DOI: 10.1289/ EHP4860

Note to readers with disabilities: *EHP* strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in *EHP* articles may not conform to <u>508 standards</u> due to the complexity of the information being presented. If you need assistance accessing journal content, please contact <u>ehp508@niehs.nih.gov</u>. Our staff will work with you to assess and meet your accessibility needs within 3 working days.

Supplemental Material

Transfer and Metabolism of the Xenoestrogen Zearalenone in Human Perfused Placenta

Benedikt Warth, Karin Preindl, Pius Manser, Peter Wick, Doris Marko, and Tina Buerki-Thurnherr

Table of Contents

Table S1. Calculated recoveries \pm standard deviation [%] in the three investigated matrices obtained from spiking experiments.

 Table S2. Impurities of added ZEN (experiment 1-3).

Figure S1. MRM (multiple reaction monitoring) chromatograms of reference standards spiked into blank medium and a fetal medium sample after 360 minutes; quantifier transitions in blue: zearalenone-14-sulfate (ZEN-14-Sulf) m/z 397.1 -> 317.2; α -zearalenol (α -ZEL) m/z 319.2 -> 275.2; zearalenone (ZEN) m/z 317 -> 175; qualifier transition in green: ZEN-14-Sulf m/z 397.1 -> 175.1; α -ZEL m/z 319.2 -> 160.1; ZEN m/z 317 -> 131.

Figure S2. Perfusion profiles and fetal-maternal ratios (FM ratio) of β -zearalenol (β -ZEL) and zearalanone (ZAN). β -ZEL and ZAN, present as contaminants in ZEN (around 1%; 3 µg/L), were measured from the maternal and fetal perfusates by UPLC-MS/MS at several time points during 6 h of perfusion with 318 µg/L ZEN. FM ratios were calculated for each time point and FM ratios of antipyrine and creatinine were added for comparison. Data represent mean ± SD of three independent placentae perfused with medium containing ZEN. p < 0.05 is considered statistically significant (* denotes differences between maternal and fetal concentrations in β -ZEL or ZAN perfusions; # and \$ denote differences in the FM ratio between metabolites and antipyrine or metabolites and creatinine, respectively). Perfusion data comparing maternal and fetal concentrations were analyzed by unpaired Student's t-test.

Excel Table S1. Concentrations of measured analytes in perfusion medium [µg/L].

Perfusions without ZEN (C1-3), perfusions with addition of ZEN (ZEN1-3).

Additional File- SupplementalMaterial_Data.zip

	placental tissue	fetal plasma per	fusion medium
α-ZAL	95 ± 8	98 ± 7	91 ± 15
α-ZEL	84 ± 12	91 ± 3	94 ± 10
α-ZEL-14-GlcA	91 ± 5	96 ± 3	108 ± 7
β-ZAL	95 ± 6	95 ± 8	77 ± 26
β-ZEL	93 ± 11	99 ± 15	74 ± 22
β-ZEL-14-GlcA	97 ± 1	88 ± 11	112 ± 12
ZAN	84 ± 11	95 ± 2	87 ± 4
ZEN	88 ± 2	89 ± 11	100 ± 13
ZEN-14-GIcA	92 ± 10	82 ± 4	96 ± 12
ZEN-14-Sulf	89 ± 2	90 ± 1	97 ± 3
α-zearalanol (α-ZAL);	α-zearalenol (α-ZEL);	α-zearalenol-14-glucuronide	e (α-ZEL-14-GlcA);
β-zearalanol (β-ZAL);	β -zearalenol (β -ZEL);	β-zearalenol-14-glucuronide	e (β-ZEL-14-GlcA);
zearalanone (ZAN);	zearalenone (ZEN);	zearalenone-14-glucuronid	e (ZEN-14-GlcA);
zearalenone-14-sulfate	(ZEN-14-Sulf)		

 Table S1 calculated recoveries ± standard deviation [%] in the three investigated matrices

 obtained from spiking experiments

Table S2 Impurities of added ZEN (experiment 1-3)

1.40	1.05
1.09	1.04
1.48	0.95
32 + 0 2	1.01 ± 0.05
	.32 ± 0.2

 β -zearalenol (β -ZEL); zearalanone (ZAN)



Figure S1: MRM (multiple reaction monitoring) chromatograms of reference standards spiked into blank medium and a fetal medium sample after 360 minutes; quantifier transitions in blue: zearalenone-14-sulfate (ZEN-14-Sulf) m/z 397.1 -> 317.2; α -zearalenol (α -ZEL) m/z 319.2 -> 275.2; zearalenone (ZEN) m/z 317 -> 175; qualifier transition in green: ZEN-14-Sulf m/z 397.1 -> 175.1; α -ZEL m/z 319.2 -> 160.1; ZEN m/z 317 -> 131



Figure S2: Perfusion profiles and fetal-maternal ratios (FM ratio) of β -zearalenol (β -ZEL) and zearalanone (ZAN). β -ZEL and ZAN, present as contaminants in ZEN (around 1%; 3 µg/L), were measured from the maternal and fetal perfusates by UPLC-MS/MS at several time points during 6 h of perfusion with 318 µg/L ZEN. FM ratios were calculated for each time point and FM ratios of antipyrine and creatinine were added for comparison. Data represent mean ± SD of three independent placentae perfused with medium containing ZEN. p < 0.05 is considered statistically significant (* denotes differences between maternal and fetal concentrations in β -ZEL or ZAN perfusions; # and \$ denote differences in the FM ratio between metabolites and antipyrine or metabolites and creatinine, respectively). Perfusion data comparing maternal and fetal concentrations were analyzed by unpaired Student's t-test.