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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 ($n=3041$). 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 ($n=2441$).

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. non-detect: $\beta=0.07$ (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 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.

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1. Introduction

Triclosan (2,4,4'-tricloro-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 (Bhargava and Leonard, 1996). At low doses, triclosan impairs bacterial growth by inhibiting enoyl-acyl carrier protein reductase, an enzyme necessary for bacterial lipid biosynthesis (McMurry et al., 1998), while at high doses, triclosan is bactericidal, possibly due to cell membrane damage (Villalain et al., 2001). 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.) (Adolfsson-Erici et al., 2002). 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 (Riley and

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Lamont, 2013), 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 (Aiello et al., 2007). In addition, triclosan may be an endocrine disruptor (Stoker et al., 2010; Zorrilla et al., 2009; Paul et al., 2010), contribute to the development of antibacterial resistant organisms (Levy, 2001), or alter the human microbiome (Lankester et al., 2013). 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 (Calafat et al., 2008). 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 (Giuliano et al., 1987). The importance of the role that these microorganisms play in human health is known (Geuking et al., 2014; Ianiro et al., 2014). 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 (Kilkkinen et al., 2002), 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.

2. 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 (Centers for Disease Control and Prevention (CDC), 2005–2006; Centers for Disease Control and Prevention (CDC), 2007–2008). 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 ($n=3323$). Valid urinary measures were available for a final sample of $n=3041$ 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 (Calafat et al., 2008; Ye et al., 2005). Enterolactone was measured using HPLC-MS/MS with atmospheric pressure ionization (Parker et al., 2012). 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 (Centers for Disease Control and Prevention (CDC), 2005–2006; Centers for Disease Control and Prevention (CDC), 2007–2008).

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/$\sqrt{2}$. For the independent variable triclosan, concentrations were non-detectible in approximately 20% of samples, and were not normally distributed among those with detectible 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/$\sqrt{2}$ or using multiple imputation (Berglund, 2015). Additional covariates included established predictors of enterolactone concentration (Rybak et al., 2013; Kilkkinen et al., 2001). 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); $\geq$ 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$25th percentile); medium: >9.0–<19.9; high: ≥ 19.9 ($\geq$75th percentile)), frequency of bowel movements ($<$1/day; 1/day; >1 per day), and urinary cotinine (0 (non-detect); <12; ≥ 12 ng/mL (Jarvis et al., 2008)). After accounting for missing covariate data, the sample size for fully adjusted models was $n=2441$.

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/$\sqrt{2}$, 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