ORIGINAL ARTICLE: ENVIRONMENT AND EPIDEMIOLOGY

Urinary triclosan concentrations and diminished ovarian reserve among women undergoing treatment in a fertility clinic

Lidia Mínguez-Alarcón, Ph.D., M.P.H.,[a] Georgios Christou, M.D.,[a,d] Carmen Messerlian, Ph.D.,[a] Paige L. Williams, Ph.D.,[b,c] Courtney C. Carignan, Ph.D.,[a] Irene Souter, M.D.,[d] Jennifer B. Ford, R.N.,[a] Antonia M. Calafat, Ph.D.,[e] and Russ Hauser, M.D.,[a,b,d] for the EARTH Study Team

[a] Department of Environmental Health, [b] Department of Epidemiology, and [c] Department of Biostatistics, Harvard T. H. Chan School of Public Health, Boston, Massachusetts; [d] Vincent Obstetrics and Gynecology, Massachusetts General Hospital, Boston, Massachusetts; and [e] National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia

Objective: To investigate the association between urinary triclosan concentrations and antral follicle count (AFC), a well-accepted marker of ovarian reserve, among women from a fertility center.
Design: Prospective cohort study.
Setting: Hospital fertility center.
Patient(s): A total of 109 women.
Intervention(s): Urinary triclosan concentrations quantified by online solid phase extraction-high performance liquid chromatography-isotope dilution tandem mass spectrometry.
Main Outcome Measure(s): AFC through transvaginal ultrasonography on the third day of an unstimulated menstrual cycle or on the third day of a progesterone withdrawal bleed.
Result(s): The geometric mean of the specific gravity–adjusted urinary triclosan concentrations for the 225 samples provided by the 109 women was 13.0 μg/L (95% confidence interval [CI], 8.9, 19.1). Women had median (with interquartile range) AFC of 13 (8, 18). The specific gravity–adjusted urinary triclosan concentrations were inversely associated with AFC (−4%; 95% CI, −7%, −1%). Women with triclosan concentrations above the median had lower AFC compared with those whose triclosan concentrations were equal to or below the median, with an adjusted difference of −3.2 (95% CI, −3.9, −1.6) among those with a body mass index <25 kg/m^2 and −1.8 (95% CI, −3.2, −0.3) among those who were <35 years old.
Conclusion(s): Specific gravity–adjusted urinary triclosan concentrations were inversely associated with AFC in women seeking care at a fertility center. This association was modified by age and body mass index, with the younger and leaner women showing larger decreases in AFC. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Antral follicle count, infertility, ovarian reserve, triclosan

Discuss: You can discuss this article with its authors and with other ASRM members at https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/16783-24017

Triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) is a lipid-soluble, chlorinated aromatic compound with broad-spectrum antibacterial properties that has been used for over 40 years as an ingredient in personal care products such as detergents, soaps, lotions, toothpaste, and shampoos (1, 2). Triclosan can be used as a plastic additive impregnated into toys, medical devices, household, veterinary, and industrial products (1, 2). Due to its widespread use, there is the potential for the general population to be exposed to triclosan

Received March 9, 2017; revised May 10, 2017; accepted May 12, 2017.
L.M.-A. has nothing to disclose. G.C. has nothing to disclose. C.M. has nothing to disclose. P.L.W. has nothing to disclose. C.C.C. has nothing to disclose. I.S. has nothing to disclose. J.B.F. has nothing to disclose. A.M.C. has nothing to disclose. R.H. has nothing to disclose.
Supported by National Institutes of Health grants R01ES022955, R01ES009718, and R01ES000002 from the National Institute of Environmental Health Sciences (NIEHS). The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC). Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the US Department of Health and Human Services.
Reprint requests: Lidia Mínguez-Alarcón, Ph.D., M.P.H., Department of Environmental Health, Harvard T. H. Chan School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115 (E-mail: lminguez@hsph.harvard.edu).

Fertility and Sterility® Vol. ■, No. ■, ■ 2017 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2017.05.020

through dermal and mucosal contact with consumer products, and through ingestion of contaminated food or water (3–5). Triclosan has a half-life in plasma of 19 hours with the major fraction of it being eliminated primarily in urine within the first 24 hours (3–5). The detection of triclosan in urine in nearly 75% of the 2003–2004 National Health and Nutrition Examination Survey (NHANES) participants confirms the ubiquity of the exposure (4). Although triclosan has also been detected in breast milk (6, 7), urine is the optimal matrix for measuring nonpersistent, semivolatile environmental chemicals that are biotransformed to hydrophilic, polar metabolites such as triclosan (8).

The use of triclosan was not highly regulated in the United States until very recently due to its U.S. Food and Drug Administration (FDA) classification of generally recognized as safe and effective (GRAS/GRAE). Concerns related to health effects of other organochlorines that were previously banned partially led the FDA in September 2016 to issue a final rule: triclosan and another 18 ingredients used in over-the-counter consumer antiseptic soaps are misbranded and are new drugs for which approved new drug applications are required for marketing (9). Similar policies have been implemented in Canada and in the European Union (10–12).

In several experimental studies using various animal models, triclosan has been implicated as an endocrine disruptor. Perinatal and pubertal exposed rats showed decreased levels of thyroxine, with the effect being more prominent among the animals treated with the highest doses of triclosan (13, 14). In vitro studies, triclosan enhanced ovarian and breast cancer cell growth and also impaired human endometrial stromal cell proliferation, migration, and decidualization (15–17). Female reproductive system development and endocrine function are adversely affected by triclosan in both mice and rats. Triclosan increases estrogen hormone levels and modulates estrogen's actions on target organs such as the uterus (18). Additionally, triclosan causes disruption of blastocyst implantation in mice and alters ovine placental estrogen synthesis, leading to adverse pregnancy outcomes (19).

Human studies exploring the effect of triclosan exposure on reproductive health are limited. In a case-control study among subfertile men, triclosan affected the negative feedback loop of luteinizing hormone secretion due to its presumptive adverse impact on Leydig cells (20). Among participants in the Maternal-Infant Research on Environmental Chemicals (MIREC) study, women with higher urinary triclosan concentrations had an increased time to pregnancy, an indicator of fecundability, when compared with women who had lower urinary triclosan concentrations (21). However, this negative association was not confirmed among women in the Longitudinal Investigation of Fertility and the Environment (LIFE) study (22). To date, the potential effect of triclosan on ovarian reserve has not been examined.

Antral follicle count (AFC) is a well-accepted marker of ovarian reserve used primarily in clinical settings to assess fecundability in women with suspected infertility and make decisions regarding their treatment options (23). We prospectively explored whether urinary triclosan concentrations are associated with AFC among women seeking care at a fertility center.

MATERIALS AND METHODS

Study Population

The study participants were women enrolled in the Environment and Reproductive Health (EARTH) Study, an ongoing prospective cohort established in 2004 to evaluate environmental and dietary determinants of fertility (24). Women aged between 18 and 45 years at enrollment who planned to use their own gametes were eligible to participate in the study. Approximately 60% of women contacted by the research nurses were enrolled. This prospective analysis included women who provided at least one spot urine sample for the measurement of triclosan concentrations before the measurement of their AFC at the Massachusetts General Hospital (MGH). Fertility Center between the years of 2007 and 2016 ($n = 118$).

Due to insurance coverage limitations, women undergoing infertility evaluation and treatment have their AFC measurement only once a year. Therefore, the urine sample collections preceded the AFC measurement up to a year for each woman. Of these, 8 women (7%) with a diagnosis of polycystic ovary syndrome (PCOS) as noted in their medical records were not included in this analysis because these observations were given a code, not a count. We also excluded one woman who was missing a baseline infertility diagnosis, resulting in a final study sample of 109 women for this analysis.

The study was approved by the human studies institutional review boards of MGH, the Harvard T.H. Chan School of Public Health, and the Centers for Disease Control and Prevention (CDC). The participants signed an informed consent after the study procedures were explained by trained research study staff and all questions were answered.

Assessment of the Exposure

Each woman provided a spot urine sample at study entry, and twice during each subsequent treatment cycle, corresponding to days 3–9 of the early/mid follicular phase of the cycle and in the preovulatory phase, and again at the time of oocyte retrieval or intrauterine insemination. All urine samples collected before the AFC scan date (ranging from 1 to 10 urine samples per woman) were included in the analysis. Urine was collected in a sterile, clean polypropylene specimen cup at the MGH Fertility Center.

Specific gravity (SG) was used to adjust the triclosan concentrations for urinary dilution. We measured the SG at room temperature and within several hours (typically within 1 hour) of the urine collection using a handheld refractometer (National Instrument Company) that was calibrated with deionized water before each measurement. The urine was then divided into aliquots, frozen, and stored at −80°C. The samples were shipped on dry ice overnight to the CDC where they were stored at or below −40°C until analysis.

The concentration of total (free plus conjugated) triclosan in 100 μL of urine was determined using an online solid-phase extraction coupled to high-performance liquid chromatography−isotope dilution−tandem mass spectrometry, an approach described elsewhere (25). The limit of detection