Laboratory Methods

A spot urine sample was collected in phthalate- and triclosan-free urine containers. Immediately following collection, urine samples were transported at room temperature to the laboratory for storage and processing Maximum transport time was about one hour. Analysis of free and total triclosan and triclocarban in urine were done by liquid chromatography- tandem mass spectrometry. Using Agilent LC 1260- AB Sciex 5500, both analytes were measured simultaneously using electrospray ionization in the negative polarity. Each analyte was monitored by multiple reaction monitoring using triclosan-d6 and C-triclocarban as internal standard for triclosan and triclocarban, respectively. The transitions used for all analytes are shown in Table 1.

Each urine sample was thawed and centrifuged at 3000 rpm for 10 minutes before it was prepared for LC-MS/MS analysis by solid phase extraction using Waters Oasis HLB cartridge (1cc). Each SPE cartridge was washed with 5 column volumes of methanol to get rid of its reported BPA contamination. The cartridges were then activated with water before 500 µL of urine was loaded. The column was washed with 5% methanol before each analyte was eluted by methanol. The methanol eluates were evaporated under a stream of nitrogen gas after which they were reconstituted in 10% methanol for column injection.

A 25 µL aliquot of the extract was used for each of the replicate injections of the sample. Chromatographic separation of the analytes was achieved by gradient elution using water with 0.05% ammonium acetate (pH=7.8) as mobile phase A and methanol with 0.05% ammonium acetate (pH=7.8) as mobile phase B. The elution gradient employed was- 0-0.5 min= 80%B; 3 min= 100%B; 3-7 min= 100%B; and 7.01-10 min= 80% B.

Free triclosan and triclocarban were directly measured from each urine sample while total triclosan and triclocarban were measured from a split sample of each urine that was processed through enzymatic deconjugation. Enzymatic deconjugation was done by treating 1mL split urine with H pomatia β-glucuronidase (500 U/assay) and H pomatia sulfatase (50U/assay) at pH 5.5. Each enzyme-treated sample was incubated at 37°C overnight to ensure complete enzymatic hydrolysis. The resulting hydrolysate was then prepared for LC-MS/MS analysis for solid phase extraction. Conjugated triclosan and triclocarban were calculated from the difference between the total and free triclosan and triclocarban levels, respectively.

The limit of detection (LOD) for both analytes is 0.05 µg/L. Quantitation of each analyte was done by isotope dilution method using a 10-point calibration curve. Each analyte has a limit of quantitation (LOQ) of 0.1 µg/L.

To ensure that the analysis was free of triclosan and triclocarban contaminations from any of the sample collection materials and the materials and equipment used in the analysis, field blank testing was performed by simulating the sample collection, extraction and analytical run using synthetic human urine (UTAK Laboratories, Inc). No triclosan and triclocarban were detected ≥ its LOD in any of the sample collection and extraction materials along with the instrument used in the analysis.