



HHS Public Access

Author manuscript

Environ Res. Author manuscript; available in PMC 2016 October 01.

Published in final edited form as:

Environ Res. 2015 October ; 142: 66–71. doi:10.1016/j.envres.2015.06.017.

Triclosan and prescription antibiotic exposures and enterolactone production in adults

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Abstract

Background—The gut microbiome plays an important role in the development of disease. The composition of the microbiome is influenced by factors such as mode of delivery at birth, diet and antibiotic use, yet the influence of environmental chemical exposures is largely unknown. The antimicrobial compound triclosan, found in many personal care products and widely detected in human urine, is an environmental exposure for which systemic microbiotic effects may be of particular interest. To investigate the relationship between triclosan and gut microflora, we assessed the association between triclosan and enterolactone, an intestinal metabolite that is produced via bacterial transformation of dietary lignans (seeds, nuts) and has known susceptibility to oral antibiotics.

Methods—We examined urinary triclosan and enterolactone for 2005–2008 U.S. National Health and Nutrition Examination Survey subjects, aged ≥20 years (n = 3,041). We also examined the association between prescription antibiotic use and enterolactone to confirm its susceptibility to changes in bacterial composition of the body. Associations between natural log-transformed enterolactone and 1) detected vs. not detected (<2.3 ng/mL) triclosan, 2) triclosan quintiles (Q1–Q5), and 3) any vs. no antibiotics were estimated with multiple linear regression, adjusting for sex, age, race, body mass index, poverty income ratio, education, fiber intake, bowel movement frequency, cotinine and creatinine (n=2,441).

Results—Triclosan was detected in 80% of subjects (range: <2.3 – 3620 ng/mL), while enterolactone was detected in >99% of subjects (range: <0.1 – 122,000 ng/mL). After adjustment, enterolactone was not associated with triclosan (detect vs. nondetect: $\beta = 0.07$ (95% CI: –0.15, 0.30); Q5 (104.5 ng/mL) vs. Q1 (none): $\beta = 0.06$ (95% CI: –0.21, 0.34)). In sex-stratified analyses, triclosan was associated with higher enterolactone in women (detect vs. non-detect: $\beta = 0.31$ (95% CI: –0.07, 0.70), but not men $\beta = -0.18$ (95% CI: –0.47, 0.11). However, any antibiotic use (n=112), as compared to no antibiotic use, was associated with significantly lower

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Human Subjects

The National Health and Nutrition Examination Survey has been approved by the National Center for Health Statistics Research Ethics Review Board.

enterolactone ($\beta = -0.78$ (95% CI: $-1.22, -0.36$)), with no sex-specific effects. This association was driven by inverse associations with the following antibiotic classes: macrolide derivatives, quinolones, sulfonamides, and lincomycin derivatives.

Conclusions—Antibiotics, but not triclosan, are negatively associated with urinary enterolactone. Antibiotics may reduce enterolactone by killing certain gut bacteria. At levels detected in the U.S., triclosan does not appear to be acting similarly, despite broad antimicrobial properties. Additional study of determinants of triclosan exposure and enterolactone production may be needed to better understand positive associations among women.

Keywords

triclosan; enterolactone; NHANES; antimicrobial; intestinal metabolite

Introduction

Triclosan (2,4,4'-trichloro-2'-hydroxy-diphenyl ether) is a synthetic compound classified both as a drug and a pesticide. It is widely used in consumer goods for its broad-spectrum antimicrobial properties (1). At low doses, triclosan impairs bacterial growth by inhibiting enoyl-acyl carrier protein reductase, an enzyme necessary for bacterial lipid biosynthesis (2), while at high doses, triclosan is bactericidal, possibly due to cell membrane damage (3). Triclosan is contained in personal care products, such as toothpastes, deodorants and soaps, as well as impregnated into materials such as plastics and textiles for use in various consumer goods (kitchenware, clothing, etc.) (4). Certain uses of triclosan may be more effective than others in terms of antibacterial action and human health: toothpaste with triclosan decreases plaque and gingival inflammation (5), but triclosan-containing soap may be no more effective than normal soap in preventing infectious illness or in reducing bacteria levels on the hands of users (6). In addition, triclosan may be an endocrine disruptor (7–9), contribute to the development of antibacterial resistant organisms (10), or alter the human microbiome (11). Accordingly, there is a need for improved understanding of the risks and benefits of triclosan use.

The 2003–2004 National Health and Nutrition Examination Survey (NHANES) estimated more than 70% of U.S. residents had detectible triclosan in their urine (12). Contact with triclosan is likely limited to oral mucosa and skin surfaces, so the presence of the compound in urine suggests absorption and thus systemic exposure. As a result of this exposure, triclosan may be exerting unrealized effects on microorganisms throughout the body, such as the bacteria that colonize the gut, which are known to be susceptible to even parenterally administered antibiotics (13). The importance of the role that these microorganisms play in human health is known (14, 15). We should thus examine whether triclosan affects the human gut microbiome.

Among many functions, the microbiota of the gut are involved in the intra-luminal metabolism of some dietary components, the products of which can then be absorbed and utilized by the body. An example of such a metabolite is enterolactone, produced in the intestine via bacterial conversion of dietary lignans (found in nuts, seeds, fruits, etc.). Enterolactone production, as measured by its concentrations in serum or urine, is reduced by

some oral antibiotics (16), consistent with its dependence on the microbiome and implying that it may be a useful marker of bacterial function in the intestine. Accordingly, we conducted a cross sectional investigation of urinary enterolactone levels in relation to triclosan exposure, as well as prescription antibiotic medication use. This approach capitalizes on existing, publically available data, and allows us to test known (antibiotics and enterolactone) and unknown (triclosan and enterolactone) relationships, ultimately informing future hypotheses about the systemic antimicrobial behavior of triclosan.

Materials and methods

The National Health and Nutrition Examination Survey (NHANES) is a program of cross sectional surveys of adults and children that characterizes the health and nutritional status of the United States population using interview and physical examination data (17, 18). For each two year “cycle” of NHANES, a random one-third subset of subjects is also selected to contribute biologic specimens for biomarker measurement. For the present analysis, we included men and non-pregnant women, aged ≥ 20 years, who were included in the one-third subset of subjects assessed for both urinary enterolactone and triclosan in the 2005–2006 and 2007–2008 NHANES cycles ($n = 3,323$). Valid urinary measures were available for a final sample of $n = 3,041$ subjects.

A single spot urine sample was collected from each subject. Triclosan was measured using online solid-phase extraction coupled to high-performance liquid chromatography-isotope dilution-tandem mass spectrometry, as described elsewhere (12, 19). Enterolactone was measured using HPLC-MS/MS with atmospheric pressure ionization (20). Urinary creatinine was measured using a Jaffé rate reaction; this and all laboratory methods, along with documentation of human subjects ethics review, are described in greater detail on the NHANES website (17, 18).

We used multivariable linear regression to estimate the association between triclosan and enterolactone. Enterolactone was right-skewed and was natural log-transformed in all analyses to improve model fit (normality of residuals, heteroscedasticity, etc.). Enterolactone values below the LOD ($<LOD$) were imputed as $LOD/2$. For the independent variable triclosan, concentrations were non-detectable in approximately 20% of samples, and were not normally distributed among those with detectable levels. Therefore, triclosan was modeled either as detected ($\geq LOD$) vs. not detected ($<LOD$), or using 5 categories, where values below the LOD were in the lowest category, and the detected values were divided into quartiles. Associations were also examined by modeling natural log-transformed triclosan as a continuous variable, as there were no strong violations of linearity in these models following transformation. In continuous analyses, triclosan values $<LOD$ were imputed as either $LOD/2$ or using multiple imputation (21). Additional covariates included established predictors of enterolactone concentration (22, 23). These covariates included continuous age and body mass index (kg/m^2), as well as education ($<$ high school; high school/GED; some college or Associate of Arts (AA); college), poverty income ratio (PIR, the ratio of income to the family's appropriate poverty threshold) (low: ≤ 1.85 ; medium: $>1.85-3.5$; high: > 3.5), dietary fiber intake (grams) as estimated from 1-day dietary interview (low: ≤ 9.0 ($\leq 25^{th}$ percentile); medium: $>9.0 - <19.9$; high: ≥ 19.9 ($\geq 75^{th}$ percentile)).

75th percentile)), frequency of bowel movements (< 1/day; 1/day; > 1 per day), and urinary cotinine (0 (non-detect); < 12; ≥ 12 ng/mL (24)). After accounting for missing covariate data, the sample size for fully adjusted models was n = 2,441

To account for urinary dilution in both exposure and outcome measures, we modeled urinary creatinine in two ways: 1) we modeled concentrations of enterolactone and triclosan (ng/mL) along with urinary creatinine (natural log transformed mg/dL) as a covariate, and 2) we adjusted enterolactone and triclosan concentrations directly by dividing urinary concentrations by urinary creatinine. For the latter, analyses were performed using natural log-transformed, creatinine-adjusted enterolactone concentrations (ln-ng/mg); triclosan values < LOD were imputed as LOD/ 2, and triclosan categories were derived based on the creatinine adjusted distribution (ng/mg). We also conducted a sensitivity analysis in which extremely dilute (creatinine < 30 mg/dL) and concentrated (> 300 mg/dL) samples were excluded (n = 311).

Oral antibiotics have been shown to reduce levels of serum enterolactone, and we wanted to verify that we could detect a similar association in these data. Prescription medications were reported among NHANES subjects in response to the question, “In the past month, have you used or taken medication for which a prescription is needed?” Among responses, antibiotics were identified using drug codes as provided in the publically available NHANES dataset (per the Multum Lexicon Drug Database (<http://www.multum.com/>)). “Any antibiotic” use was defined as anyone reporting use of anti-infective drugs with any of the following classifications: penicillins, quinolones, macrolide derivatives, cephalosporins, sulfonamides, urinary anti-infectives, lincomycin derivatives, miscellaneous antibiotics, tetracyclines, leprostatics, aminoglycosides, or glycopeptides. In addition to “any antibiotic” use, we also assessed the association between enterolactone concentrations and specific antibiotic classes for classes with 5 or more users. An individual may have reported using multiple drugs. Due to small sample size, analysis of specific antibiotic classes were adjusted for creatinine only.

Weighted proportions, means, and geometric means were calculated using survey procedures in SAS 9.3 (SAS Institute Inc., Cary, NC) and NHANES survey weights. Univariate t-tests and multivariable linear regression were conducted using PROC SURVEYREG (SAS 9.3). Stratum specific associations were estimated for categories of sex and fiber intake.

Results

The subjects included in this analysis had a mean age of 47 years (Table 1). Over 60% were overweight or obese, and most were of non-Hispanic White ethnicity (72%). Self-reported antibiotic use was rare at 5%. Geometric mean urinary triclosan and enterolactone concentrations were slightly higher than those reported from previous NHANES cycles, at 16.9 (95% CI: 15.6, 18.3) and 260 (95% CI: 236, 286) ng/mL, respectively (12, 25) (Table 2). In univariate analyses across covariate categories, urinary triclosan concentrations were higher among younger subjects (vs. middle age), males, Mexican Americans and Other Hispanics (vs. non-Hispanic Whites), those with high PIR (vs. low), and no exposure to cotinine (vs. ≥ 12 ng/mL) (Table 1). There was also a suggested increase in triclosan with

increasing education. Triclosan was lower among subjects with low reported fiber intake (vs. medium). Enterolactone was detected in nearly all subjects (< LOD: n = 5 (0.2%)), and concentrations were higher among older subjects (vs. middle age), and among those with college education or greater, high PIR (i.e., socioeconomic status) (vs. low), and no exposure to cotinine (vs. 12 ng/mL). Low enterolactone concentrations were observed among the obese (vs. normal BMI), and among those reporting more than one bowel movement per day (vs. 1/day), low fiber intake (vs. medium intake), and antibiotic use.

If we did not account for creatinine in any way, enterolactone concentrations were positively associated with triclosan concentrations (e.g., triclosan detect vs. non-detect: $\beta_{\text{crude}} = 0.31$ (95% CI: 0.11, 0.51)), as concentrations of both compounds expectedly increased with increasing urinary concentration (i.e., creatinine). In multivariable regression models, however, this association was substantially attenuated. Adjusting for creatinine alone, this association decreased to $\beta = 0.10$ (95% CI: -0.12, 0.33). In the fully adjusted model of triclosan categories, estimates tended to be in the positive direction, but did not reach statistical significance and there was no evidence of a linear dose response (Table 3). Restricting analyses to those who did not report antibiotic use, or excluding subjects with extreme creatinine levels, did not substantially affect results. Directly dividing urinary concentrations shifted results away from the null in the positive direction, but were not statistically significant (not shown). Continuous models were not sensitive to either method for handling concentrations <LOD (LOD/ 2 substitution or multiple imputation).

After adjustment for creatinine and other confounders, enterolactone was significantly lower in subjects who reported antibiotic use in the previous month (n = 112) versus those that did not (n = 2,329) ($\beta_{\text{adjusted}} = -0.78$ (95% CI: -1.22, -0.36)). Results were similar across multiple modeling approaches, including crude models, and models using creatinine-divided enterolactone concentrations. When specific classes of antibiotics were assessed, not all antibiotics were negatively associated with urinary enterolactone (Table 4). The negative association between antibiotics and enterolactone was driven by quinolones, macrolide derivatives, sulfonamides, and lincomycin derivatives. Penicillins, cephalosporins, and tetracyclines were not associated with enterolactone. Urinary anti-infectives and “miscellaneous antibiotics” were associated with higher levels of enterolactone. Results were similar when modeling creatinine as a covariate, or when modeling creatinine-adjusted enterolactone concentrations, with the exception that sulfonamides shifted into the non-significant range when the latter approach was implemented. Univariate associations (no creatinine adjustment) were also similar.

Stratified analyses examining the associations between either triclosan or antibiotics with enterolactone did not reveal different relationships according to fiber intake. Similarly, there were no differences in the associations between antibiotics and enterolactone among men (detect vs. non-detect: $\beta = -0.83$ (95% CI: -1.64, -0.01)) and women ($\beta = -0.79$ (95% CI: -1.51, -0.07)). However, an interaction between triclosan and sex was observed, suggesting a trend towards a positive association in women (detect vs. non-detect: $\beta = 0.31$ (95% CI: -0.07, 0.70), but not in men ($\beta = -0.18$ (95% CI: -0.47, 0.11) (interaction p-value = 0.02).

Discussion

In this cross sectional analysis of urinary triclosan and enterolactone concentrations, triclosan exposure is not associated with enterolactone production in a manner that is similar to prescription antibiotics. This finding suggests that, despite triclosan's broad antimicrobial properties, the dose of triclosan experienced by typical U.S. adults is not high enough to affect the bacteria involved in the pathways that transform dietary lignans to enterolactone. These findings are meaningful given the documented susceptibility of enterolactone to prescription antibiotics in these and other data (16). However, consideration of other effects of triclosan is still warranted. For example, bacterial species at other sites, such as the oropharynx, female genital tract, or sebaceous glands, may be more susceptible to these levels of triclosan exposure; other products of gut microbiome function may be more susceptible to triclosan exposure than enterolactone.

The pathways involved in the metabolism of lignans to enterolactone are complex, and involve multiple biochemical conversions, including deglycosylation, demethylation, dehydroxylation and dehydrogenation (25, 26). Previous studies have demonstrated potentially important roles for multiple bacterial species, including *Peptostreptococcus productus*, *Eggerthella lenta*, *Eubacterium*, *Butyribacterium methyltrophicum*, *Clostridium scindens*, and *Lactonifactor longoviformis* (26–30). A recent epidemiologic study observed that subjects with lower enterolactone concentration in serum also had lower fecal bacteria counts, especially for bacteria in the *Lactobacillus-Enterococcus* group (31). The target of low-dose triclosan is enoyl-acyl carrier protein reductase, which is essential for lipid biosynthesis in many, but not all, bacterial species (2). Therefore, it is plausible that the bacterial species involved in enterolactone production are resistant to low-dose triclosan, in that they can synthesize lipids even in the absence of enoyl-acyl reductase (32). However, since it is likely that multiple bacterial species are involved in enterolactone production, such an assertion is speculative. Furthermore, triclosan is likely to have other mechanisms, including disruption of bacterial cell membranes (3, 33). In this regard, our results are surprising and warrant more detailed investigation.

Consistent with previous findings (16), we did observe that enterolactone concentrations were inversely associated with prescription antibiotics, providing additional evidence that enterolactone production is susceptible to perturbations in intestinal microflora. Although our sample size was limited for specific antibiotic classes, we observed inverse associations with macrolide derivatives, quinolones, sulfonamides, and lincomycin derivatives. Further, our findings are also consistent with those of Kilkkinen and colleagues (16), who reported the strongest suppression of enterolactone production occurred in users of macrolides, and the weakest among users of penicillins and cephalosporins. The susceptibility of enterolactone to antibiotic use broadly demonstrates the capacity for antibiotics to transiently alter the microbiome. It is also important to consider the effects that low enterolactone levels may have on human health, independent of microbiome effects. Enterolactone is a biologically active compound, noted for estrogen-like activity (34) and the capacity to inhibit lipid peroxidation (35, 36), among other functions. High levels of enterolactone have been shown to improve fertility (37) and breast cancer survival in postmenopausal women (38), as well as reduce cardiovascular disease related mortality in

men (39). Conversely, it is plausible that low levels of enterolactone, as induced by antibiotic use, may confer increased risk for adverse outcomes in certain individuals.

The strengths of our study include the ability to control for multiple potentially confounding factors. We identified numerous demographic and lifestyle characteristics that were associated with triclosan and enterolactone, and adjusted our models accordingly. While some characteristics were associated in a discordant manner (e.g., older age was associated with low triclosan exposure and high enterolactone levels), others were concordantly associated, suggesting complex confounding. Of note, characteristics such as low cotinine, high fiber intake, high PIR, and high education level were all, to some degree, associated with both higher levels of triclosan and higher levels of enterolactone. These suggest that triclosan exposure and enterolactone production may be associated with socioeconomic status (SES) and tendencies for engaging in (or avoiding) healthy or hygienic behaviors. Given that our results were mostly null, but in the positive direction, it is possible that our results may be influenced by residual confounding related to the tendency for both our exposure and outcome to correlate with “healthy” behaviors, despite efforts to control for such factors.

When stratified by sex, women, but not men, demonstrated higher enterolactone levels in association with exposure to triclosan. There was no difference between men and women with respect to antibiotic use and enterolactone. Given the absence of a sex-specific effect in response to antibiotics, it is unlikely that the association between triclosan and enterolactone in women is due to any unique feature of gut function or enterolactone metabolism in women. Rather, it is possible that the sources of triclosan exposure in women differ from the sources of exposure in men due to differential use of household and personal care products (40), and also correlate with dietary sources of enterolactone. However, we did not have the product use information to further explore this hypothesis.

This study is not a comprehensive investigation into the effects of triclosan on human microbiota. The use of enterolactone as a marker of intestinal microflora function is novel, yet crude, and we acknowledge that it can only provide limited information with regard to overall gut health. We also note that a single spot urinary measurement of triclosan is representative of short term exposure and may not accurately characterize an individual’s typical exposure. A recent study of pregnant women suggested that one spot urine sample may be fairly reliable for characterizing exposure to triclosan over the course of pregnancy (41), but reliability in other adults is not well described in the literature. The single measure used here does not allow us to distinguish between isolated and chronic exposure to triclosan, the latter of which may arguably have a more substantial effect on gut microflora.

Conclusions

In light of enterolactone’s confirmed susceptibility to prescription antibiotics, we cautiously assert that the internal dose of triclosan, as measured in a representative U.S. sample, does not have *widespread* antibiotic properties and does not act within the gut in a manner similar to prescription antibiotics. These findings do not preclude other unmeasured effects of triclosan. The effects of this compound on the microbiome still warrant further study.

Acknowledgments

Funding Source

This study was funded [in part] by the National Institute of Environmental Health Sciences, National Institutes of Health.

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Highlights

- Human exposure to antimicrobial triclosan is widespread.
- Enterolactone is made by bacterial conversion of dietary lignans in the human gut.
- Enterolactone decreases with antibiotic use, but not in association with triclosan.
- Antibiotics and triclosan may not be acting similarly upon intestinal microflora.

Table 1

Study sample characteristics: survey weighted means and percent distributions from adults (age ≥ 20 years) in 2005–2006 and 2007–2008 NHANES cycles (n = 3,041)

Characteristics	n	Weighted Geometric Mean (95% CI) (ng/mL)		
		% or mean (95% CI)	Triclosan	Enterolactone
Age, years (<i>mean</i>)	3041	47 (46, 48)		
20–39	1018	37 (34, 40)	23.1 (20.7, 25.7)**	247 (220, 277)
40–59 [†]	993	40 (37, 43)	15.2 (13.3, 17.4)	232 (197, 275)
60+	1030	23 (21, 26)	12.2 (10.4, 14.5)	341 (298, 392)**
BMI ^I , kg/m ² (<i>mean</i>)	3010	29 (28, 29)		
< 18.5	49	2 (1, 2)	14.3 (7.5, 27.4)	238 (123, 461)
18.5 – 25.0 [†]	835	31 (29, 33)	17.4 (15.4, 19.8)	295 (255, 341)
25.1–30	1018	32 (30, 34)	16.3 (14.0, 19.1)	280 (248, 317)
30+	1108	35 (33, 37)	17.1 (15.3, 19.2)	217 (194, 241)**
Sex				
Male	1526	49 (46, 51)	18.9 (16.6, 21.5)*	265 (237, 297)
Female [†]	1515	51 (49, 54)	15.2 (13.6, 16.9)	255 (222, 292)
Race/Ethnicity				
Mexican American	565	8 (6, 10)	25.6 (20.4, 32.0)**	288 (251, 330)
Other Hispanic	206	4 (2, 5)	27.1 (18.2, 40.4)*	211 (151, 295)
Non-Hispanic White [†]	1484	72 (67, 76)	15.9 (14.5, 17.5)	266 (235, 301)
Non-Hispanic Black	678	11 (8, 14)	15.6 (13.4, 18.1)	246 (206, 295)
Other, Multi-Race	108	5 (4, 6)	16.8 (11.7, 24.3)	202 (142, 290)
Poverty Income Ratio ^I				
Low (< 1.85)	1121	27 (25, 30)	14.8 (12.4, 17.6)*	199 (170, 232)**
Medium (<1.85–3.5)	744	26 (23, 28)	15.5 (13.3, 18.1)	269 (236, 307)
High (> 3.5) [†]	958	47 (43, 50)	18.9 (17.0, 21.1)	300 (262, 343)
Cotinine ^I , ng/mL				
< LOD (<0.01) [†]	529	18 (16, 21)	18.5 (15.4, 22.3)	340 (280, 412)
LOD – < 12.0	1589	54 (50, 57)	19.4 (17.7, 21.3)	284 (254, 318)
12	774	28 (25, 31)	12.4 (11.1, 13.9)**	188 (162, 219)**
Bowel Movements ^I				
< 1/Day	383	14 (12, 15)	14.6 (12.0, 17.7)	351 (283, 435)
1/Day [†]	1444	53 (51, 56)	17.1 (15.4, 19.0)	322 (286, 362)
>1/Day	965	33 (30, 36)	17.3 (14.5, 20.6)	167 (146, 191)**
Fiber Intake ^I , grams				
Low (0–9)	740	23(21, 25)	13.1 (11.6, 14.8)**	208 (178, 243)*

Characteristics	n	Weighted		Geometric Mean (95% CI) (ng/mL)	
		% or mean (95% CI)	Triclosan	Enterolactone	
Medium (9.1 – <19.9) [†]	1448	51 (49, 54)	17.6 (16.0, 19.3)	258 (232, 288)	
High (≥ 19.9)	726	26 (23, 28)	19.5 (17.1, 22.1)	311 (265, 364)	
Antibiotic Use					
Yes	133	5 (4, 6)	18.1 (13.6, 24.1)	114 (75, 176)**	
No [†]	2908	95 (94, 96)	16.8 (15.4, 18.4)	270 (247, 296)	
Education ¹					
Less than HS	848	18 (15, 20)	14.9 (12.7, 17.4)	225 (191, 264)**	
HS/GED	752	25 (23, 28)	14.4 (12.5, 16.6)*	206 (173, 247)**	
Some College/AA	835	31 (28, 34)	18.7 (16.1, 21.8)	262 (235, 293)*	
College or more [†]	604	26 (22, 29)	19.0 (16.4, 22.1)	357 (301, 422)	

Abbreviations: BMI: body mass index; LOD: limit of detection; HS: high school; GED: General Education Development; AA: Associate of Arts Degree

¹ Of 3041 subjects, data were missing on the following: body mass index (BMI) (n = 31), poverty income ratio (PIR) (n = 218), cotinine (n = 149), constipation (n = 249), fiber intake (n = 127), education (n = 2)

[†] Referent group for univariate t-test of natural log transformed biomarker concentration (values below limit of detection (LOD) imputed as LOD/2)

* p < 0.05 for univariate t-test;

** p < 0.01 for univariate t-test

Table 2

Distributions of urinary triclosan and enterolactone concentrations, adults 20 years, 2005–2006 and 2007–2008 NHANES cycles (n = 3,041)

Characteristics	Triclosan	Enterolactone
Geometric Mean (ng/mL)	16.9 (15.6, 18.3)	260 (236, 286)
Creatinine-adjusted Geometric Mean (ng/mg)	17.1 (15.8, 18.4)	263 (238, 290)
Median (Min, Max) ¹ (ng/mL)	12.1 (<LOD, 3620)	366 (<LOD, 122,000)
N (%) < LOD	637 (20)	5 (0.2)

¹Median determined from unweighted distribution

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Table 3Association of urinary triclosan and enterolactone¹ (n = 2,441)

Triclosan Exposure (ng/mL)	Creatinine-Only Adjusted ² β (95% CI)	Fully Adjusted ³ β (95% CI)
ln-triclosan ⁴	0.03 (-0.02, 0.09)	0.01 (-0.04, 0.06)
Non-detect	0	0
Detect	0.10 (-0.12, 0.33)	0.07 (-0.15, 0.30)
Q1 (Non-detect)	0	0
Q2 (2.3 - <7.4)	0.10 (-0.12, 0.33)	-0.02 (-0.33, 0.28)
Q3 (7.4 - <21.6)	-0.07 (-0.38, 0.25)	0.20 (-0.06, 0.46)
Q4 (21.6 - < 104.5)	0.24 (-0.02, 0.50)	0.07 (-0.27, 0.41)
Q5 (104.5+)	0.09 (-0.23, 0.43)	0.06 (-0.21, 0.34)

¹ Enterolactone concentrations are natural-log transformed² Restricted to n = 2,441 with complete covariate data³ Adjusted for sex, age, race/ethnicity, body mass index (kg/m²), poverty income ratio, dietary fiber intake, bowel movements per day, education, urinary cotinine and ln-urinary creatinine⁴ Values < LOD imputed as LOD/ 2

Table 4Associations of urinary enterolactone¹ (ng/mL) and antibiotic use

	n	β (95% CI)
Any Antibiotic Use		
Fully Adjusted ²	112	-0.78 (-1.22, -0.36)
Creatinine-Only Adjusted ³	133	-0.78 (-1.27, -0.31)
Antibiotic Class ⁴		
Penicillins	41	-0.09 (-0.75, 0.57)
Quinolones	22	-1.69 (-2.95, -0.44)
Macrolide Derivatives	20	-1.93 (-3.38, -0.49)
Cephalosporins	11	0.27 (-0.84, 1.37)
Sulfonamides	11	-1.21 (-2.30, -0.13)
Urinary Anti-infective	9	1.16 (0.50, 1.83)
Lincomycin Derivatives	8	-3.53 (-4.14, -2.89)
Miscellaneous Antibiotics	7	0.90 (0.21, 1.60)
Tetracyclines	7	0.18 (-0.81, 1.18)
Leprostatics	2	--
Aminoglycosides	2	--
Glycopeptides	0	--

¹Enterolactone concentrations are natural-log transformed²Subjects include n = 112 antibiotic users vs. n = 2,329 with no antibiotic use³Subjects include n = 133 antibiotic users vs. n = 2,908 with no antibiotic use⁴Users of antibiotic class vs. n = 2,908 with no antibiotic use; all estimates are adjusted for urinary creatinine.