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Fate of Sucralose through Environmental and Water Treatment Processes and Impact on Plant Indicator Species

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Supporting Information

ABSTRACT: The degradation and partitioning of sucralose during exposure to a variety of environmental and advanced treatment processes (ATP) and the effect of sucralose on indicator plant species were systematically assessed. Bench scale experiments were used to reproduce conditions from environmental processes (microbial degradation, hydrolysis, soil sorption) and ATPs (chlorination, ozonation, sorption to activated carbon, and UV radiation). Degradation only occurred to a limited extent during hydrolysis, ozonation, and microbial processes indicating that breakdown of sucralose will likely be slow and incomplete leading to accumulation in surface waters. Further, the persistence of sucralose was compared to suggested human tracer compounds, caffeine and acesulfame-K. In comparison sucralose exhibits similar or enhanced characteristics pertaining to persistence, prevalence, and facile detection and can therefore be considered an ideal tracer for anthropogenic activity. Ecological effects of sucralose were assessed by measuring sucrose uptake inhibition in plant cotelydons and aquatic plant growth impairment. Sucralose did not inhibit plant cotelydon sucrose uptake, nor did it effect frond number, wet weight, or growth rate in aquatic plant, *Lemna gibba*. Though sucralose does not appear toxic to plant growth, the peristent qualities of sucralose may lead to chronic low-dose exposure with largely unknown consequences for human and environmental health.

INTRODUCTION

Sucralose (1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside) is an artificial sweetener used in over 80 countries worldwide.[1] Due to its intense sweetness (>600× as sweet as table sugar), sucralose is commonly mixed with bulking ingredients and sold as SPLENDA by McNeil Nutritionals.[2] According to McNeil Nutritionals, the average expected lifetime intake of sucralose is 1.3 mg per kg of bodyweight per day (mg/kg/day), and the maximum expected daily intake is 2.4 mg/kg/day,[2] well within the United States Food and Drug Administration (FDA) acceptable average daily intake level of 5 mg/kg/day.[3] Ninety-five percent of consumed sucralose is excreted as the parent compound,[4] thus, if every American consumed their average expected intake, over 10 million kg of sucralose would be discharged annually, which averaged over total US water use[5] yields an average concentration of 17.6 μg/L in wastewater influent. Actual concentrations of sucralose found in wastewater effluent have already been measured as high as 10.8 μg/L[6] and are potentially accumulating in surface waters with unknown effects for human and environmental health.

Little is known about the postconsumption fate of sucralose. Many chlorinated molecules (i.e., polychlorinated biphenyls, aldrin, chlordane, heptachlor, dioxins) have been found to be persistent, bioaccumulative, and/or toxic (PBT).[7,8] Sucralose is known to be persistent through metabolic processes and in the environment[6] but is not known to bioaccumulate (K_{ow} = 0.3).[9] At sucralose doses of up to 3% of dietary intake (significantly greater than the FDA limit), adverse effects include cecal enlargement, pelvic mineralization, and epithelial hyperplasia in rats as well as gastrointestinal disturbance in rabbits.[10,11] A more recent study on rats showed adverse effects within the FDA limit including a decrease in beneficial fecal microflora bacteria and increase in fecal pH.[12] Additionally Splenda, administered at dosages of 1.1−11 mg/kg, was shown to enhance the expression of intestinal glycoprotein and cytochrome P450s CYP3A4 and CYP2D1, which are known to limit the bioavailability of orally administered pharmaceuticals by extrusion from the intestine and accelerated metabolism, leading to decreased absorption.[12]

Effects of sucralose on environmental health are also unclear. In plants, sucralose has been shown to interfere with sucrose uptake in sugar cane[13] disrupting the substrate-binding site of the sucrose uptake gene, ShSUT1. Sucrose transport was inhibited by 22% at an equivalent concentration of sucralose although sucralose is not translocated across the cell membrane.[13] The implications of low concentration exposure to plant nutrient uptake and growth are yet to be reported.

Microbial degradation and hydrolysis of sucralose has been documented. Microbial studies show partial sucralose degradation likely through cometabolic processes[14,15] reporting that about 55% was mineralized within a 4-week time frame. Wet soils, anaerobic sewage, and lake water showed slow and minute

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levels of sucralose degradation. Sucralose hydrolysis has been reported at elevated temperature and acidic pH.[16] During hydrolysis, the glycosidic linkage is broken producing 1,6-dichloro-1,6-dideoxy-D-fructose (DDF) and 4-chloro-4-deoxy-D-galactose (CG).[9] After one year at pH 3, only 1% of aqueous sucralose hydrolyzes. At near neutral pH, hydrolysis is not detectable.[9] The effects of any degradation byproduct and intermediates are unknown, though CG is known to be similar in structure to the known neurotoxin, 6-chloro-6-deoxyglucose.[17]

Previous studies on persistence assessed the amount of sucralose found in wastewater influent and effluent, surface waters, and coastal and marine waters. Studies in Europe have shown that sucralose does not undergo degradation through water treatment processes and can be found in surface waters downstream of cities.[6,18−20] Another study found sucralose in coastal and marine waters of the southeastern United States extending even into the Gulf Stream.[21] Only one study has quantified sucralose removal from a municipal wastewater treatment plant upgraded with postozonation followed by sand filtration showing removal of up to 31%.[22] While sucralose persistence is documented, the studies do not systematically assess the fate of sucralose through individual wastewater treatment processes or its environmental impact.

Due to its persistence sucralose has been proposed as a tracer of anthropogenic activity.[19,20] Caffeine has historically been used as a tracer even though it is nonconservative. More recently Beurge[19] proposed that acesulfame-K, another artificial sweetener, as a tracer due to its prevalence in Swiss surface and wastewaters.

While the prevalence of sucralose is increasing, its fate in advanced treatment and environmental processes as well as potential ecological impact are largely unexplored and unquantified. This study systematically evaluated the extent of degradation or mineralization of sucralose through ATPs including chlorination, UV, ozone, and adsorption processes as well as the effects of environmentally mediated processes (i.e., microbial degradation, soil sorption). To evaluate potential ecological impact, the effect of sucralose exposure on sucrose uptake in plant cotelydons was assessed, and aquatic plant toxicity tests were performed. Further, sucralose persistence was compared to caffeine and acesulfame-K for direct comparison of its appropriateness as an anthropogenic tracer.

EXPERIMENTAL SECTION

Chemicals. Caffeine, acesulfame-K, methoxyphenol, and TRIS buffer were purchased from Sigma-Aldrich. Calcium chloride, sodium hypochlorite (5.65−6%), ferric chloride, phosphate buffers, acetic acid, and carbonate buffers were from Fisher Scientific, and ammonium acetate was obtained from JT Baker. Tert-butanol and potassium oxalate were from Acros Organics. Phenanthroline was from Alfa Aesar. All chemicals were ≥99% purity. Radiolabeled sucrose (^{14}C) was purchased from American Radiolabeled Chemicals Inc. with a specific activity of 500−700 mCi/mmol. SPLENDA Sucralose was donated by Tate and Lyle Corporation. Activated Carbon (Calgon F-400) was donated by Calgon Carbon Corporation. Elliot Silt Soil Loam, Pahokee Peat Soil, Elliot Humic Acid, and Peat Humic Acid were obtained from the International Humic Substance Society (IHSS).

Liquid Chromatography − Mass Spectrometry (LC-MS). Analysis of sucralose, acesulfame-K, and caffeine was done using LC-MS (Varian 500-MS, 212-LC pumps, with electrospray ionization) with a Waters reverse phase, dC18 column (2.1 × 150 mm, 3 μm particle size) with in-line guard column, and Prostar autosampler (20 μL sample loop). Elution gradients were run using deionized water or 20 mM ammonium acetate and acetonitrile at a flow rate of 200 μL/min. Refer to Table S1, Supporting Information for elution programs and mass spectrometer conditions.

A standard curve was run before and after each set of analyses with check standards added every 8 samples. Standards and experimental samples were made by dilution of a concentrated stock for each analyte. All samples were unmodified and directly injected for analysis unless otherwise mentioned as in the case of sorption and microbial degradation experiments.

Experimental Setup. Chlorination. One μM compound solutions were spiked with sodium hypochlorite to reach 25 μM chlorine (measured by absorbance at 292 nm, $\varepsilon = 362\ M^{-1}\ cm^{-1}$). In-line direct injection of sample into LC-MS without a quenching agent was used for kinetic analysis.

Ozonation. Ozone was supplied by a Triogen ozone generator and was directed through 50 mM phosphate buffer to remove impurities before being bubbled into chilled deionized water. Ozone concentrations were maintained between 0.5−0.6 mM. Aqueous ozone was placed directly into a quartz cell in a 2:1 ratio with phosphoric acid, and the concentration was measured immediately (absorbance at 258 nm, $\varepsilon = 3000\ M^{-1}\ cm^{-1}$). One μM solutions of analyte were spiked with ozone, to reach 100 μM ozone, and vortexed. At given time intervals, samples were spiked with $NaNO_2$ (1 mM) to quench, vortexed, and run directly using LC-MS.

Ultraviolet (UV) Exposure. One μM samples in glass vials were randomly spaced in a Rayonet photochemical reactor (RMR-600) fitted with ten 350 nm bulbs (Rayonet, RPR-3500) and six 254 nm bulbs (Sankyo-Denki, G8T5). Covered positive controls were included to assess impact of reactor conditions. Controls in quartz tubes were also used to ensure that the glass vials were not absorbing relevant UV light. A Fisher Scientific UV light meter (RS232) was used to measure the illuminance within the photoreactor, and ferrioxalate actinometry (procedure as found in Hatchard and Parker[23]) was used to measure the light intensity.

Sorption Isotherms. Adsorption experiments were performed with granular activated carbon (GAC), Elliott Silt Loam Soil (ESL), Pahokee Peat Soil (PPS), and sand. GAC was pulverized and sieved to obtain particles between 500−850 μm. All samples contained 2−5 mg of GAC or 5 g ± 0.01 g of soil/sand, were amended with 50 mL solution, and placed on an orbital shaker for at least 2 weeks. Equilibration time was established to be at least one week (Figure S1, Supporting Information) indicating that two to four weeks is sufficient for analysis. Before analysis, samples were settled, and the supernatant was filtered through a 0.2 μm PVDF syringe tip filter.

Hydrolysis. pH values 2−10 were tested using phosphate, acetic acid, carbonate, phosphate, TRIS, and carbonate buffers respectively. Buffer solutions were prepared at 0.01 M with 1 μM of sucralose. The experiments were conducted at 5 °C, 25 °C, 37 and 65 °C for 5 days.

Microbial Degradation. Soil samples were obtained locally from a clearing bordering the Mill River, New Haven, CT. The soil was collected from 1-5 cm of soil depth, air-dried, sieved, and amended with 1 mg/kg sucralose and, when noted, 10 mg/kg sucrose to reach 0.2 g/g water content (80% of 1/3-bar water

content). The soils were left open to the atmosphere and were amended with water weekly to obtain initial soil moisture. Twice autoclaved soil was used as a negative control for soil sorption. Samples were taken at 7-day intervals by removing a small soil sample, adding deionized water (0.4 mL/mg soil), mixing (1 h), and centrifuging (30 min, 13.2 rpm). The supernatant was removed and filtered through a 0.2 μm PVDF syringe tip filter (extraction efficiency = 86%).

Plant Uptake. Seeds of beets (*Beta vulgaris*), corn (*Zea mays*), soy (*Glycine max*), and castor bean (*Ricinis communis*) were obtained through local vendors, soaked in deionized water for 24 h, and allowed to germinate for 36−48 h on moistened filter paper. The cotyledons were then immersed in solutions of ^{14}C-sucrose (20 μM) and sucralose (2 or 20 μM) for up to 24 h. Radioactivity of the surrounding solution was measured using a liquid scintillation analyzer, LSA (Packard, Tri-Carb 2900TR). 100 μL samples (4 replicates) were taken at fixed time intervals and placed in CytoScint. Radioactivity loss was correlated with radioactive uptake in corn and soy seedling by immersion of rinsed cotyledons in fluor and shaking until a homogeneous liquid phase was obtained.

Aquatic Plant Toxicity. *Lemna gibba* G-3 culture was obtained from the Canadian Phycological Culture Center and maintained in Hunter's media as described by Brain et al.[24] Prior to experimentation, plants were acclimatized to their respective test media (Hunter's media with and without 29 mM sucrose) for one week. Seven day static renewal experiments were conducted according to Brain et al.[24] After acclimatization, two *Lemna* plants, each with four fronds, were transferred into flasks containing sterilized test solution. Flasks were arranged in a randomized complete block design and maintained in a growth chamber (25 °C) under constant cool white fluorescent light (6800 lx). Frond number and fresh weight were measured on day seven. Growth rate was calculated by dividing the number of doubling events by the time: growth rate = $((\log(F_T/F_0))/\log 2)/t$; where F_T is the number of fronds at time, t; and F_0 is the starting number of fronds.[24]

Statistical Analysis: Aquatic Plant Toxicity test. A one-way analysis of variance (ANOVA) was conducted using Sigmaplot 11.0 to identify differences between treatment levels and controls (α = 0.05). ANOVA assumptions of normality and equal variance were confirmed using the Shapiro-Wilk and Levene Median tests, respectively. When data failed to conform to parametric assumptions, a nonparametric Kruskal−Wallis one-way analysis of variance on ranks was performed. Plant growth response variables in controls grown with or without 29 mM sucrose were compared using a simple *t* test.

■ ENVIRONMENTAL PROCESSES ANALYSIS

Microbial Degradation. Experiments on flooded soils showed little or no sucralose degradation concurring with Labare et al.[14] which reported less than 4% mineralization. On dry soils, sucralose was degraded by 45% at 4 weeks (Figure 1) consistent with Labare et al.[14] The observed difference can be attributed to variations in soils and microbes. Sorption isotherms of the autoclaved soil samples indicate that less than 5% of sucralose loss can be attributed to soil sorption (Figure S2, Supporting Information). Additionally, the soils were amended with sucrose to evaluate the effect of a sucralose analog on cometabolism. Soils amended with sucralose displayed a higher initial rate of degradation than soils amended with sucralose and sucrose. However, after four weeks, the percent of sucralose degraded was identical for the two systems.

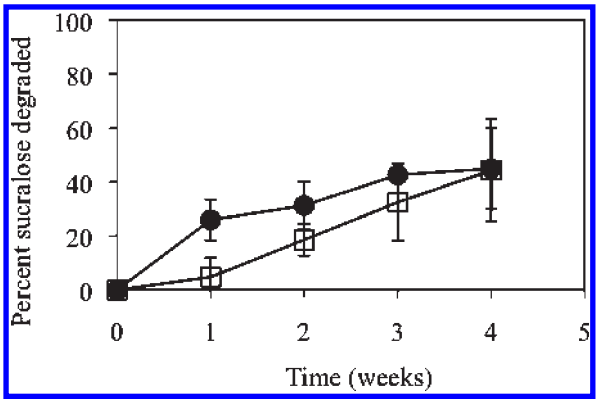

Figure 1. Microbial degradation of sucralose in soil amended with ● 1 mg/kg sucralose; □ 1 mg/kg sucralose, and 10 mg/kg sucrose. Averaged degradation rate was found to be 1100 μg/kg-d. Error bars represent 95% confidence interval (n = 3).

Previous studies found an intermediate from soil microbe degradation while no intermediate or byproduct was identified in aerobic sewage.[14,15,25] The proposed intermediate, either the aldehyde or uronic acid of sucralose, was not found in this study possibly due to the lower concentrations of sucralose and consequent byproducts used in this study compared to Labare et al.[14] where no detection limit for the byproduct was mentioned. Labare reported intermediate production is dependent on soil conditions which could also potentially explain no observation of the proposed transformation intermediates.

Hydrolysis. Sucralose hydrolysis was performed for pH 2−10 and temperatures 5−65 °C over five days to assess imminent byproduct formation (Figure 2). Significant degradation occurred at pH 10 and elevated temperature (37 and 65 °C). Within an environmentally relevant pH (4−8) and 5 days, sucralose did not hydrolyze regardless of temperature. Hydrolysis products were not found likely due to the short time frame. Though previous tests by Tate and Lyle[6,16] reported that sucralose should hydrolyze within the given conditions (pH 2, 62 °C, 120 h, 30.4% degradation) these tests have not been repeated and cannot be validated here.

Analysis on LC-MS indicated that degradation at pH 10 with elevated temperatures resulted in dechlorination rather than hydrolysis. Two products were found with mass weights of 359/361 m/z, with a pattern indicating a dichlorinated structure, and 323 m/z with a monochlorinated mass spectral pattern (Figure 2B). These products are more polar, based on lower HPLC retention times, and correspond well with to the dichlorinated and monochlorinated structure of sucralose after dechlorination through dehydrohalogenation (β-elimination) promoted through the basic conditions.

Soil Sorption. Soil sorption isotherms (Figure 3) show that sucralose affinity for both loam and peat soils is low. Sucralose sorption to peat soil, high in organic matter, was greater than sandier loam soil, as expected.[26] These results are expected as sucralose is highly water-soluble and hydrophilic indicating that sucralose is not likely to partition into sewage sludge and soils. This leads to transport through water treatment plants to surface and ground waters.

Comparing the adsorption of sucralose to acesulfame-K and caffeine provides a reference for soil affinity. For both systems, sucralose sorption was significantly less than that of acesulfame-K

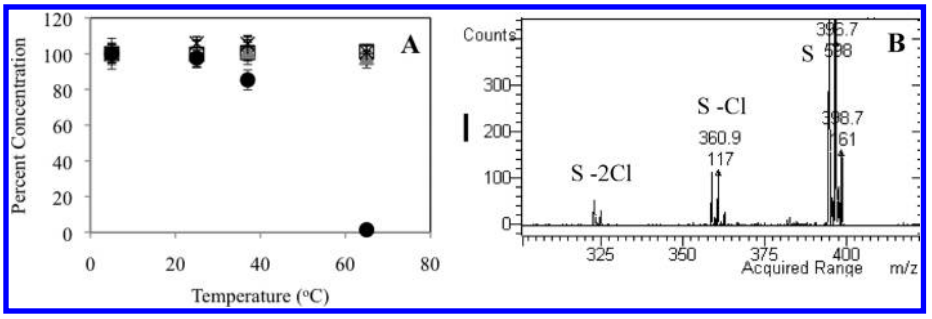

Figure 2. A) Concentration of sucralose after 5 days at pH (◇ 2; □ 4; ▲ (gray) 6; + 7; x 8; ● 10) and temperatures (5, 25, 37, 65 °C). B) Averaged mass spectrum of dechlorinated products of resulting from dehydrohalogenation of sucralose (S = sucralose, S−Cl = dichlorinated sucralose, S−2Cl = monochlorinated sucralose). Error bars represent 95% confidence interval (n = 3).

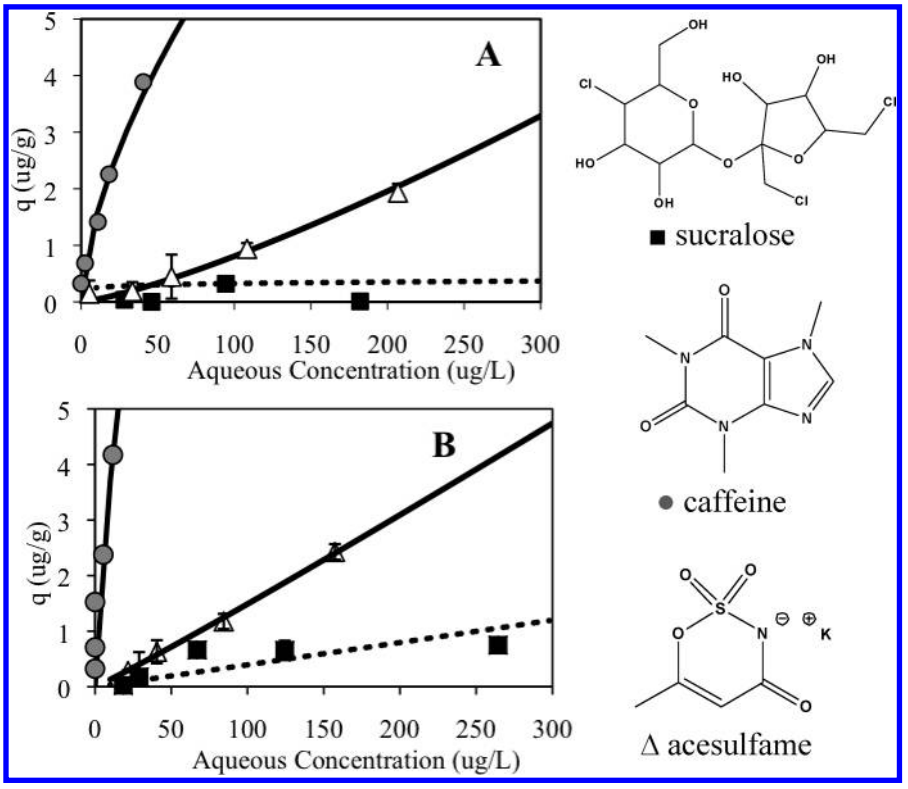

Figure 3. Sorption isotherms for sucralose, caffeine, and acesulfame on Elliott Silt Loam Soil (A) and Pahokee Peat Soil (B) where q is the mass of analyte sorbed at equilibrium per dry mass of sorbent. Isotherms fit a Freundlich model with coefficients for (A) 1/n: 0.113, 1.28, 0.625 ; K_f: 0.421, 15.32, 27.21 $(mg/g)(L/mg)^{1/n}$ and for (B) 1/n: 1.01, 1.05, 0.734 ; K_f: 4.05, 16.85, 109.32 $(mg/g)(L/mg)^{1/n}$, respectively.

and caffeine indicating that sucralose is potentially a better aquatic tracer of anthropogenic activity.

■ WATER TREATMENT PROCESSES

Granular Activated Carbon (GAC). GAC may be used to adsorb water contaminants in either water or point-of-use treatments. Sucralose affinity to GAC was less than caffeine and greater than, though comparable to, acesulfame (Figure 4). Using the Freundlich isotherm model, the difference between acesulfame and sucralose is only noticeable at high (above environmentally relevant) equilbrium concentrations. Comparing sorption to other compounds with similar 1/n values (K_f = 78.6 $(mg/g)(L/mg)^{1/n}$), sucralose sorption to GAC is less likely than chlordane, naphthalene, and toluene with K_f's 190, 132, and 97 $(mg/g)(L/mg)^{1/n}$) respectively.[27]

Chlorination. There was no evidence of hypochlorite reacting with 1 μM sucralose at a concentration of 25 μM over 5 h. This chlorine concentration is common for water treatment though the concentration affecting sucralose is expected to be much lower due to the presence of other compounds presenting additional demand in real world applications. Given its lack of any electron rich sites for oxidation and its already trichlorinated structure, the resistance of sucralose to chlorination is expected. Caffeine also was not oxidized within the 5-h time frame, but acesulfame does degrade up to 20% ($k = 1.47 \times 10^{-5}$ s^{-1}). Sucralose degradation through chlorination was still not observed after 24 h.

Ozonation. Significant degradation of 1 μM acesulfame-K, caffeine, and sucralose was found after one hour at an ozone dose 100 μM (Figure 5a). No acesulfame-K or caffeine was detected at 5 min while 6% sucralose remained after one hour. As with chlorine, the effective ozone concentration in wastewater treatment is expected to be less due to competing compounds. Oxidation by ozone can occur through two mechanisms: 1) direct reaction with ozone or 2) radical mediated oxidation.[28] Given that sucralose does not have any evident sites for direct oxidation by ozone, it is hypothesized that sucralose degradation occurred through the latter radical pathway. Upon addition of 0.5 mM t-butanol as a radical quench, sucralose degradation was completely hindered while acesulfame-K and caffeine were still completely degraded, though at slower rates, 5 and 20 min respectively (Figure 5b). It can be concluded that the harsher and less selective degradation pathway of radical mediated oxidation is necessary for the breakdown of sucralose. Samples of ozone treated sucralose analyzed using mass spectrometry indicated that sucralose was likely mineralized.

Though sucralose was significantly degraded with hydroxyl radicals produced from ozone, the amount of degradation that will actually occur during ozonation as part of the water treatment process will likely be minimal as other organic materials will effectively act as radical scavengers. This would agree with the findings of Hollender et al.[22] showing only 31% sucralose removal in a water treatment plant upgraded with ozonation.

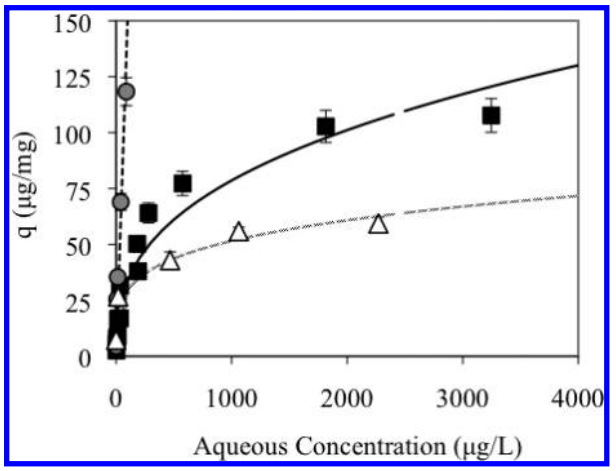

Figure 4. Sorption isotherm of ■ sucralose; ● (gray) caffeine; Δ acesulfame-K on GAC. The Freundlich coefficients were found to be K_f = 78.6; 51.5; 1040 (mg/g)(L/mg)$^{1/n}$ and 1/n = 0.364; 0.238; 0.835, respectively. Error bars represent 95% confidence interval (n = 3).

Ultraviolet Light. Sucralose and caffeine did not degrade after 5 h of UV exposure in a photochemical reactor, while acesulfame-K was found to degrade by about 35% ($k = 2.38 \times 10^{-5}$ s^{-1}, Figure S3, Supporting Information). The light intensity was measured using chemical actinometry of potassium ferrioxalate (9.2×10^{16} photons/s), and illuminance was measured by light meter (263 lx). The addition of natural organic matter (NOM) did not result in additional degradation for any of the compounds, though photosensitization was seen using methoxyphenol (Figure S4, Supporting Information), and as expected as found by Canonica et al.[29] These results indicate that only acesulfame has the potential to degrade in pure and natural waters through exposure to UV light.

■ ANALYSIS OF POTENTIAL ECOLOGICAL EFFECTS

Plant Uptake Studies. Sucralose did not inhibit sucrose uptake in a variety of monocot and dicot plant cotelydons at the concentrations and time periods evaluated. Plant cotyledons naturally produce sucrose with sucrose uptake within the cotyledon occurring at a high rate.[30] The effect of sucralose on sucrose uptake in plant cotyledons was studied to evaluate inhibition of sucrose uptake and transport. Since high sucrose uptake rates have been exhibited in cotyledons of castor bean,[30] soybean,[31] and corn[32] as well as in the taproots of beets[33] and carrots,[32] uptake inhibition studies were performed on these cotyledons at 1:1 and 10:1 sucrose to sucralose on a mass basis. Neither sucralose concentration impacted the rate of sucrose uptake over a 24-h period (Figure S5, Supporting Information). Previous sucrose and glucose uptake studies looked at competitive inhibition over a time frame of 3−24 hours[32,33] finding that uptake inhibition occurs within 3 h. Though these findings suggest that sucralose does not interfere with the uptake of sucrose by these species of plant cotyledons, there could still be impacts on sucrose transport in more mature plants and root systems or for plant growth over a longer time as evidenced by reported disruption at the substrate-binding site of the sucrose uptake gene, ShSUT1.[13]

Aquatic Plant Toxicity. Aquatic plant toxicity tests were performed on the aquatic macrophyte *Lemna gibba,* a widely employed aquatic plant model for toxicity studies of a variety of industrial chemicals.[34] Other anthropogenic compounds evaluated

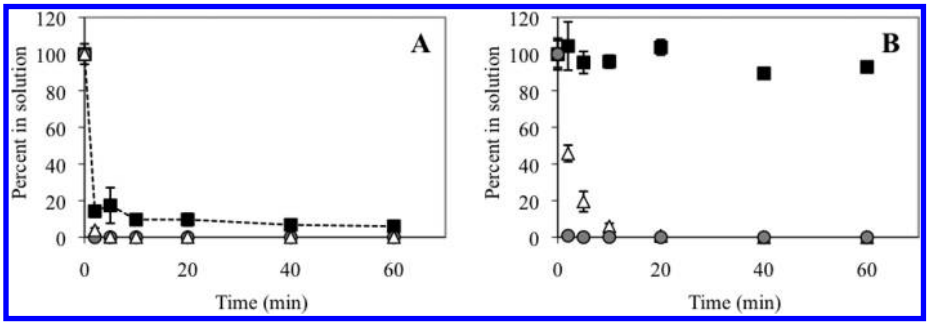

Figure 5. Results of ozonation for ■ sucralose; ● (gray) caffeine; Δ acesulfame-K; A) Decreasing amount of contaminant after one hour of exposure to 100 μM ozone to 1 μM of contaminant (initial first order reaction rates: 0.00427 s^{-1}, 0.0209 s^{-1} for sucralose and acesulfame-K, respectively, caffeine was immeasurable). B) Results when t-butanol, a hydroxyl radical quench is added to the ozone and contaminant (first order reaction rates: 0.0129 s^{-1}, 0.0398 s^{-1} for acesulfame-K and caffeine respectively). Sucralose did not degrade in the presence of t-butanol indicating that sucralose degradation by ozone is through radical mediated reactions. Error bars represent 95% confidence interval (n = 3).

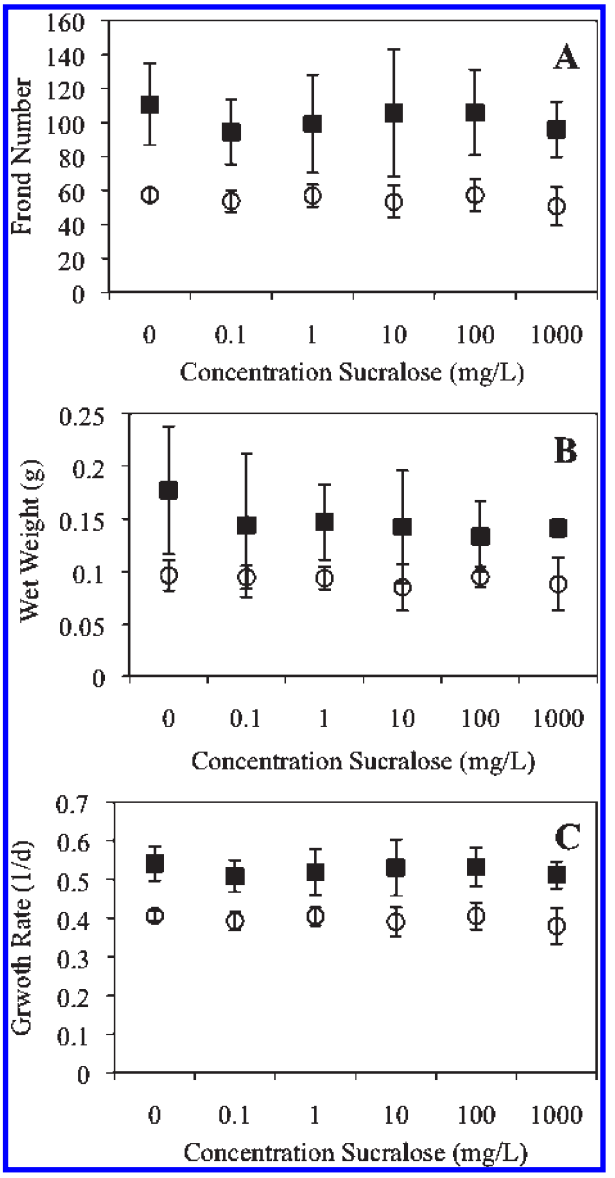

Figure 6. *Lemna gibba* growth rate frond number (A), wet weight (B), and growth rate (C) following a 7 d exposure to ○ sucralose only; ■ sucralose and 29 mM sucrose. Error bars represent 95% confidence interval ($n = 3$, for the sucralose and sucrose 0.01 mg/L treatment level, $n = 2$) for each experimental treatment level from a single experimental setup.

in the same manner have significantly increased or decreased the growth of *L. gibba* over a range of concentrations.[35,36] *L. gibba* growth was not adversely affected by sucralose over a 7-day time frame (Figure 6) as there was no statistically significant difference ($P > 0.05$) in frond number, wet weight, or growth rate between control and sucralose exposed *L. gibba* under the conditions tested. Control growth in both of these experiments were consistent with previously reported *L. gibba* growth rates.[35] Also in agreement with previous studies,[37] a significant increase in frond number, wet weight, and growth rate ($P < 0.001$) was observed when *L. gibba* was cultured in the presence of 29 mM sucrose. The coadministration of sucralose and 29 mM sucrose, however, did not significantly inhibit the growth and reproduction of *L. gibba*.

In previous studies with *L. gibba*, EC_{50}s for a range of antibiotics spanned from 51 to 5620 μg/L.[34] In contrast, this study found that sucralose did not exhibit any adverse effects on growth rate, wet weight, or frond number at concentrations of at least 1000 mg/L. The absence of toxic effects from sucralose on this aquatic macrophyte to such high levels supports the lack of phytotoxicity observed from our previous experiments on plant cotelydons.

IMPLICATIONS

The persistent behavior of sucralose suggests that it may be a viable aquatic environmental tracer of anthropogenic activity, especially when compared to other common candidates, caffeine and acesulfame. The suitabilty of these compounds as anthropogenic tracers stems from their unambiguous sole production and consumption from human activity. Compared to the alternatives, sucralose's relative abundance in wastewater effluent and surface waters, unique mass spectrum, and highly conservative nature means that its identification in water will be facile and unambivalent, providing an ideal chemical marker for wastewater contamination in surface and groundwaters. Sucralose can thus be used to indicate the presence of other hydrophilic anthropogenic contaminants such as certain pharmaceuticals or personal care products that are at lower concentrations or are more difficult to identify. In addition to its use as a conventional tracer, sucralose, in combination with other proposed tracers can be used to form a timeline of contamination. For example, acesulfame-K was implemented as a food additive in the EU in 1983, US in 1988, and Canada in 1994, whereas sucralose was implented in 2004, 1999, and 1991, respectively. The presence or lack of these potential tacers could indicate a time frame and provide more information as to the source and degree of contamination.

The resistance of sucralose to degradation through natural and water treatment processes may have implications regarding water taste, human health, and potential water reuse applications. New information is also included about the impact, or lack thereof, of sucralose on plant indicator species for aquatic toxicity. This highlights the discussion of a rare collection of molecules that are highly persistent but do not bioaccumulate and have little to no reported toxicity at environmentally relevant concentrations. Is persistence reason enough for concern or regulation?

While there are no known health effects of sucralose at low concentrations, the risks of chronic low dose exposure due to ambient concentrations of sucralose is unknown. Long-term exposures to low levels of persistent anthropogenic chemicals may result in chronic risks, but sucralose and its degradation products have not been sufficiently or systematically evaluated in this context.

ASSOCIATED CONTENT

Supporting Information. Selected supplementary method and figures regarding microbial degradation, UV irradiation, plant uptake, sorption kinetics, and models. This material is available free of charge via the Internet at http://pubs.acs.org.

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