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China National Center for Food Safety Risk Assessment

Dietary and Internal Exposure Assessment to Zearalenone in a Typical Area of China

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Introduction

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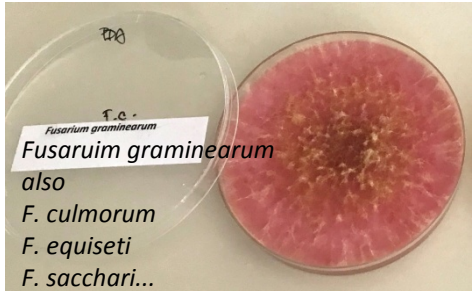
Introduction



Fusaruim graminearum
also
F. culmorum
F. equiseti
F. sacchari...

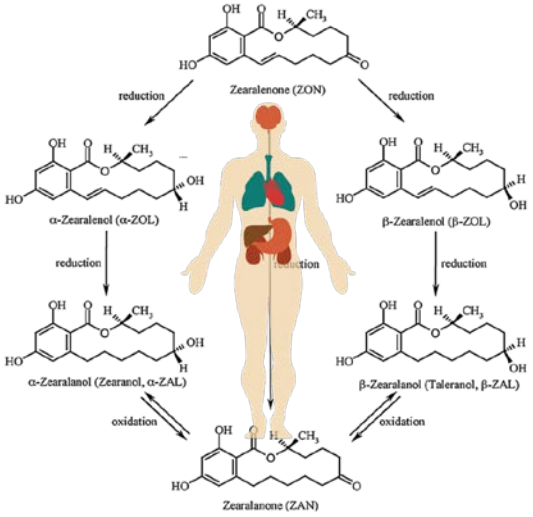
Due to the health risk caused by ZEN
JECFA's PMTDI: 0.5 mg/kg bw/day (2000)
EFSA's TDI: 0.25 mg/kg bw/day (2011)

ZEN and its metabolites high estrogenic activity

Hypoestrogenic syndromes In human

ZEN has also been found hepatotoxic, immunotoxic , genotoxic

IARC Group 3 carcinogen



This study aimed to characterize the exposure to ZEN by **two assessment approaches**

Introduction

Exposure assessment of mycotoxins

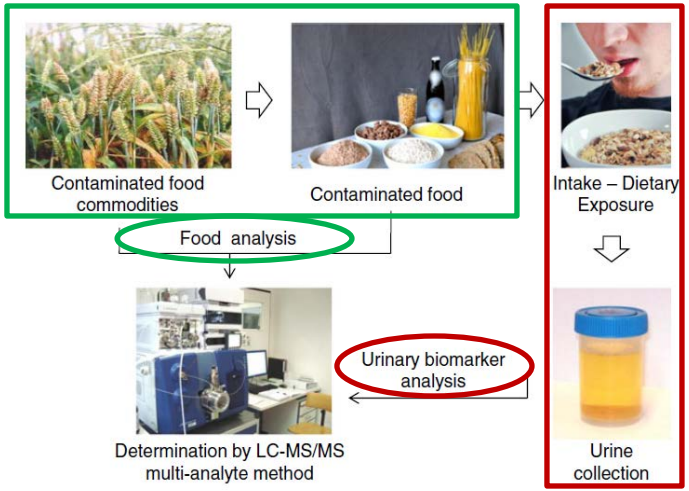
traditional food analysis compared with innovative biomarker approach

Contaminate level in food (parent form in food)

⇩

Dietary exposure assessment

e.g. total diet study
duplicate diet study



Biomonitoring (biomarker in urine, blood, feces etc.)

⇩

Internal exposure assessment

Benedikt Warth et al. 2013. Anal Bioanal Chem . 405:5687–5695

Key NOAEL

Uncertainty factors

⇨

TDI = NOAEL/Ufs

⇨

Safety:

Exposure < TDI ✓

Exposure ≥ TDI ✗

⇨

Management Decision

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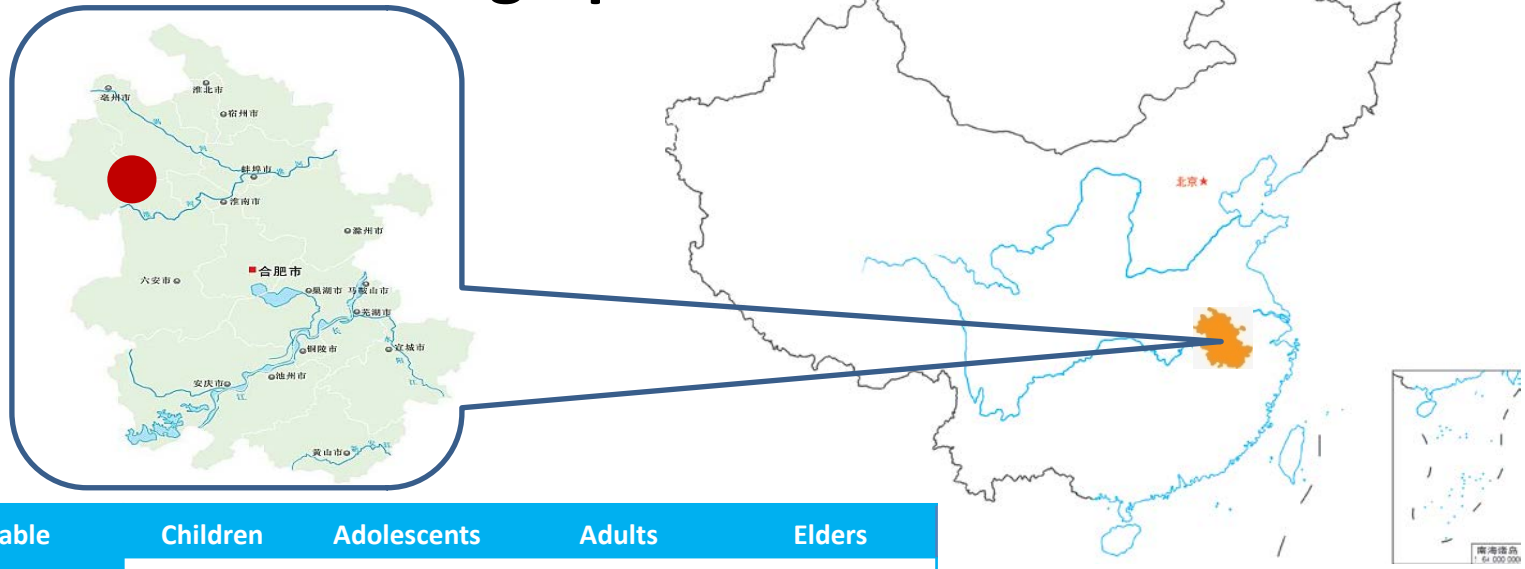
Introduction

Demographic characteristics

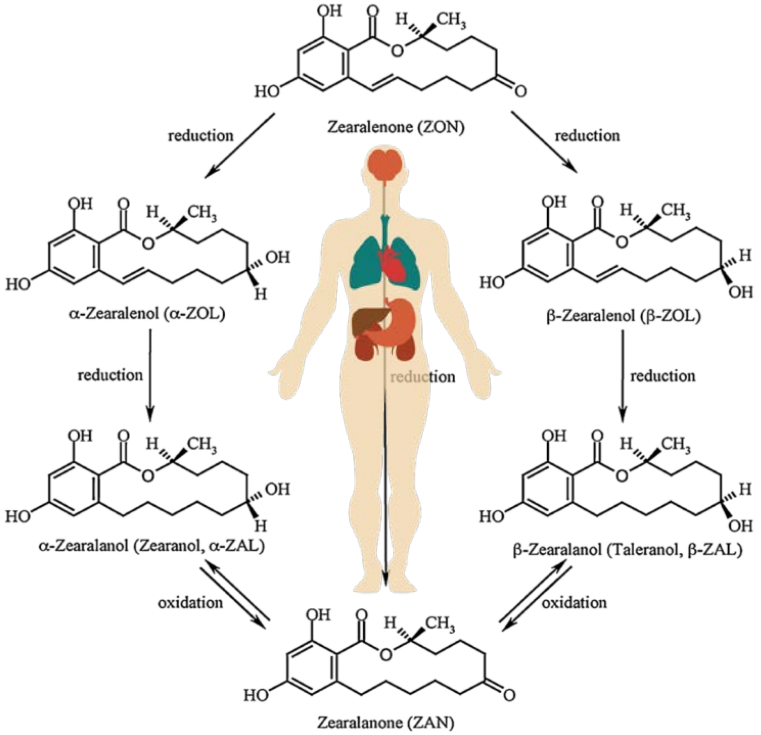


Variable	Children	Adolescents	Adults	Elders
Male	12	18	40	26
Female	21	13	41	28
Total	33	31	81	54
Age (years)	6.8±2.3	14.5±1.3	42.3±14.0	70.4±3.7
Body weight (kg)	25.0±7.2	53.1±10.2	62.2±9.0	58.7±10.2
BMI (kg/m^2)	17.7±4.3	20.2±3.3	22.6±2.9	22.4±3.0

- ✓199 healthy volunteers from 58 families
- ✓Aged 4-80
- ✓96 males and 103 females

Analytical methods

Analytical methods



Target analytes

Food method:

ZEN, α-ZOL, β-ZOL, α-ZAL, β-ZAL, ZAN

Urine method: Target metabolites

ZEN, α-ZOL, β-ZOL, α-ZAL, β-ZAL, ZAN — **Free ZENs**

ZEN-14-GlcA
ZAN-14-GlcA
α-ZOL-14-GlcA
β-ZOL-14-GlcA
……

enzymatic hydrolysis →

ZEN
ZAN
α-ZOL
β-ZOL
……

Total ZENs

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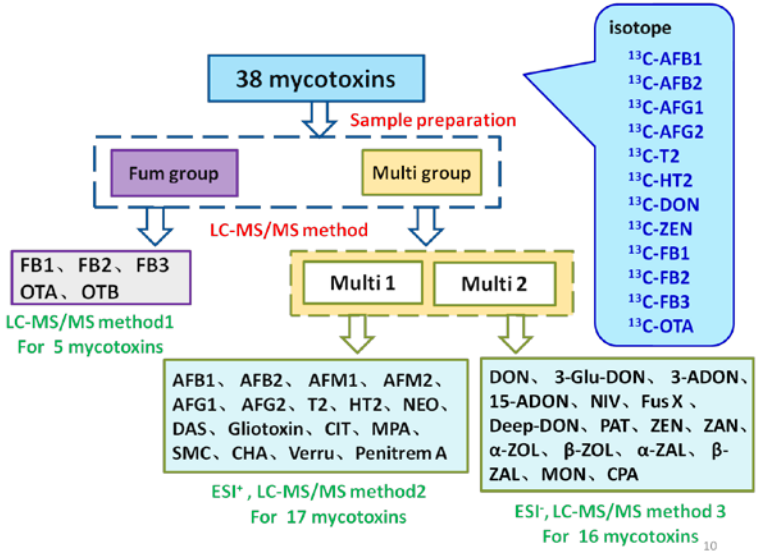
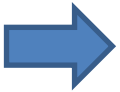
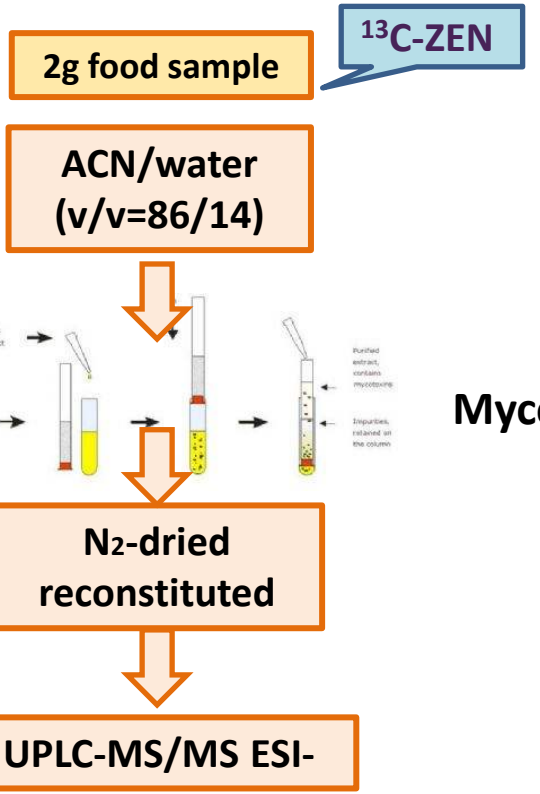
Analytical method for food

Food sample preparation







MycoSep 226

China Total Diet Study (TDS) method for mycotoxins
A method set for 38 mycotoxins
13 food categories
each categories with 1-5 cooking ways in TDS

Wu Y., Zhao Y., Li J., 2017. The fifth China Total Diet Study. First ed. Science Press, Beijing.

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Analytical methods

Urine sample preparation 96-well PRiME HLB μElution method

- **Dilution**

  2.5-fold with phosphate buffer (75 mM, pH 6.8)

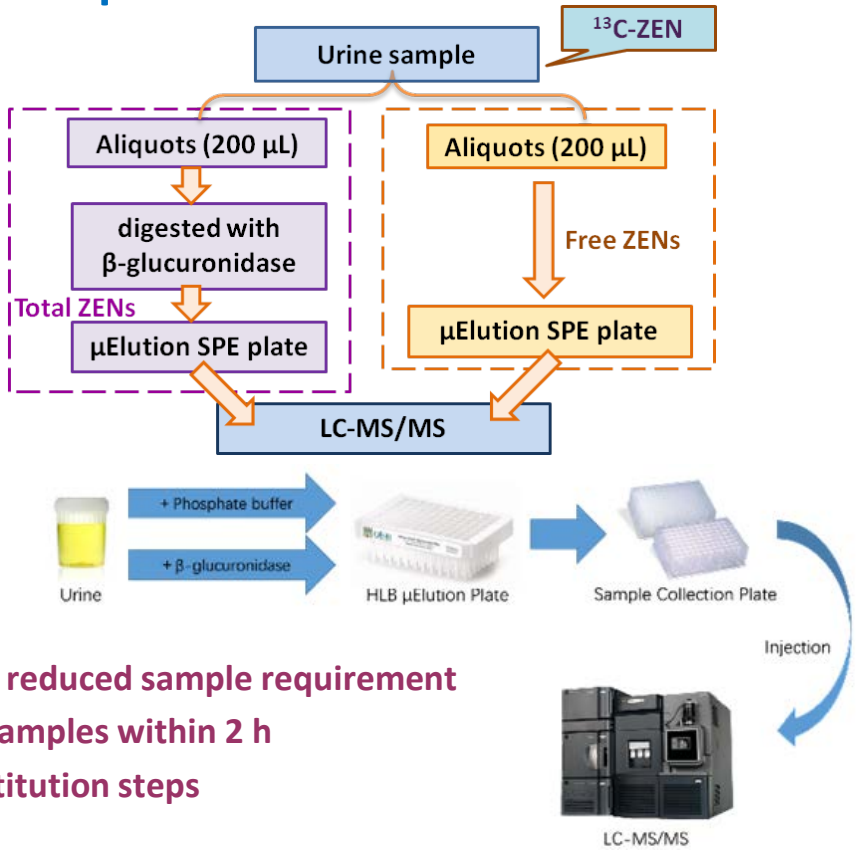
- **Loading buffer**
- **Washing solvent**

  1.200 μL of water was used as the washing solvent to remove salts and other water-soluble interferences

  2. 200 μL of **50% MeOH**

- **Elution buffer**

  200 μL of **100% methanol**



- ✓ 2 mg sorbent in each well greatly reduced sample requirement
- ✓ simultaneous preparation of 96 samples within 2 h
- ✓ without evaporation and reconstitution steps

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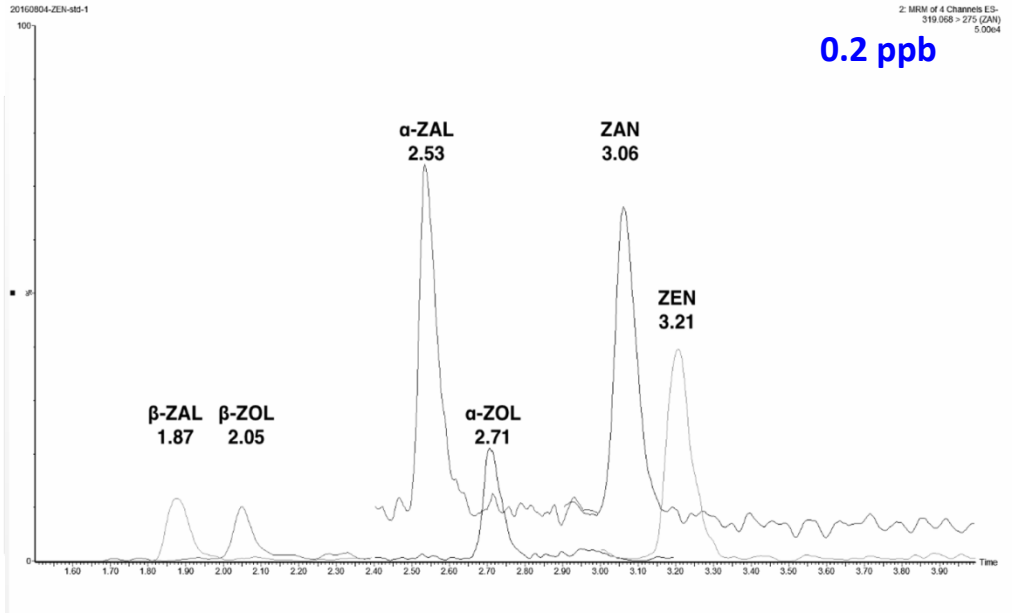
Analytical methods

Instrument

UPLC： Waters Acquity Ultra Performance LC system (UPLC)

MS/MS ： XEVO-TQ-MS (triple quadrupole mass spectrometry)

UPLC –MS/MS conditions



- Column: Waters CORTECS C18 UPLC column (2.1 mm×100 mm, 1.6 μm) or equivalent.
- Mobile phase: A: Water; B: ACN/ methanol (v/v=20/80).
- gradient program: started with 50% B, 50%-70% at 0-5 min, 70%-90% at 5-6 min, 90% at 6-7 min, and then reduced to 50% within 0.1 min and held for 1.9 min, with total runtime of 9 min.

Analytical methods

Method validation

Guidelines

European Medicines Agency (EMEA)
US Food and Drugs Administration (FDA)

- linearity
- specificity
- accuracy (method recovery, R_M): 87.9%-95.7%
- precision (intra and inter-day variability): Intra-day<4.3%; Inter-day <6.0%
- sensitivity (LOD and LOQ) :

LODs/LOQs of the analytical methods for food and urine sample (ng/mL)

Sample	ZEN	ZAN	α-ZOL	β-ZOL	α-ZAL	β-ZAL
Food	0.04/0.1	0.04/0.1	0.1/0.3	0.1/0.3	0.2/0.6	0.2/0.6
Urine	0.02/0.05	0.03/0.1	0.04/0.13	0.06/0.2	0.04/0.13	0.02/0.07

Li C., Deng C, Zhou S, et al. 2018. Anal Bioanal Chem. 410, 5301–5312.

Dietary exposure to ZEN

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Dietary exposure to ZEN

Duplicate diets analysis

Staple food

ZENs analysis

Food consumption

Dietary xposure

24-h duplicate diets study

+

✓the 24-hour duplicate portions of cereal meal were collected from 58 families.

✓the consumption of the food was recorded precisely for each family member.

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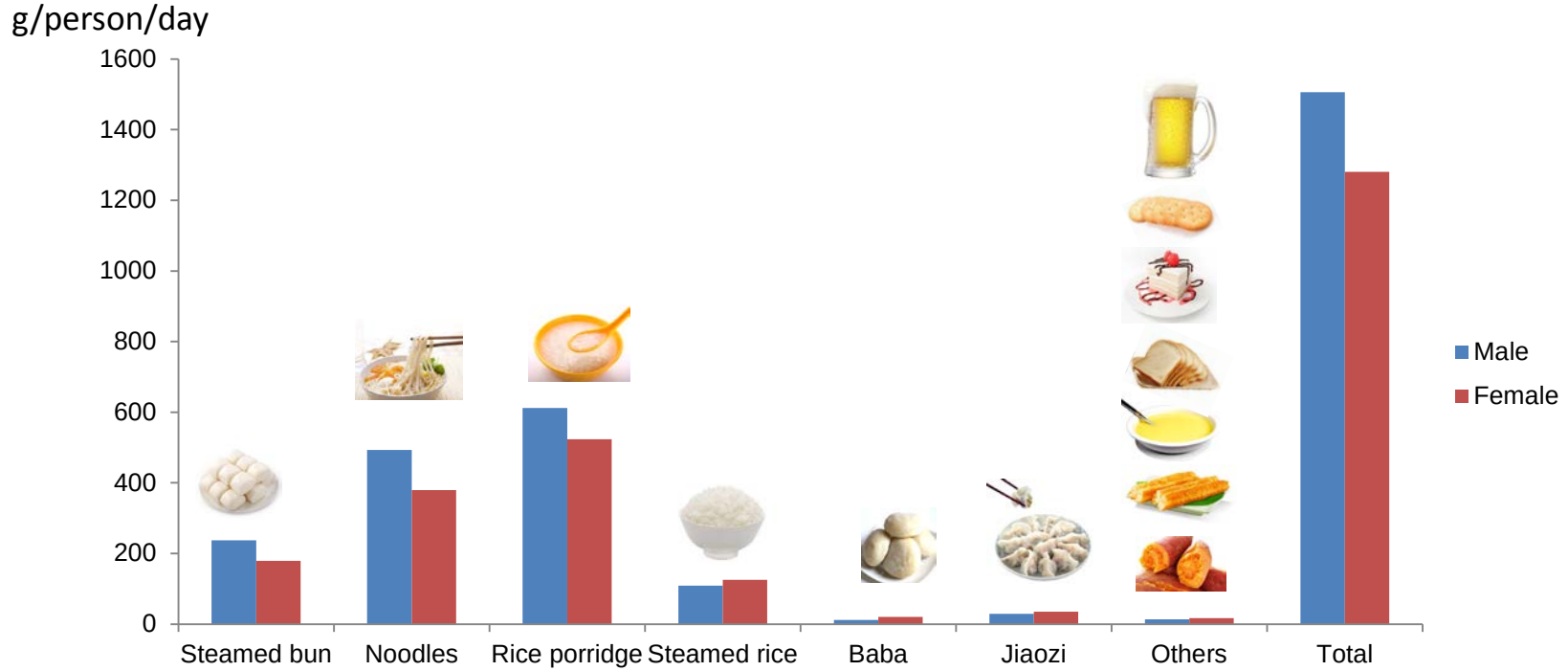
Dietary exposure to ZEN

Food consumption

In total, 363 staple food samples of 21 categories were collected.



Comparison of the mean consumption in food categories

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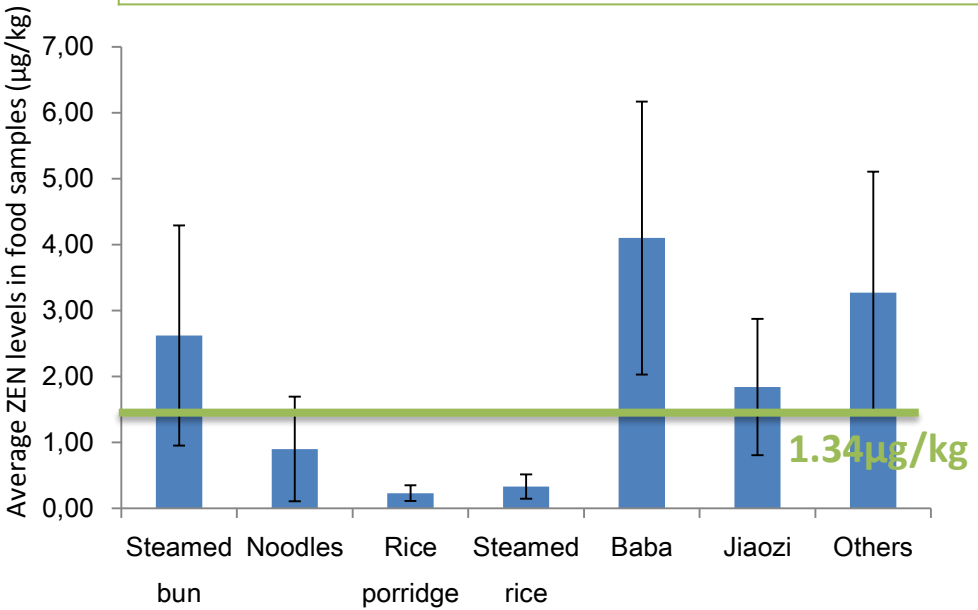
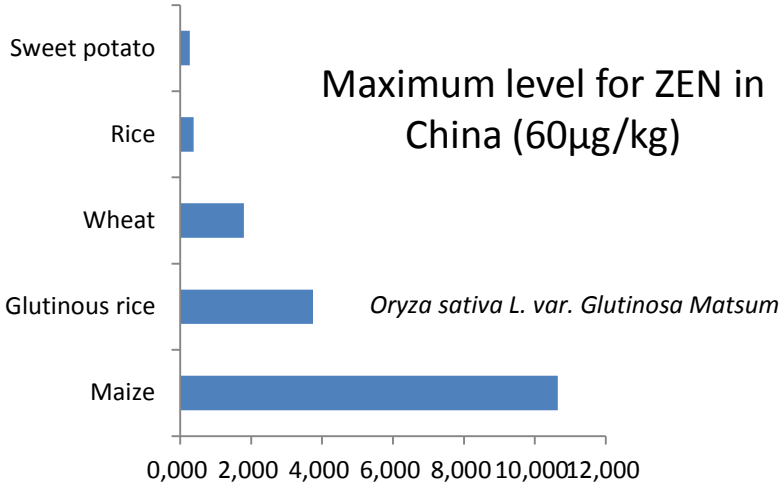
Dietary exposure to ZEN

ZEN levels in food samples

ZAN, α-ZOL, α-ZAL and β-ZAL were not detected in all food samples.

	Positive	Mean ± SD (μg/kg)	Median (μg/kg)	Range (μg/kg)
ZEN	227 (62.5%)	1.34 ± 2.50	0.54	ND~28.6
β-ZOL	2 (0.6%)	S1 (cookie, 1.07 μg/kg), S2(cookie, 0.84 μg/kg)		





Average ZEN levels in different cereal sources (μg/kg)

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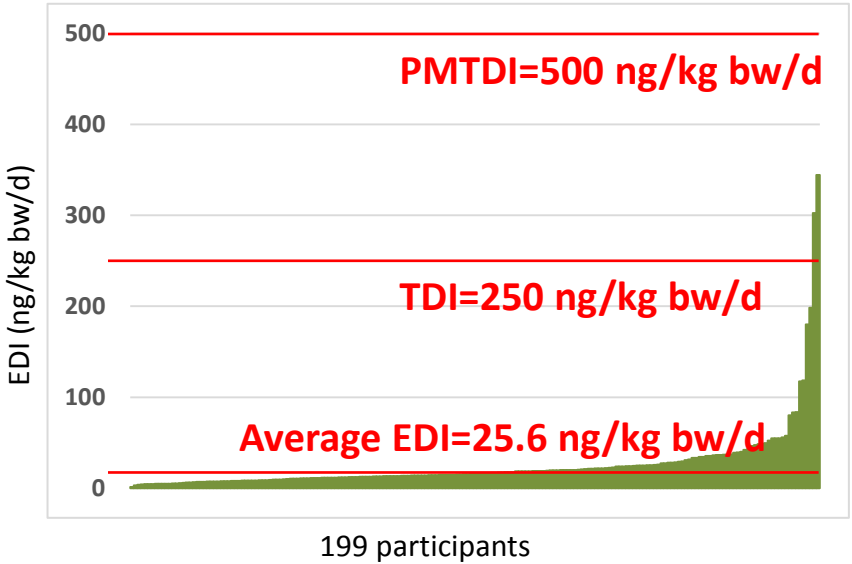
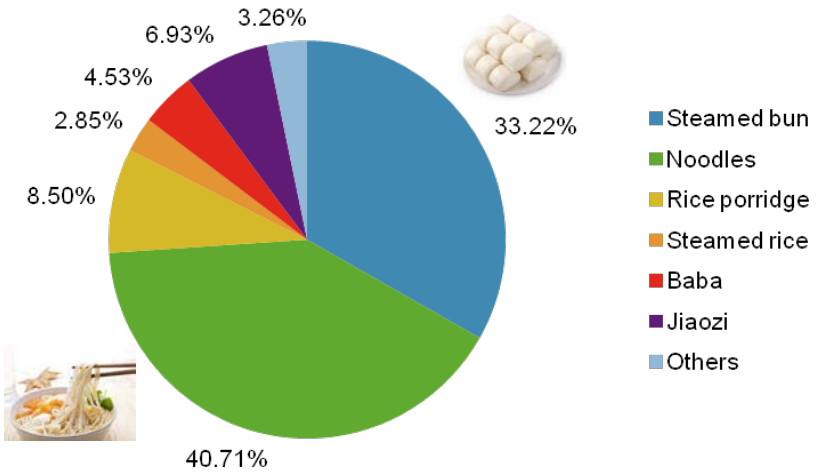
Dietary exposure to ZEN

Estimated daily intake (EDI)

$$\mathrm{EDI} = \frac{\sum(ZEN\ level\ in\ food \times food\ consumption)}{\text{body weight}}$$

	Mean ± SD (ng/kg bw/d)	P50 (ng/kg bw/d)	P75 (ng/kg bw/d)	P95 (ng/kg bw/d)	Range (ng/kg bw/d)	Exceeding	
						TDI (EFSA)	PMTDI (JECFA)
EDI	25.6 ± 38.6	15.7	25.1	56.2	1.43~344	2 (1.0%)	0





Internal exposure to ZEN

Internal exposure to ZEN

199 morning urine samples before and after β-glucuronidase hydrolyzing were analyzed

- unhydrolyzed urines : Only free ZEN (fZEN)
- hydrolyzed urines : ZEN, α-ZOL and β-ZOL (total ZENs, tZENs)

Compound	Free				Total			
	positive n (%)	mean (μg/L)	median (μg/L)	range (μg/L)	positive n (%)	mean (μg/L)	median (μg/L)	range (μg/L)
ZEN	42 (21.1)	0.02	ND	ND- 0.33	175 (87.9)	0.38	0.22	ND-3.80
α-ZOL	0	ND	ND	ND	51 (25.6)	0.09	ND	ND-2.65
β-ZOL	0	ND	ND	ND	48 (24.1)	0.14	ND	ND-2.84

Level below LOQ was set to half of the LOQ, and level below LOD was set to half of the LOD.ND: not detected (< LOD)

Internal exposure to ZEN

fZEN/tZENs ratios by different populations

	N (M/F)	Total		Male		Female	
		Mean±SD	Median	Mean±SD	Median	Mean±SD	Median
Children	5 (3/2)	0.2303±0.1872	0.3333	0.1307±0.1755	0.0303	0.3796±0.0655	0.3796
Adolescents	12 (4/8)	0.2702±0.5546	0.0632	0.1342±0.1717	0.0606	0.3383±0.6744	0.0639
Adults	16 (8/8)	0.2851±0.1466	0.3333	0.3057±0.0987	0.3333	0.2645±0.1880	0.3333
Elders	9 (7/2)	0.1926±0.3157	0.3319	0.1442±0.1496	0.0851	0.3621±0.0406	0.3621
Total	42 (22/20)	0.2545±0.3157	0.3319	0.1993±0.1496	0.2368	0.3153±0.4276	0.3333

Only samples positive for fZEN >1/2 LOD and tZENs >1/2 the sum of LOD of total ZEN (0.06) were included to calculate the fZEN/tZENs ratio.

✓In total, fZEN was only 25% of tZENs.

✓Females had slight **higher** fZEN/tZENs ratio than males, but the difference was not significant.

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Internal exposure to ZEN

Probable daily intake (PDI)

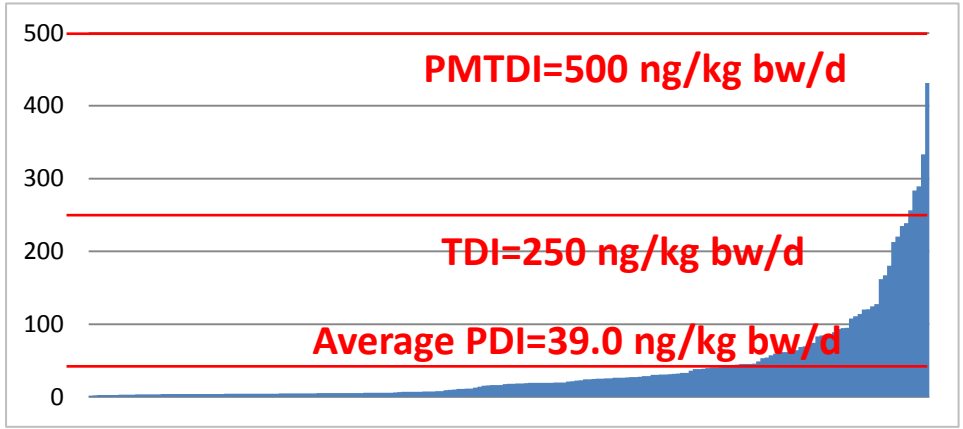
$$\mathrm{PDI} = \frac{C_{tZENs} \times V}{W \times E}$$

W: body weight E = excretion rate (ER, %), 36.8% forZEN

V: morning urine volume age<18 (500mL), age>18(1500mL)

World Mycotoxin J. **2013**, 3: 299-308.

	Mean ± SD (ng/kg bw/d)	P50 (ng/kg bw/d)	P75 (ng/kg bw/d)	P95 (ng/kg bw/d)	Range (ng/kg bw/d)	Exceeding	
						TDI (EFSA)	PMTDI (JECFA)
Internal ER = 36.8%	39.0 ± 63.3	18.3	41.3	108	2.17~431	5 (2.5%)	0



199 participants, PDI was calculated with ER of 36.8%

Dietary and internal exposure

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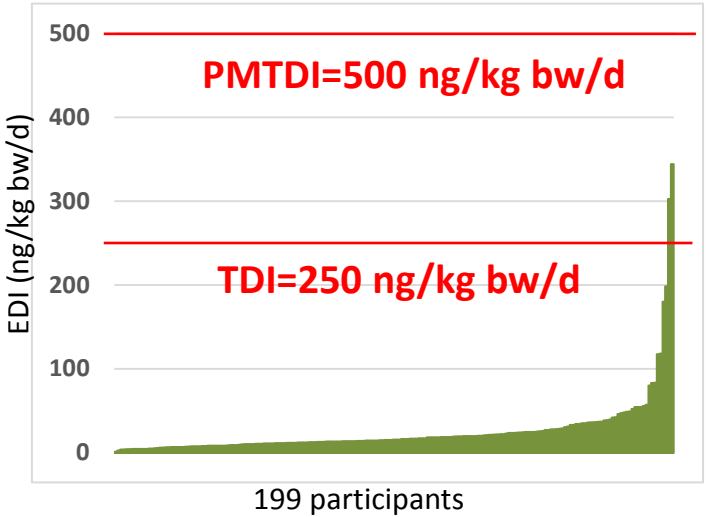
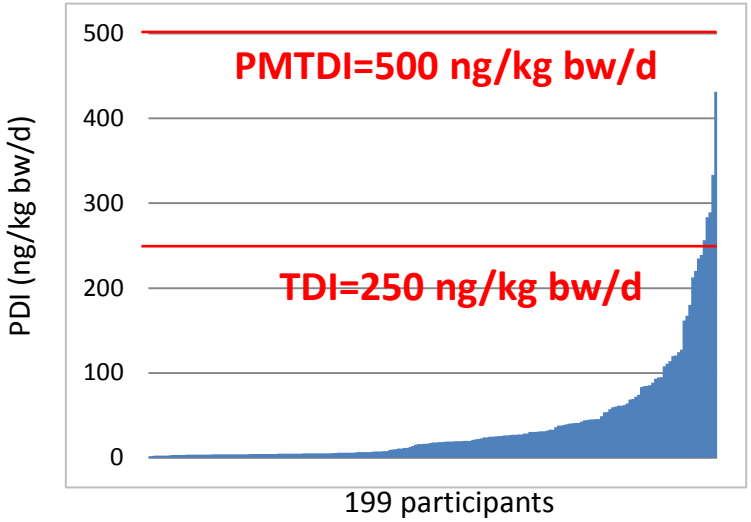
Dietary and internal exposure

EDI and PDI are **correlative** with Spearman correlation coefficient of 0.344 (P<0.01)



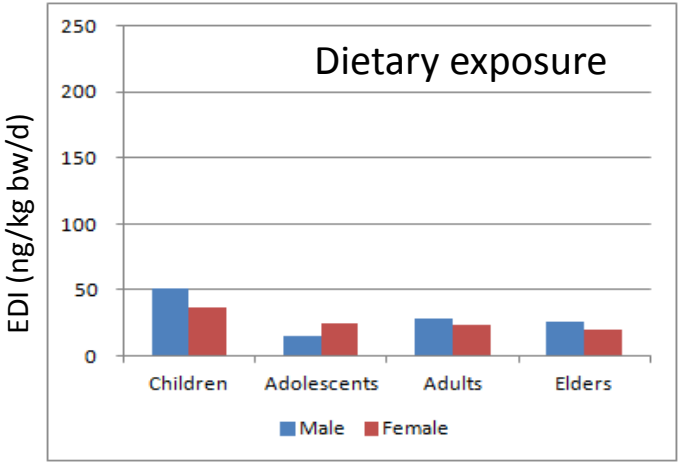


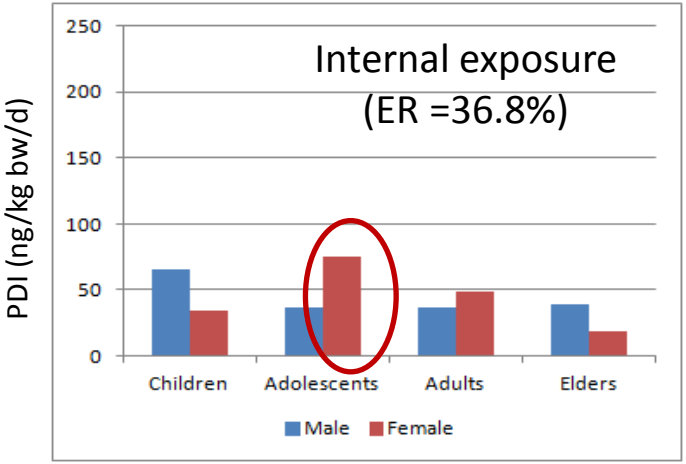
PDI was 1.5 times higher than EDI. The 50% differences might come from:

- ✓Only cereal foods were collected, so the EDI could be underestimated.
- ✓Morning urine might be more concentrated with higher excretion level
- ✓Masked ZENs co-occuring with ZEN in diet could also convert into α-/β- ZOL and α-/β- ZAL in metabolism contributing internal exposure.

Dietary and internal exposure

EDI and PDI levels in different population groups





Much higher PDI than EDI was found **in female adolescents**

As an estrogenic toxin, ZEN metabolism pattern of female adolescents could differ from other groups.

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Summary

From duplicate diet study:

- ZEN was the predominant contaminant in food
- Wheat is the primary source of ZEN exposure

From biomonitoring study:

- ZEN gluco-conjugate was the most sensitive biomarker in this study

Dietary and internal exposure assessment:

- There was significant correlation between EDI and PDI
- PDI was 1.5 times higher than EDI, but both of them were at safe levels comparing to TDI.
- Much higher PDI than EDI was found in female adolescents, it suggest that the excretive capabilities between ages and genders are different.

This work was supported by the CFSA “523” High Level Talents Development Project.

Thank you for your attention