Partitioning, Persistence, and Accumulation in Digested Sludge of the Topical Antiseptic Triclocarban During Wastewater Treatment

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Supplemental Information

Sample Preparation. Aliquots (approximately 100 mL) of influent samples were diluted (250 mL final volume) and centrifuged at 10,000 x *g* for 20 min to remove solids. The supernatant was concentrated by solid phase extraction using Oasis HLB cartridges (3 cm^3, 60 mg; Waters Corp., Milford, MA) that had been pre-equilibrated with 3 mL of a 1 + 1 mixture of acetone and methanol, followed by 3 mL of methanol and 6 mL of reagent water. Concentrated analytes were eluted with organic solvents (3 mL; 1 + 1 mixture of methanol and acetone containing 10 mM acetic acid). Extracts were dried and reconstituted in the eluent used for high pressure liquid chromatography (HPLC). Finally, concentrates were filtered, diluted as needed with water containing 10 mM acetic acid, spiked to a final concentration of 50 µg/L with $^{13}C_6$-TCC as the internal standard, and analyzed by LC/ESI/MS as described previously (1). Sample particulates were extracted overnight with organic solvents (3 mL; 1 + 1 mixture of methanol and acetone), dried, and processed as described previously (1). Effluent samples (1000 mL) were centrifuged at 2,000 x *g* for 20 min. Supernatant and sediment fractions were processed separately as described above. Each batch of samples analyzed included blanks and quality control samples to exclude the possibility of background contamination, instrument drift, and analyte carry-over between samples.

LC/ESI/MS and LC/ESI/MS/MS Analysis. Concentrated organic extracts were introduced into a Shimadzu HPLC system consisting of a SIL-10ADvP autosampler, a DGU-14A eluent degaser, two LC-10ADvp gradient pumps, and an SCL-10Avp system controller (Shimadzu Corp., Columbia, MD). Using the autoinjector, 10-µL aliquotes were injected into the system, chromatographically separated on an Ultra IBD C18 column (5 µm particle size, 2.1 x 150 mm; Restek Corp., Bellefonte, PA) and analyzed and quantified using an LCMS 2010A single quadrupole mass spectrometer (Shimadzu Corp., Columbia, MD). Settings of the mass

spectrometer for quantification of TCC were described elsewhere (1,2). All TCC concentrations reported for sludge were determined by single quadrupole mass spectrometry and confirmed qualitatively and quantitatively by tandem mass spectrometry (3) on a Surveyor HPLC and autosampler system (Thermo Electron, San Jose, CA) coupled to a Quantum Ultra triple quadruple tandem mass spectrometer (Thermo Electron; San Jose, CA) using the characteristic transitions for TCC (*m/z* 313→160), TCC-d_7 (*m/z* 320→163) and $^{13}C_6$-TCC (*m/z* 319→160).

Considerations regarding the appropriateness of the sampling strategy. The challenge of accurately tracking contaminant masses in a full-scale treatment facility is reflected in both the paucity of studies conducted to date and the sizable standard deviations associated with mass estimates obtained in this work (Figure 3). More common is the practice of determining and reporting analyte concentrations detected in influent, effluent and sludge of the wastewater treatment facilities investigated (2,4-6).

Large plants like the one examined here typically process wastewater and solids by splitting the total flow and processing the resultant fractions in various parallel process streams. This strategy allows for conducting maintenance work during uninterrupted operation, and adds stability and safety to the overall treatment process. Yet, it also complicates an accurate assessment of the overall performance of the plant due to the existence of multiple parallel treatment flows. Here, this issue was dealt with by obtaining composite samples reflecting the average concentration of TCC during processing of wastewater and sludges in multiple vessels operated in parallel where applicable. Fortunately, for the most critical measurements, i.e., the samples representing TCC mass entering and exiting the plant, this approach was not needed.

Further consideration was given to the time of sampling and the number of samples required for obtaining a mass estimate of reasonable accuracy. For water-miscible compounds, sampling of input and output ideally should be offset by the hydraulic residence time of the wastewater moving through the plant (plug-flow scenario with interspersed mixing pools).

However, for more sorptive compounds, such as TCC, the fate of the chemical is linked to the residence time and path of the particles it is attached to (continuous stirred tank scenario). From this discussion, it must be concluded that—analytical challenges aside—accurate tracking of TCC mass is more challenging in the aqueous stream than in the solid stream. This is because the long residence time of solids in the plant (e.g., average sludge retention time of 19 days for the anaerobic digestion process) and their continuous recycling (e.g., average sludge retention time of 8 days in the activated sludge unit) ensure normalization or quenching of input extremes (i.e., short-term maxima in minima). Accordingly, much of the sampling effort had to concentrate on the aqueous stream, whereas a relatively smaller number of composite samples was needed to confidently establish concentrations in digested sludge. Data reported in this study are based on the acquisition of the following number of samples whose TCC content was determined: ~180 influent samples obtained using automatic samplers; 22 effluent samples obtained using automatic samplers; and 3 composite samples of digested sludge. Accurate estimation of the TCC mass entering and exiting the plant requires a consideration of both the concentration found in individual samples and the flow volume at the time of sampling. To obtain a reasonable estimate of the absolute mass in the samples taken and thus the mass moving through the plant, two strategies were used: (i) samples were analyzed individually and measured concentrations multiplied with flow volumes observed at the time of sampling (flow-proportionate samples; e.g., Figure 2A), or (ii) representative time-proportionate composite samples were mixed from time-discrete samples (e.g., Figure 2B). Despite the rainfall event identified in Figure 2A, there was only a modest fluctuation in the average daily flow rates (511 to 647 ML/d) due to the separation of domestic sewage and stormwater in the collection system. Accordingly, as shown in Figure 2B, daily fluctuations in TCC loading were moderate. For analysis of the mass of TCC in the solid waste stream, composite samples of digested sludge (yielding the data presented in Figure 2D) were analyzed repeatedly in triplicate and also subjected to spiking experiments as detailed below.

Quality of the analytical data for the mass balance. When sorption is an important mechanism governing the fate of the target compound, as is the case with TCC, then the quality of a given mass balance hinges on one's ability to successfully recover the analyte from the matrix and thereafter accurately quantify it in the complex organic extract. This study sought to address such concerns as best as technically feasible, by employing accelerated solvent extraction and the isotope dilution technique in conjunction with tandem mass spectrometric detection and quantification (3). By its very nature, this approach accounted for incomplete recovery of analyte (TCC) from difficult matrixes (i.e., sludge). All TCC concentrations reported were adjusted for recovery of the isotope-labeled surrogate standard spiked into the sample at known amounts prior to processing as is customary when using isotope dilution. The accuracy of this technique was determined in spiking studies using the composite sludge samples that served to estimate the residual mass of TCC in digested solids. Since the mass estimates for these samples were critical for the mass balance, these samples were analyzed repeatedly by LC/ESI/MS (n=15) and LC/ESI/MS/MS (n=6), with both methods yielding comparable results. Tandem mass spectrometry served as a confirmatory assay, and repeat analysis demonstrated proper homogenization of the sample materials. In addition, a total of 18 spiking experiments were carried out with these materials. Aliquots of digested sludge were spiked with (nonlabeled) TCC at a level approximately equivalent to 50% and 100% of the TCC mass detected prior to spiking, and allowed to equilibrate overnight. Average recoveries for both scenarios were 91 ± 8% (50% spiking level) and 93 ± 17% (100% spiking level). Comparable analyte recovery rates were found when aliquots of sludge samples were suspended in an acetone/methanol mixture (1 + 1) and extracted at ambient temperature for 18 hours on an automatic shaker. Additional yet unpublished long-term monitoring data from the plant demonstrate that the concentrations reported here for influent, effluent and digested sludge are both typical and reproducible.

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