The supernatants were purified with MultiSep 226 columns (Romer Labs, Union, MO, USA), eluted with 3 mL methanol, dried under nitrogen, and reconstituted in 1 mL acetonitrile-0.2% formic acid solution (v/v = 20/80) for LC-MS/MS analysis. In this method, food samples were not subjected to a hydrolysis (by acid or enzyme) that could cleave conjugated forms of metabolites. Only the free forms of ZEN, α-/β-ZEL, α-/β-ZAL, and ZAN were measured.

Both free and total amounts of ZEN, α- ZEL, β-ZEL, ZAN, α-ZAL, and β-ZAL in urine were analyzed using a method published previously (Li et al., 2018). Briefly, urine samples were thawed and centrifuged prior to the addition of ^{13}C-ZEN internal standard. The pH was adjusted to 6.8 with phosphate buffer. Then, β-glucuronidase (1000 U/mL urine) was added into the urine and incubated in a shaking water-bath overnight at 37 °C for complete hydrolysis of glucuronides (see supplementary material). For analysis of free ZEN and its metabolites, this enzymatic hydrolysis step was omitted. An Oasis ® PRiME HLB μElution Plate was used for high throughput cleanup, allowing dozens of samples to be prepared simultaneously prior to LC-MS/MS analysis. The method was validated in accordance with the guidelines defined by the EMEA (European Medicines Agency, 2011) and FDA (FDA, 2018), showing satisfactory recoveries ranged 94.1–116% for all the analytes (Li et al., 2018).

The LC-MS/MS measurements of food and urine samples were performed on an ACQUITY UPLC™ I-Class system coupled to a Xevo® TQ-S tandem quadrupole mass spectrometer (Waters, MA, USA). For chromatographic separation, a CORTECS™UPLC® C18 column (2.1 × 100 mm, 1.6 μm) from Waters set at 40℃ was applied. A linear gradient of methanol/acetonitrile (80/20, v/v, solvent B) and water (solvent A) was used as mobile phase as follows: 50–66% B in 0–4 min, 66–90% B in 4–4.1 min, 90% B for 4.1–6.0 min, 90–50% B in 6.0–6.1 min and left to equilibrate for 1.9 min before the next run. The flow rate was 0.4 mL/min. MS/MS analysis was in ESI negative multi reaction monitoring (MRM) mode. Other detailed MS/MS parameters were also described (Li et al., 2018). The limit of detection (LOD) of the analytes ranged 0.04–0.20 μg/kg in food and 0.02–0.06 ng/mL in urine, respectively, as summarized in Table 1.

2.6. Statistical analysis

For statistical analysis, concentrations below LOD (undetectable) were set to LOD/2 of the respective compound. The variables were analyzed with ANOVA, Kruskal-Wallis test, and Mann-Whitney rank test to investigate the differences among different subgroups (age, gender). The correlation between EDI and PDI was evaluated using Spearman test. Statistical analysis was performed using SPSS, version 22 (SPSS, Chicago, IL, USA). A p-value < 0.05 was considered statistically significant.

Table 1
Limits of detection of the analytical methods for food and urine samples.

Sample	ZEN	ZAN	α-ZEL	β-ZEL	α-ZAL	β-ZAL
Food (μg/kg)	0.04	0.04	0.10	0.10	0.20	0.20
Urine (ng/mL)	0.02	0.03	0.04	0.06	0.04	0.02

3. Results and discussion

3.1. Demographic characteristics

The study cohort consisted of 199 participants; 96 were male and 103 were female. The male participants were between 4 and 78 years old, and the female subjects were between 4 and 80 years old. Four different age groups were considered: 37 children (4–12 years), 28 adolescents (13–18 years), 83 adults (18–65 years), and 51 elders (> 65 years). The demographic characteristics of the subjects are shown in Table S1.

The study population in Anhui province located at lower basins of the Yangtze and Huaihe Rivers in east China was likely to be at relatively high risk of exposure to mycotoxins. In this region, outbreaks of head blight and ear rot, *Fusarium* fungal diseases of wheat and maize producing mycotoxins are frequent. Two previous studies reported similar results in Anhui province: ZEN contaminated 70% of the wheat with mean level of 34.2 μg/kg (Xiong et al., 2009) and contaminated 68.7% of the wheat with mean level of 25.7 μg/kg (Xu et al., 2019). Both did not investigate ZEN masked forms. Our study was carried out during September, the harvest season of wheat. Moreover, the population preferred the local homemade food, thus, their exposure to ZEN mainly came from local contamination.

3.2. Duplicate diet analysis

3.2.1. Food consumption

The consumption data was obtained via practical measurements at duplicate diet collection sites. In total, 244 cereal food samples in 15 categories were collected. For each food category, the average daily consumption was calculated as follows:

$$Consumption_j = \frac{\sum_{i=1}^{n} Consumption_i}{n}$$

where $Consumption_j$ is the average daily consumption of the food category j (g/person/day), $Consumption_i$ is the individual consumption of food category j for each participant (g/person/day), and n is the number of participants in this study (n = 199 for the entire population, n = 96 for the males, and n = 103 for the females). The detailed information is shown in Table S2. Steamed bun, noodles, rice porridge, steamed rice, baba (local pancake), and jiaozi (local dumpling) accounted for 97.4% of the staple food consumption. Other food items rarely consumed, such as steamed twisted roll, cookies, cornmeal porridge, and crispy rice were classified as “others” (Fig. 1 and Table S2). Wheat and rice were the predominant cereal sources. Comparing the mean daily consumption of food categories between genders, males tended to consume more staple foods, while the diet of female participants was more diversified.

3.2.2. ZEN occurrence in food

The contaminated levels of ZEN, ZAN, α-ZEL, β-ZEL, α-ZAL and β-ZAL in 244 staple food samples were analyzed. The results indicated that 59.8% (146/244) of samples were ZEN positive, with an average (± SD) concentration of 1.21 ± 2.15 μg/kg. β-ZEL only existed in a cookie sample at 1.07 μg/kg. This cookie sample also had the highest ZEN concentration of 19.3 μg/kg, below the EU maximum limit for ZEN in processed cereal-based foods (50 μg/kg). ZAN, α-ZEL, α-ZAL, and β-ZAL were not detected in any of the food samples. The occurrence of ZEN in separate food categories is summarized in Fig. 2(A). Cookie (19.3 μg/kg), crispy rice (13.5 μg/kg) and cornmeal porridge (10.6 μg/kg) were found to be the most heavily contaminated, but they were

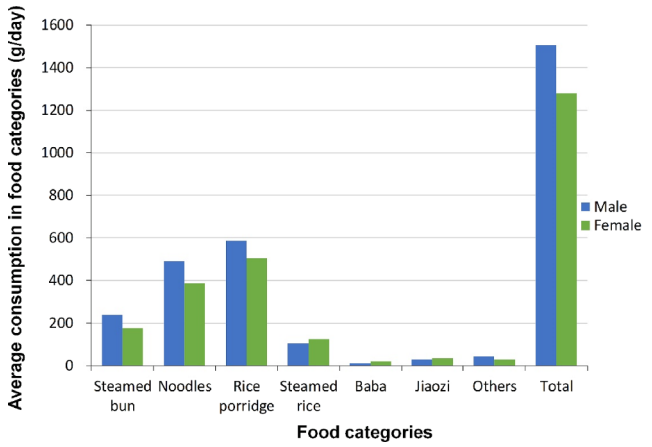

Fig. 1. Comparison of the mean consumption in food categories between males and females.

rarely consumed. Only one sample of each category was consumed and collected in this study (Table S3). For the commonly consumed food categories, the highest mean level of ZEN was found in baba (3.52 ± 3.06 μg/kg), a local sticky pancake made of glutinous rice (*Oryza sativa L. var. Glutinosa Matsum)*. According to the cereal sources, the diet samples were divided into maize, wheat, rice, glutinous rice, and sweet potato groups. Among these five groups, the mean level of ZEN was in the order of maize > glutinous rice > wheat > rice > sweet potato ($p < 0.01$), as showed in Fig. 2(B). Therefore, from the perspective of contamination level, foods made from maize, glutinous rice, and wheat need more attention regarding ZEN contamination.

3.3. Dietary exposure assessment

3.3.1. ZEN dietary exposure

The individual estimated daily intake (EDI, in ng/kg bw/day) of ZEN was calculated from all the staple foods consumed during a day as follows:

$$\mathrm{EDI} = \frac{\sum_{i=1}^{p} T_i \times F_i}{W}$$

where T_i is the concentration of ZEN in the foodstuff i ($i = 1,\ldots, p$) (ng/g); F_i is the consumption of the foodstuff i in a day (g/d); and W is the individual body weight (kg). The EDIs of ZEN for different populations are presented in Table 2, ranging from 1.43 to 344 ng/kg bw/day, with a mean level of 25.6 ± 38.6 ng/kg bw/day. This was slightly higher than the national average level of 21.8 ng/kg bw/day from 5th China total diet study (Wu et al., 2018). Only two of the participants (1.0%) had ZEN dietary exposure exceeding the EFSA's TDI of 0.25 μg/kg bw/day. They were from the same family and both consumed a large amount of noodles containing a relatively high level of ZEN (13.7 μg/kg). The mean level of EDI was slightly higher for males (28.1 ± 49.9 ng/kg bw/day) than for females (23.2 ± 23.8 ng/kg bw/day), but the difference was not statistically significant ($p = 0.588$). Among the four age groups, children had the highest EDI ($p < 0.05$) of 37.5 ± 56.3 ng/kg bw/day, equivalent to approximately 15.0% of EFSA's TDI (0.25 μg/kg bw/day) and 7.5% of the PMTDI set by JECFA (0.5 μg/kg bw/day).

3.3.2. Food contribution to dietary exposure

The contributions of food categories to the dietary ZEN intake were investigated (Fig. 3). The steamed bun (34.0%), noodles (35.9%), and jiaozi (7.7%), which are mainly made from wheat, contributed 77.6% of ZEN dietary exposure. The rice products, including rice porridge (8.4%), steamed rice (2.6%) and baba (5.4%), contributed only 16.4%. Although the ZEN level in maize-derived food was relatively high, the consumption of maize was rather low. In this study, only one maize-derived food was consumed and collected. As a result, ZEN exposure from maize was very low, accounting for 2.1% of the total, within the population.

It is worth noting that only cereal foods were collected in this study, and the conjugated forms of masked ZEN (Phase II metabolites) were not measured in food analysis. Both might lead to an underestimation of ZEN dietary exposure.

3.4. Human biomonitoring

3.4.1. Urinary ZEN biomarkers

The high sensitivity of our analytical method guaranteed reliable human biomonitoring of ZEN and its metabolites. Both free and total (free + conjugated) amounts of ZEN and its metabolites (α-ZEL, β-ZEL, ZAN, α-ZAL, and β-ZAL) in 199 urine samples were obtained by analysis before and after enzymatic hydrolysis.

Only ZEN was detected in free form (fZEN). Of the 199 samples, 42 (21%) were found positive for fZEN, only 12 (6%) of which had quantifiable levels, with the mean and highest concentration of 0.022 ng/mL and 0.335 ng/mL, respectively. Among the four age groups, adolescents had the highest urinary fZEN at 0.0459 ng/mL on average ($p < 0.01$). These results are consistent with those of previous studies that did not perform enzymatic hydrolysis. The parent compound ZEN and its metabolites were scarcely detected in Germany, Bangladesh, Haiti (Gerding et al., 2014; Gerding et al., 2015), Belgium (Ediage et al., 2012; Heyndrickx et al., 2015; Huybrechts et al., 2015), Cameroon (Abia et al., 2013; Ediage et al., 2013), Thailand (Warth et al., 2014), Nigeria (Ezekiel et al., 2014), China (Henan Province) (Li et al., 2018), and China (Nanjing City) (Fan et al., 2019), all showing positive rates lower than 10%. One exception is a US study, in which free forms of ZEN and its metabolites were detectable in 78.5% of 163 girls, aged 9 and 10 years (Bandera et al, 2011). Another study in Portugal reported detectable fZEN in about half of 95 urine samples (Martins et al, 2019).

After β-glucuronidase digestion, free and conjugated forms were detected as total ZEN, α-ZEL, β-ZEL, ZAN, α-ZAL, and β-ZAL. Total ZEN, α-ZEL and β-ZEL were detected in 175 (87.9%), 51 (25.6%), and 48 (24.1%) urine samples. Total ZAN, α-ZAL and β-ZAL were not detected. The average total (free + conjugated) amounts of ZEN (tZEN), α-ZEL (tα-ZEL), and β-ZEL (tβ-ZEL) were 0.383 ng/mL, 0.089 ng/mL, and 0.142 ng/mL, respectively. The positive rates and concentrations of the analytes greatly increased after enzymatic treatment. Therefore, if the urine sample is not processed via enzymatic treatment, the assessment of ZEN exposure will be dramatically underestimated. ZEN and its phase I metabolites, α-ZEL and β-ZEL, are excreted in urine mainly in their glucuronide forms via Phase II metabolism. Recently, Phase II metabolism by sulfation of ZEN and its reduced metabolites has been evidenced in Caco-2 cells (Pfeiffer et al., 2011) and in human placenta (Warth et al., 2019). However, ZEN sulfates have not been found in human urine so far, even though pure ZEN at a rather high level was ingested by a volunteer (Mirocha et al., 1981). Therefore, β-glucuronidase was used in this study, and the results can represent most of ZEN urinary metabolites.

From the urinary results, ZEN-glucuronides were the major metabolites in human urine. Hence, the tZEN (free + conjugates of ZEN) could be the most sensitive biomarker for human biomonitoring in urine. The total amount of ZEN biomarkers (tBM) calculated as the sum of tZEN, tα-ZEL, and tβ-ZEL was employed to estimate the internal exposure. The tBM ranged from 0.060 to 6.35 ng/mL, with a mean concentration of 0.615 ng/mL. No significant gender and age differences were observed for tBM levels, but the urinary tZEN levels were different ($p < 0.05$) among age groups, with the highest level in adolescents followed by children, adults, and elders. Both fZEN and tZEN levels in adolescents were significantly higher than other age groups. The results of urinary ZEN biomarkers are summarized in

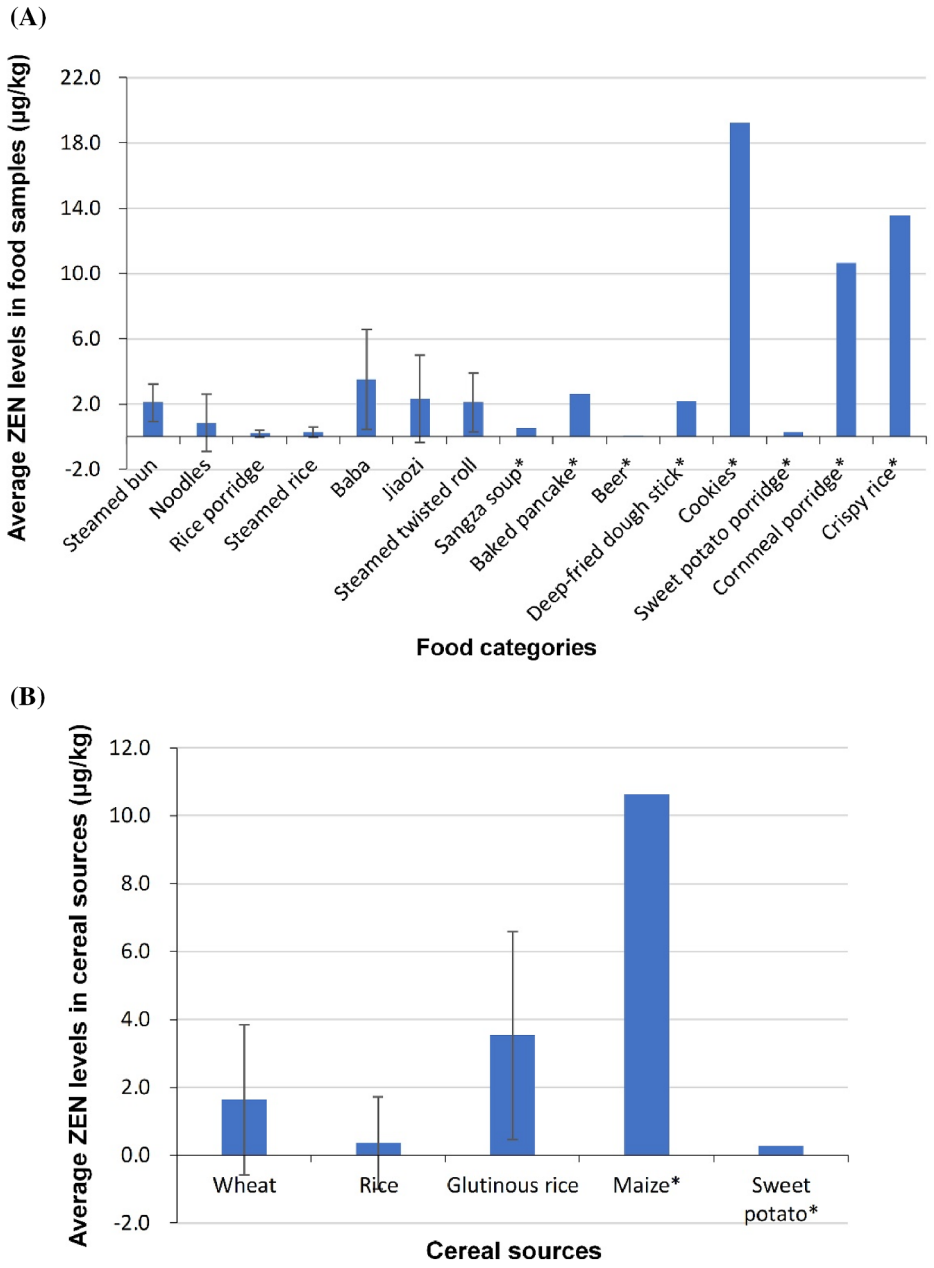

Fig. 2. Mean concentrations of ZEN in food categories (A) and cereal sources (B). Error bars represent the standard deviation. *There was only one maize-based sample (cornmeal porridge sample) and one sweet potato-based sample (sweet potato porridge) in the food samples.

Table 2
Estimated daily intake of ZEN for different populations (ng/kg bw/day).

Age	Number (M/F)	Total			Male			Female		
		Mean ± SD	Median	P90	Mean ± SD	Median	P90	Mean ± SD	Median	P90
Children 3-12y	37 (16/21)	37.5 ± 56.3	20.1	83.4	51.6 ± 82.4	20.1	230	26.7 ± 18.4	20.1	55.9
Adolescents 13-18y	28 (15/13)	19.3 ± 11.8	16.5	39.5	14.8 ± 8.35	12.2	30.5	24.5 ± 13.4	19.6	45.4
Adults 19-65y	83 (40/43)	25.3 ± 44.4	13.2	39.5	27.8 ± 55.0	14.0	39.8	22.9 ± 32.0	13.1	47.3
Elders > 65y	51 (25/26)	20.8 ± 13.1	17.4	45.6	21.4 ± 11.8	18.6	44.2	20.2 ± 14.5	14.9	53.2
Total	199 (96/103)	25.6 ± 38.6	15.7	46.3	28.1 ± 49.9	16.0	43.8	23.2 ± 23.8	15.7	48.4

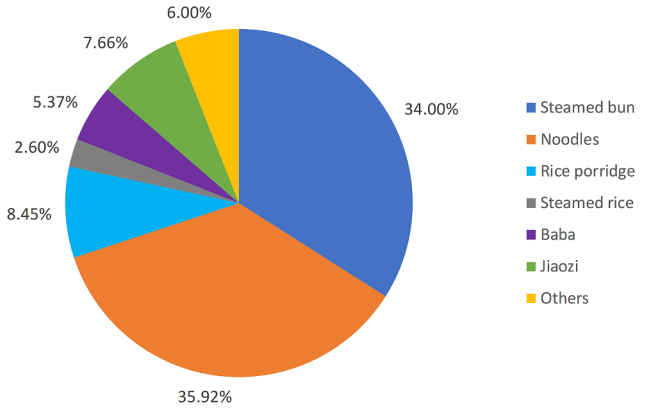

Fig. 3. Contribution of cereal sources of exposure to ZEN.

Table 3, and the detailed concentrations of fZEN and tBM for different ages and genders are shown in Table 4.

Results of recent human biomonitoring studies with and without enzymatic hydrolysis of urine samples were compared in Table 5. For the hydrolyzed urine samples, the positive rates of ZEN were much higher than those in unhydrolyzed samples. The average tBM was about eight times higher in our study than in 252 Swedish adults (tBM at 0.080 ng/mL) (Wallin et al., 2015), three times higher than in 52 residents in Southern Italy (tBM at 0.224 ng/mL) (Solfrizzo et al., 2014) and 62 residents in Bangladesh (Ali and Degen, 2019), more than two times higher than in 60 German adults (tBM at 0.2–0.3 ng/mL) (Ali and Degen, 2018), and 1.6 times higher than in 301 residents in Henan, China (tBM at 0.374 ng/mL) (Li et al., 2018). While tBM in this study was similar to that in a study of 54 female adults in South Africa (Shephard et al., 2013), it was three times lower than the tBM in a study of 163 girls in the USA (Bandera et al., 2011). These tBM levels reflect ZEN exposure in different cohorts.

In our study, urinary levels of tα-ZEL were found to be lower than those of tβ-ZEL, with a ratio of 0.63 (tα-ZEL/tβ-ZEL). The sum of tα-ZEL and tβ-ZEL accounted for only 37.6% of the tBM, which is much lower than tZEN (62.3% of the tBM). The tα-ZEL/tβ-ZEL ratio and (tα-ZEL + tβ-ZEL)/tBM ratio were in good agreement with the ratios in Henan, China (0.43 and 0.49, respectively) (Li et al, 2018), despite having a tBM level 1.6 times higher. In these two studies, β-ZEL was the major metabolite over α-ZEL. In another study carried out in Nanjing, China, only fZEN and fZAN were detected in less than 10% of the samples, using a direct analytical method (Fan et al., 2019). Free ZAN were more than two times higher than fZEN, showing a potential regional difference in ZEN metabolism in China. The tα-ZEL/tβ-ZEL ratio in our study was lower than that in Europe (0.84–3.2), Africa (1.01 and 1.44), and US (1.8). It even reached 15.2 in Bangladesh. In these countries, α-ZEL was observed to be the major metabolite in urine. More consistent results of the ZEN urinary excretion pattern were found in geographically close populations, such as in German and Sweden, or in Anhui and Henan. This might be attributed to the similar foods, cooking methods, and ethnic or genetic factors. It is also worth noting that unlike the majority of studies, a study of 42 women in Tunisia showed only α-ZAL was quantified in hydrolyzed urine with a relatively high concentration of 1.69 ng/mL (Belhassen et al, 2014). In the USA, ZEN, α/β-ZEL, α/β-ZAL, and ZAN were all detected in urine samples even though they had not been run through enzyme hydrolysis (Bandera et al, 2011). In addition to the factors mentioned above, the variability of these results might also be related to different occurrence of ZEN and its masked forms in food, but contamination in the matched food consumed was seldom reported.

Free ZEN positive samples were used to investigate the association between the levels of fZEN and tBM. On average, the fZEN/tBM ratio was 0.254. This indicates almost 74.6% of the detected biomarkers were conjugated forms. The fZEN/tBM ratios between different ages and genders are illustrated in Table 6. No age- or gender-related differences of fZEN/tBM ratios were found. Since matched unhydrolyzed and enzymatic hydrolyzed urine samples have seldom been analyzed for ZEN and its metabolites, this is the first fZEN/tBM ratio data from a large population study.

3.4.2. ZEN internal exposure

Probable daily intake (PDI) represents exposure to ZEN calculated

Table 3
ZEN biomarkers detected in 199 urine samples after enzymatic digestion.

Compound	> LOD (n)	> LOQ (n)	Mean (ng/mL)	Median (ng/mL)	P90 (ng/mL)	Range (ng/mL)
tZEN	175	112	0.383	0.218	1.14	0.0100–3.77
tα-ZEL	51	9	0.0892	0.0200	0.0650	0.0200–2.66
tβ-ZEL	48	15	0.142	0.0300	0.100	0.0300–2.84
tBM	180	111	0.615	0.294	1.66	0.0600–6.35

For the calculation of means, medians, and P90, toxin concentrations < LOD were set to LOD/2, and toxin concentrations between LOD and LOQ were set to LOQ/2 of the respective compound.

Table 4
Concentrations of free and total ZEN biomarkers in 199 urine samples (ng/mL).

Participants n (M/F)		Total			Male			Female		
		Mean ± SD	Median	P90	Mean ± SD	Median	P90	Mean ± SD	Median	P90
Children	fZEN	0.0163 ± 0.0284	0.010	0.025	0.0128 ± 0.0060	0.010	0.025	0.0189 ± 0.0375	0.010	0.022
37 (16/21)	tBM	0.818 ± 1.05	0.427	2.22	1.10 ± 1.40	0.617	3.87	0.603 ± 0.638	0.374	2.00
Adolescents	fZEN	0.046 ± 0.065	0.010	0.163	0.0312 ± 0.0550	0.010	0.143	0.0629 ± 0.0734	0.025	0.185
28 (15/13)	tBM	0.689 ± 0.725	0.422	2.03	0.499 ± 0.550	0.397	1.56	0.908 ± 0.856	0.478	2.52
Adults	fZEN	0.0175 ± 0.0272	0.010	0.025	0.0194 ± 0.0317	0.010	0.025	0.0158 ± 0.0224	0.010	0.025
83 (40/43)	tBM	0.634 ± 1.099	0.162	1.82	0.581 ± 0.930	0.075	1.90	0.686 ± 1.25	0.262	1.83
Elders	fZEN	0.0202 ± 0.0469	0.010	0.025	0.0266 ± 0.0645	0.010	0.025	0.0141 ± 0.0182	0.010	0.0145
51 (25/26)	tBM	0.394 ± 0.548	0.145	0.973	0.564 ± 0.694	0.307	1.92	0.230 ± 0.283	0.0975	0.578
Total	fZEN	0.022 ± 0.041	0.010	0.025	0.022 ± 0.044	0.010	0.025	0.022 ± 0.044	0.010	0.025
199 (96/103)	tBM	0.615 ± 0.933	0.294	1.66	0.650 ± 0.935	0.311	1.97	0.582 ± 0.935	0.262	1.63

For the calculation of means, medians, and P90, toxin concentrations < LOD were set to LOD/2, and toxin concentrations between LOD and LOQ were set to LOQ/2 of the respective compound.

Table 5
ZEN and its metabolites in human urine.

Countries	n	Positive n (Analyte)	Mean/median	range, ng/mL	References
Without enzymatic hydrolysis					
Germany	101	4 (ZEN-14-GlcA)	< LOQ	< LOQ	Gerding et al, 2014
Bangladesh	95	0	< LOD	< LOQ	Gerding et al, 2015
Germany	50	0	< LOD	< LOQ	Gerding et al, 2015
Haiti	142	4 (α-ZEL)	1.46/1.42	0.52–2.49	Gerding et al, 2015
Belgium	40	4 (ZEN)	–	< LOD–12.6	Ediage et al., 2012
		4 (β-ZEL)	–	4–24.8	
Belgium	32	0	< LOD	< LOQ	Huybrechts et al, 2015
Belgium	239	1 (α-ZEL)	5.0/5.0	5.0–5.0	Heyndrickx et al, 2015
		2 (β-ZEL-14GlcA)	0.8/0.8	0.6–1.0	
	155	0	< LOD	< LOQ	
Portugal	95 (24 h urine)	45 (ZEN)	0.17 (median)	< LOD–3.98	Martins et al, 2019
		0 (α-ZEL)	< LOD	< LOD	
		15(ZEN-14GlcA)	0.17 (median)	< LOD–25.70	
	95 (first morning urine)	54 (ZEN)	1.3 (median)	< LOD–11.51	
		4 (α-ZEL)	2.7 (median)	< LOD–8.60	
		14(ZEN-14GlcA)	0.15 (median)	< LOD–25.70	
Cameroon	220	8 (ZEN)	0.97 (Geometric mean)	0.65–5.0	Ediage et al., 2013
		9 (α-ZEL)	0.98 (Geometric mean)	0.26–1.3	
		18 (β-ZEL)	1.52 (Geometric mean)	0.02–12.5	
Cameroon	145	4 (ZEN)	0.22	< LOD–1.42	Abia et al, 2013
		2 (α-ZEL)	< LOD	< LOQ	
		4 (ZEN-14GlcA)	0.81	3.31–31	
		7 (total)	0.74	< LOD–21.38	
Thailand	60	0	< LOD	< LOQ	Warth et al, 2014
Nigeria	120	1(ZEN)	0.3	–	Ezekiel et al, 2014
		8 (ZEN-14GlcA)	9.5	< LOD–44.5	
USA	163	90 (ZEN)	1.82	0.05–33.12	Bandera et al, 2011
		60 (α-ZEL)	0.63	0.003–10.69	
		39 (β-ZEL)	0.35	0.05–1.10	
		29 (ZAN)	0.33	0.07–3.31	
		35 (α-ZAL)	0.25	0.02–0.57	
		17 (β-ZAL)	0.29	0.04–0.60	
		128 (total)	1.86	0.03–48.22	
China (Nanjing)	260	18 (ZEN)	0.146	0.056–0.311	Fan et al, 2019
		20 (ZAN)	0.342	0.106–1.82	
China (Henan)	301	4 (ZEN)	–	< LOD–0.05	Li et al, 2018
		4 (α-ZEL)	–	< LOQ	
		3 (β-ZEL)	–	< LOQ	
		1 (ZAN)	–	< LOQ	
China (Anhui)	199	42 (ZEN)	0.022	< LOD–0.335	This study
With enzymatic hydrolysis					
Italy	52	52 (tZEN)	0.057/0.056	LOQ–0.120	Solfrizzo et al, 2014
		52 (tα-ZEL)	0.077/0.074	LOQ–0.176	
		51 (tβ-ZEL)	0.090/0.088	< LOQ–0.135	
		tα-ZEL/tβ-ZEL	0.86		
		(tα-ZEL + tβ-ZEL)/tBM	0.74		
Sweden	252	92 (tZEN)	0.03	0.007–0.42	Wallin et al, 2015
		53 (tα-ZEL)	0.03	0.029–1.83	
		45 (tβ-ZEL)	0.02	0.054–1.33	
		tα-ZEL/tβ-ZEL	1.5		
		(tα-ZEL + tβ-ZEL)/tBM	0.62		
Germany	13	13 (tZEN)	0.031/0.025	0.007–0.009	Föllmann et al, 2016
		6 (tα-ZEL)	0.016/0.005	< LOD–0.075	
		3 (tβ-ZEL)	0.008/0.037	< LOD–0.021	
		tα-ZEL/tβ-ZEL	2		
		(tα-ZEL + tβ-ZEL)/tBM	0.44		
	12	12 (tZEN)	0.042/0.037	0.007–0.009	
		4 (tα-ZEL)	0.015/0.005	0.01–0.04	
		2 (tβ-ZEL)	0.009/0.005	0.01–0.04	
		tα-ZEL/tβ-ZEL	1.67		
		(tα-ZEL + tβ-ZEL)/tBM	0.36		
Germany	60	60 (tZEN)	0.10/0.07	0.04–0.28	Ali and Degen, 2018
		60 (tα-ZEL)	0.16/0.13	0.06–0.45	
		60 (tβ-ZEL)	0.05/0.03	0.01–0.20	
		60 (total)	0.32		
		tα-ZEL/tβ-ZEL	3.20		
		(tα-ZEL + tβ-ZEL)/tBM	0.68		
South Africa	54	54 (tZEN)	0.204/0.076	0.012–3.15	Shephard et al, 2013
		50 (tα-ZEL)	0.247/0.030	0.009–3.72	
		40 (tβ-ZEL)	0.244/0.085	0.016–5.94	
		tα-ZEL/tβ-ZEL	1.01		
		(tα-ZEL + tβ-ZEL)/tBM	0.71		

(*continued on next page*)

Table 5 (*continued*)

Countries	n	Positive n (Analyte)	Mean/median	range, ng/mL	References
Nigeria	120	98 (tZEN)	0.75/0.20	0.03–19.99	Šarkanj et al, 2018
		5 (tα-ZEL)	1.27/0.87	0.52–2.52	
		7 (tβ-ZEL)	0.88/0.33	0.06–2.74	
		tα-ZEL/tβ-ZEL	1.44		
		(tα-ZEL + tβ-ZEL)/tBM	0.74		
Tunisia	42	1 (tZEN)	< LOQ	< LOQ	Belhassen et al, 2014
		8 (tα-ZAL)	1.69/1.43	0.76–3.17	
		1 (tβ-ZAL)	< LOQ	< LOQ	
Bangladesh	62 (in winter)	62 (tZEN)	0.028/0.021	0.017–0.084	Ali and Degen, 2019
		62 (tα-ZEL)	0.198/0.194	0.170–0.346	
		11 (tβ-ZEL)	0.013/0.013	< LOD–0.022	
		tα-ZEL/tβ-ZEL	15.2		
		(tα-ZEL + tβ-ZEL)/tBM	0.88		
	62 (in summer)	62 (tZEN)	0.040/0.027	0.015–0.201	
		62 (tα-ZEL)	0.182/0.183	0.100–0.286	
		55 (tβ-ZEL)	0.018/0.011	< LOD–0.090	
		tα-ZEL/tβ-ZEL	10.1		
		(tα-ZEL + tβ-ZEL)/tBM	0.83		
China (Henan)	301	214 (tZEN)	0.24/0.15	< LOQ–3.7	Li et al, 2018
		3 (tZAN)	0.017/0.015	< LOQ–0.52	
		12 (tα-ZEL)	0.035/0.020	< LOQ–2.6	
		66 (tβ-ZEL)	0.082/0.030	< LOQ–2.1	
		tα-ZEL/tβ-ZEL	0.43		
		(tα-ZEL + tβ-ZEL)/tBM	0.31		
China (Anhui)	199	112 (tZEN)	0.383/0.218	< LOQ–3.77	This study
		9 (tα-ZEL)	0.089/0.020	< LOQ–2.66	
		15 (tβ-ZEL)	0.142/0.030	< LOQ–2.84	
		111 (total)	0.615/0.294	< LOQ–6.35	
		tα-ZEL/tβ-ZEL	0.63		
		(tα-ZEL + tβ-ZEL)/tBM	0.38		

Table 6
fZEN/tBM ratios by different age groups.

Age	n (M/F)	Total		Male		Female	
		Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median
Children	5 (3/2)	0.230 ± 0.187	0.333	0.131 ± 0.176	0.0303	0.380 ± 0.0655	0.380
Adolescents	12 (4/8)	0.270 ± 0.555	0.0632	0.134 ± 0.172	0.0606	0.338 ± 0.674	0.0639
Adults	16 (8/8)	0.285 ± 0.147	0.333	0.306 ± 0.0987	0.333	0.264 ± 0.188	0.333
Elders	9 (7/2)	0.193 ± 0.152	0.143	0.144 ± 0.135	0.0852	0.362 ± 0.0406	0.362
Total	42 (22/20)	0.254 ± 0.316	0.332	0.199 ± 0.150	0.237	0.315 ± 0.428	0.333

Only samples having detectable fZEN (fZEN > LOD) (n = 42, 22 males and 20 females) were included to calculate the fZEN/tBM ratio.

from biomarker data, and it was estimated for individuals as follows:

$$\mathrm{PDI} = \frac{C_{tBM} \times V}{W \times E}$$

where C_{tBM} is the concentration of the tBM (ng/mL) (sum of the total ZEN, α-ZOL, and β-ZEL) of individuals; V is the urine excretion volume (mL) based on assumed daily urine excretion of 500 mL for children and 1500 mL for adolescents, adults, and elders (Gong et al., 2015); W is the individual body weight (kg); E is the excretion rate. An excretion rate of 36.8% (including 28.4% as total ZEN and 8.3% as α-ZEL) has been derived from a piglet study (Gambacorta et al., 2013) and was used in some human biomonitoring studies to assess human exposure, owing to very limited data on urinary excretion of ZEN in humans (Solfrizzo et al., 2014; Wallin et al., 2015; Föllmann et al., 2016). PDIs of ZEN for different populations are presented in Table 7. The average PDI was 41.6 ng/kg bw/day, equivalent to 16.6% of the TDI set by EFSA. Five participants (2.5%) exceeded the EFSA's TDI of 0.25 μg/kg bw/day. These five participants were from different families and included 3 female adults and 2 male children, one being a 4-year-old boy, whose EDI value also exceeded the TDI. Among the four population groups,

Table 7
Probable daily intake of ZEN for different age groups (ng/kg bw/day).

Age	n (M/F)	Total			Male			Female		
		Mean ± SD	Median	P90	Mean ± SD	Median	P90	Mean ± SD	Median	P90
Children 3-12y	37 (16/21)	47.5 ± 71.4	24.3	138	65.3 ± 100	23.3	279	33.7 ± 33.8	26.6	92.4
Adolescents 13-18y	28 (15/13)	54.0 ± 59.2	29.5	145	36.2 ± 37.5	29.2	109	74.8 ± 73.3	38.5	210.2
Adults 19-65y	83 (40/43)	42.7 ± 75.7	9.42	117	36.8 ± 61.4	5.66	92.4	48.2 ± 87.3	16.6	122
Elders > 65y	51 (25/26)	28.6 ± 41.8	11.8	63.8	39.4 ± 52.6	19.7	134	18.2 ± 24.5	7.47	42.2
Total	199 (96/103)	41.6 ± 65.5	18.4	120	42.1 ± 64.5	19.1	118	41.0 ± 66.7	16.6	120

Table 8
Comparison of dietary exposure and internal exposure.

Exposure	Mean ± SD	P50	P75	P90	Range	Exceeding (size, rate%)	
	(ng/kg bw/d)					TDI[b]	PMTDI[c]
EDI	25.6 ± 38.6	15.7	25.2	57.6	1.4–344.3	2 (1.0%)	0
PDI[a]	41.6 ± 65.5	18.4	42.6	120.1	2.2–431.2	5 (2.5%)	0

a: PDI was calculated with ER of 36.8%; b: TDI is 250 ng/kg bw/d set by EFSA; c: PMTDI is 500 ng/kg bw/d set by JECFA.

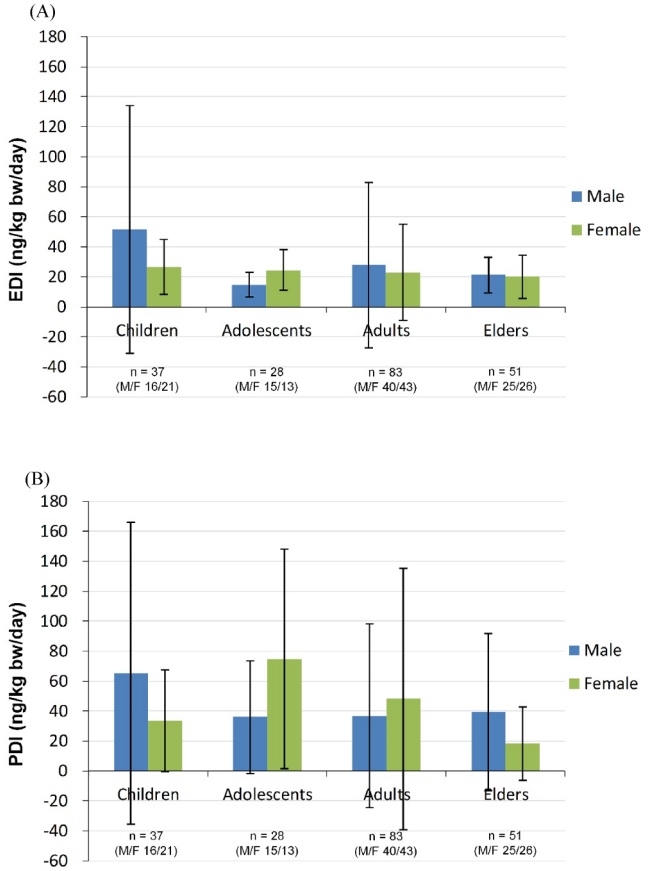

Fig.4. Comparison of dietary exposure and internal exposure. (A) Dietary exposure of ZEN, (B) internal exposure of ZEN calculated with an excretion rate of 36.8%.

Table 9
The ratio of PDI/EDI in different age groups.

Subgroup	n (M/F)	Mean ± SD	P50	P75	P90
Children	37 (16/21)	2.07 ± 2.94	0.89	2.29	7.25
Adolescents	28 (15/13)	2.89 ± 2.78	2.17	3.58	7.88
Adults	83 (40/43)	2.27 ± 3.39	0.91	2.43	7.64
Elders	51 (25/26)	1.57 ± 2.11	0.79	1.82	3.48
Total	199 (96/103)	2.14 ± 2.95	0.94	2.45	5.79

adolescents had slightly higher PDI on average, but no significant differences were found ($P = 0.069$).

3.5. Relationship between human dietary and internal exposure

To investigate the association of ZEN dietary and internal exposure, 199 subjects who had matched dietary and urinary data were included. There was a significant correlation between EDI and PDI, with a Spearman correlation coefficient of 0.344 ($p < 0.01$). Among the four age groups, the correlation in children was not significant (0.312, $p = 0.060$). In the other three age groups, the Spearman correlation coefficients were 0.506 ($p < 0.01$), 0.328 ($p < 0.01$), and 0.275 ($p < 0.05$) in adolescents, adults, and elders, respectively. Despite the overall positive correlation, they showed different trends with age. The correlation between dietary and internal exposure to ZEN appears to be stronger in adolescents than in other populations.

In this study, both the EDI and PDI were markedly below the TDI set by EFSA (only 10% and 16% of the TDI, respectively, Table 8). PDI was 1.6 times higher than EDI. Four possible causes may explain the differences. First, as mentioned above, only cereal foods were collected in our duplicate diet study. Thus, the EDI could be underestimated. Second, the morning urine might be more concentrated, with higher excretion levels resulting in higher PDI. Third, the internal exposure reflects the total exposure via all possible routes. Environmental and occupational exposure may also contribute, considering the living environment (farmland) of the subjects. Most importantly, the masked ZEN co-occurred with ZEN in crops, including the phase I metabolites ZAN, α-/β-ZEL, and α-/β-ZAL and phase II glucoside and sulfate conjugates (EFSA, 2016). In this study, phase I metabolites were included in the food analysis, but the phase II conjugates in food, which could also contribute to ZEN exposure and convert into ZEN urinary biomarkers, were not measured. Despite the lack of masked ZEN occurrence data in Chinese crops, European studies reported the sum of phase II conjugates accounted for 20.4%~51.6% (Nathanail et al., 2015) and 37% (De Boevre et al., 2013) of the total ZEN related compounds in cereals and cereal-based foods. As a result, the dietary exposure to ZEN obtained in our study was underestimated, and this might be a major cause of the difference between EDI and PDI.

From Fig. 4, the trends of PDI between ages and genders were consistent with the EDI except the internal exposure was much higher in adolescents (54.0 ng/kg bw/day), with the data shown in Table 2 and Table 7 separately. This suggests that the ZEN metabolism pattern might be different between subpopulation groups, although it is merely derived from very limited numbers of participants in each group. Considering the larger SD value of PDI than EDI, not only can the excretion of ZEN be determined by the intake of ZEN, but it can also vary between genders, ages, and individuals as well.

In order to further study the variability in ZEN excretion, we calculated the ratio of PDI/EDI for 199 individuals as an indicator of the excretion efficiency between subpopulations. The results of the PDI/EDI in populations are summarized in Table 9.

The mean PDI/EDI ratios for children (n = 37), adolescents (n = 28), adults (n = 83), and elders (n = 51) were 2.07, 2.89, 2.27 and 1.57, respectively. No significant gender difference was observed ($p = 0.871$). However, with the age-related comparison, there were differences in the PDI/EDI between age groups ($p < 0.05$). Their excretion capabilities were in order of adolescents > adults > children > elders, although the sample size of each group was insufficient to draw firm conclusions. This result suggests adolescents are more efficient in ZEN metabolism than any other age group. As a result, there is potential to overestimate exposure to ZEN in adolescents when using the same excretion rate to assess internal exposure. In addition, further validation of urinary ZEN biomarkers, especially the impacts of age and gender on the excretion of ZEN and its metabolites, is needed.

4. Conclusions

In this research, the exposure assessment of ZEN in a Chinese cohort of 199 from 74 families was studied. We employed both duplicate diet study based on food analysis and human biomonitoring approaches in the assessment of ZEN exposure. Our validated sensitive LC-MS/MS methods for ZEN and five metabolites of ZAN, α-/β-ZEL, and α-/β-ZAL in food and urine matrices generated reliable data in the exposure assessment. From the results of the duplicate diet study, ZEN was the predominant mycoestrogen contaminant in food samples. The cereal sources of maize, glutinous rice, and wheat were contaminated with relatively high ZEN concentrations. Wheat contributed to nearly 80% of the ZEN intake. The average EDI of ZEN was markedly below TDI set by EFSA. Children (n = 37) had the highest EDI level, with 15% of TDI. From the human biomonitoring, conjugates of ZEN, α-ZEL, and β-ZEL were the main metabolites of ZEN in urine. Adolescents (n = 28) had significantly higher fZEN and tZEN levels in urine. According to the human biomonitoring data, internal exposure (PDI) was 1.6 times higher than the calculated dietary exposure. Both EDI and PDI were at the safe level compared to TDI. The correlation between dietary and internal assessment has seldom been studied, especially in the case of ZEN. Thus, this is the first ZEN exposure assessment incorporating both diet study and corresponding human biomonitoring in a human cohort. Moreover, the relationship between dietary and internal exposure has been revealed for the first time. A significant correlation is observed, although it appears not very strong. Uncertainties from gender, age, and interindividual factors could hamper the accurate translation from urinary ZEN biomarkers to dietary intake. Thus, we calculated the PDI/EDI ratio to investigate differences in excretion capabilities between subpopulations. Based on the very limited number of participants, significant age-related differences were found and worthy of further investigation. Despite the limitations, mainly not including conjugated forms of masked ZEN in food analysis, this work showed a significant correlation between food-based approach and biomarker-based approach, both of which can serve as reliable and powerful tools for exposure assessment.

CRediT authorship contribution statement

Shuo Zhang: Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Shuang Zhou:** Methodology, Investigation, Validation, Writing - review & editing. **Yun Yun Gong:** Conceptualization, Writing - review & editing, Project administration. **Yunfeng Zhao:** Resources, Supervision. **Yongning Wu:** Resources, Funding acquisition.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105852.

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