Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information

Quantification of Sucralose in Groundwater Well Drinking Water by Silylation Derivatization and Gas Chromatography-Mass Spectrometry

Stefan Voss[#,%], Elisabeth Newman[%], Justin P. Miller-Schulze[%,*]

#: United States Geological Survey, California Water Science Center; Sacramento, CA

%: Department of Chemistry, California State University, Sacramento; Sacramento, CA

*: Corresponding Author

Quantification of Sucralose in Groundwater Well Drinking Water by Silylation Derivatization and Gas Chromatography-Mass Spectrometry
Voss, et al
Supplemental Information

In this Supplemental Information (SI), further details on the sample collection (Section S2.2), instrumental data analysis (Section S2.3), and EI fragmentation (Section S3.1) can be found. In addition, sample data for all samples (Table S1), matrix spike samples (Table S2), the speculated identities of the fragments discussed in S3.1 (S3A and S3VB), the NICI-GC-MS spectra of *d*6-TMS-sucralose (Figure S1) tabulated abundances of observed and speculated fragments (Table S4) are presented.

Experimental

S2.2 Sample Processing and Derivatization Details

Samples collected in this study used a stratified, random, grid-based design for well selection [1, 2], thus the wells are considered representative of the groundwater resources in the basins currently used for domestic drinking water. Well locations and results for analyses of major ions, trace elements, nutrients, pesticide constituents, volatile organic compounds, microbial indicator constituents, and groundwater age-dating tracers are available in Bennett et al [3].

Of the four batches of environmental and QA/QC samples analyzed, the third batch showed elevated levels of sucralose in all laboratory blanks and environmental samples. A t-based statistical analysis ($p = 0.013$) found that the sucralose concentrations in the method blanks in batch 3 differed significantly from the other three (batches 1, 2 and 4). Figures S1A and S1B display the concentration data in box plots for blanks and groundwater samples. As a result of this, the blanks, matrix spikes, and groundwater samples were excluded from the final analysis because they are not representative of the performance of the method itself.

Contamination of the third batch by repeatedly-used items such as micropipettors is the most likely source of contamination in the method. Further data obtained by this method (data not shown) indicates contamination at levels similar to that of batches 1,2, and 4 and so we speculate that a grossly contaminated pipette tip or other component was removed from the system between the processing of batches 3 and 4.

S2.3 Instrumental Analysis Details

For quantitative analysis, the MS was operated in selected ion mode (SIM). The ions monitored for derivatized sucralose (TMS-sucralose) were *m/z* 117, 307 – 314, 343, 345, 347, 349, 361, and 363. The dwell time per ion for the TMS-sucralose SIM window was 10 ms (sufficient to provide ca. 15 points per chromatographic peak). For qualitative EI-GC-MS analysis, the (positive mode) MS was operated in scan mode from 30-800 *m/z*, scanning at 1494 amu/sec and with a peak threshold of 100 at a sampling rate of 2^2 (4 samples per ion). Separate, qualitative analyses performed to elucidate the structure of TMS-sucralose using CI-GC-MS, negative mode MS was initially operated in scan mode from 25-900 m/z. Once it was established that the molecular ion and other fragments of interest were in the high mass range, CI-GC-MS spectra were collected in negative mode by scanning from 550-850 m/z, at 732 amu/sec with a peak threshold of 100 at a sampling rate of 2^3 (8 samples) per ion.

Results and Discussion

S3.1 Detailed Discussion of Fragmentation of TMS-sucralose for Electron Ionization GC-MS

The six-membered ring without the bridging oxygen (*m/z* 397) losing 89 amu (an O-TMS group) resulting in $C_{12}H_{25}Cl_1O_3Si_2$ (Fragment D in Table S3A) yields 308 *m/z*; this same overall loss could be produced by the six-membered ring with the bridging oxygen losing a methyl group and a TMSOH group (producing

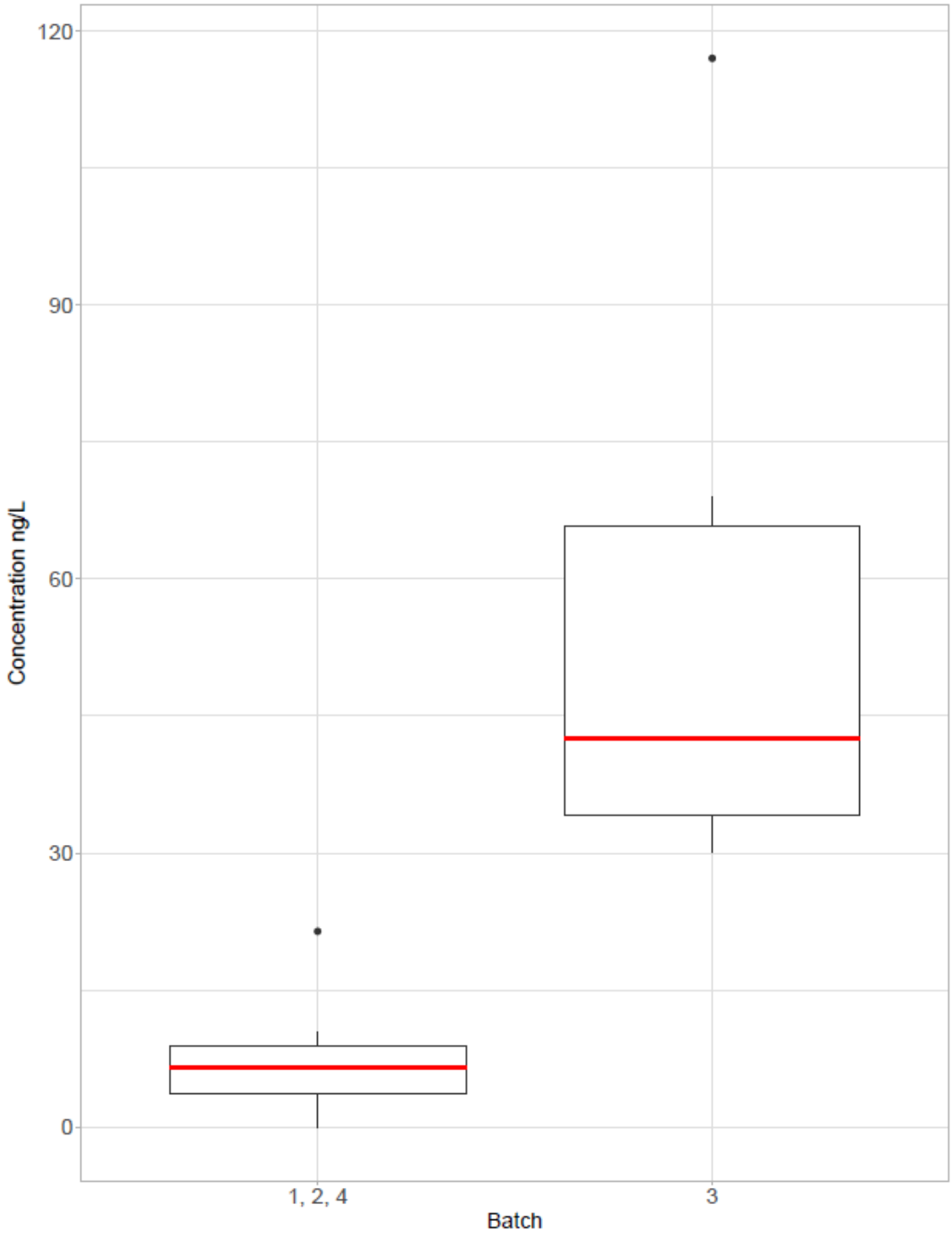
$C_{11}H_{21}Cl_1O_4Si_2$, Fragment E in Table S3A). In addition, the five-membered ring with the bridging oxygen (*m/z* 359) could lose a methyl group followed by a loss of 36 amu (HCl), resulting in $C_{11}H_{21}Cl_1Si_2O_4$ (Fragment B in Table S3A), or lose the oxygen followed by Cl, resulting in $C_{12}H_{25}Cl_1O_3Si_2$ (Fragment C in Table S3A). Loss of HCl is likely preferable as compared to loss of just chlorine in EI-GC-MS [4], but it is not possible to determine which formula is correct based on the data in Figures 1A and 1B nor the predicted isotope distributions in Table S3A. In addition, the five-membered ring losing an oxygen and a chlorine is isomeric with the six-membered ring losing an oxygen and a TMSO group. A *m/z* 307 fragment is also observed in Figures 1A and 1B and could be formed by the six-membered ring without the bridging oxygen losing a TMSOH group (Fragment A in Table S3A).

The four proposed pathways for formation of *m/z* 308, and the proposed pathway for the formation of *m/z* 307 all contain a single chlorine, as such the isotopic distributions are dominated by the "X+2" peaks for *m/z* 308 and *m/z* 307 (occurring at *m/z* 310 and *m/z* 309, respectively). The observed isotopic distributions for the cluster of peaks around *m/z* 308 and *m/z* 312 for the TMS-sucralose and TMS-*d6*-sucralose, respectively, are presented in Tables S3A and S3B along with the predicted isotopic distributions for each putative fragment. The observed abundances represent the averages of three replicate injections for the deuterated and non-deuterated compounds.

Comparison of the predicted distributions for the *m/z* 307 cluster/six-membered ring (309 in the deuterated version) and the *m/z* 308 cluster/five-membered ring (312 in the deuterated version) with the observed abundances in Tables S3A and S3B support a relatively even contribution from the *m/z* 307 and *m/z* 308 clusters. The "Sum of Fragments A and B" column in Tables S3A and S3B shows the resulting predicted isotopic abundances from the sum of the five membered ring losing the $–CH_3$ group and HCl (Fragment B) and the six-membered ring which would produce a *m/z* 307 peak (Fragment A). In both the non-deuterated (Table S3A) and deuterated (Table S3B) cases, the predicted equally weighted distributions agree well with the observed distribution. It should be noted that it is not possible from the distributions which fragment (B or C) is the five-membered component, however, that the *m/*z 308 cluster is coming from the five-membered ring is clear from the cluster around *m/z* 312 in the spectrum of the d6-TMS-sucralose. Importantly, this indicates that employing *m/z* 308 as the quantification ion and using *m/z* 312 as the corresponding internal standard ion ensures that the analogous fragment is supplying these ions for both the deuterated and non-deuterated TMS-sucralose. In addition, the predicted distributions for both the deuterated and non-deuterated TMS-sucralose fragments indicate a lack of significant contribution of internal standard to analyte and vice-versa.

Quantification of Sucralose in Groundwater Well Drinking Water by Silylation Derivatization and Gas Chromatography-Mass Spectrometry
Voss, et al
Supplemental Information

Figure S1A: Box plot detailing sucralose concentrations in blank samples in contaminated 3rd batch as compared with batches 1, 2, and 4. The red line represents median concentration, top and bottom of box represent 75th and 25th percentiles respectively, and the points above are outliers.



Quantification of Sucralose in Groundwater Well Drinking Water by Silylation Derivatization and Gas Chromatography-Mass Spectrometry
Voss, et al
Supplemental Information

Figure S1B: Box plot showing elevated sucralose concentrations in groundwater samples processed in 3rd batch of samples as compared with batches 1, 2, and 4. The red line represents median concentration, top and bottom of box represent 75th and 25th percentiles respectively, and the points above are outliers.

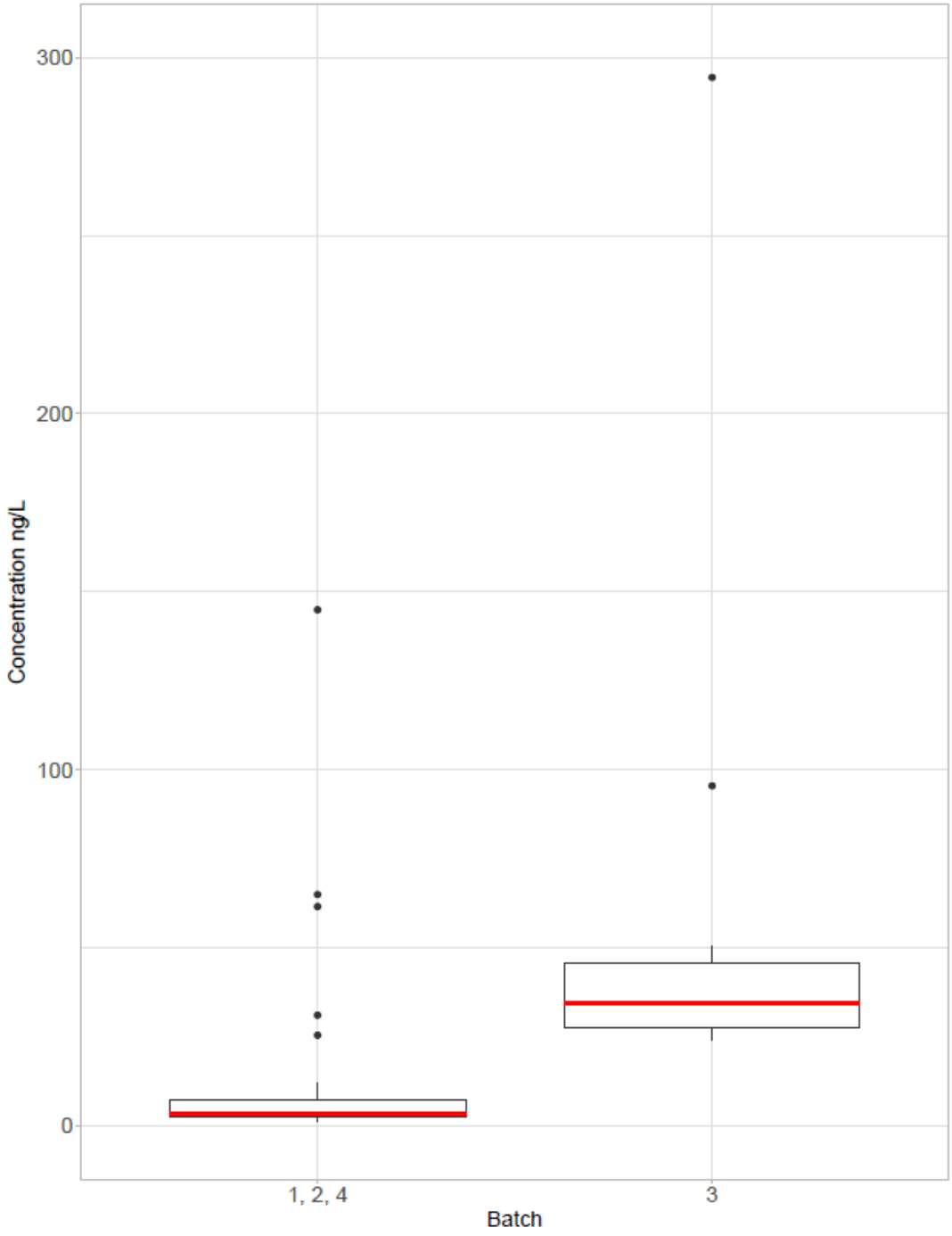


Table S1A (top): Tabulated theoretical (for different structures) and observed abundances for the m/z cluster centered around m/z = 308 in the mass spectrum of TMS-sucralose. Percent abundances are relative to the peak in largest abundance in that cluster.
Table S1B (bottom): Tabulated theoretical (for different structures) and observed abundances for the m/z cluster centered around m/z = 309 and 312 in the mass spectrum of TMS-d6-sucralose. Percent abundances are relative to the peak in largest abundance in that cluster.

Predicted							Observed		
$C_{12}H_{24}Cl_1O_3Si_2$ 6-Membered Ring -(TMSOH)		$C_{11}H_{21}Cl_1O_4Si_2$ 5-Membered Ring + O -(CH_3, HCl)	$C_{12}H_{25}Cl_1O_3Si_2$ 5-Membered Ring + O -(O, Cl)	$C_{12}H_{25}Cl_1O_3Si_2$ 6-Membered Ring -(TMSO)	$C_{11}H_{21}Cl_1O_4Si_2$ 6-Membered Ring + O -(CH_3, TMSOH)	SUM of FRAGMENTS A and B			
FRAGMENT A		FRAGMENT B	FRAGMENT C	FRAGMENT D	FRAGMENT E				
m/z	Percent Abundance	Percent Abundance	Percent Abundance	Percent Abundance	Percent Abundance	Percent Abundance	*m/z*	*Percent Abundance*	*RSD(%) Percent Abundance*
307.1	100	NA	NA	NA	NA	81.0	*307.0*	*71.7*	*0.9*
308.1	23.5	100	100	100	100	100	*308.0*	*100*	*0.0*
309.1	41.8	22.5	23.5	23.5	22.5	52.1	*309.0*	*56.8*	*1.0*
310.1	9.1	41.7	41.8	41.8	41.7	41.1	*310.0*	*42.9*	*0.5*
311.1	3.4	8.7	9.1	9.1	8.7	9.8	*311.0*	*13.3*	*1.0*
312.1	0.5	3.4	3.4	3.4	3.4	3.2	*312.0*	*3.5*	*12.7*
313.1	0.1	0.5	0.5	0.5	0.5	0.5	*313.0*	*0.8*	*24.6*
314.1	0.0	0.1	0.1	0.1	0.1	0.1	*314.0*	*NA*	*NA*

Predicted							Observed		
$C_{12}H_{22}D_2Cl_1O_3Si_2$ 6-Membered Ring -(TMSOH)		$C_{11}H_{17}D_4Cl_1O_4Si_2$ 5-Membered Ring + O -(CH_3, HCl)	$C_{12}H_{21}D_4Cl_1O_3Si_2$ 5-Membered Ring + O -(O, Cl)	$C_{12}H_{23}D_2Cl_1O_3Si_2$ 6-Membered Ring -(TMSO)	$C_{11}H_{19}D_2Cl_1O_4Si_2$ 6-Membered Ring + O -(CH_3, TMSOH)	SUM of FRAGMENTS A' and B'			
FRAGMENT A'		FRAGMENT B'	FRAGMENT C'	FRAGMENT D'	FRAGMENT E'				
m/z	Percent Abundance	Percent Abundance	Percent Abundance	Percent Abundance	Percent Abundance	Percent Abundance	*m/z*	*Percent Abundance*	*RSD(%) Percent Abundance*
308.1	NA	NA	NA	NA	NA	0.0	*308.0*	*2.1*	*0.8*
309.1	100	NA	NA	NA	NA	91.7	*309.0*	*84.0*	*2.4*
310.1	23.5	NA	NA	100	100	21.5	*310.0*	*22.2*	*2.3*
311.1	41.8	NA	NA	23.5	22.4	38.3	*311.0*	*50.9*	*0.9*
312.1	9.1	100	100	41.8	41.7	100	*312.0*	*100*	*0.0*
313.1	3.4	22.4	23.5	9.1	8.7	23.6	*313.0*	*35.3*	*2.3*
314.1	0.5	41.7	41.8	3.4	3.4	38.7	*314.0*	*41.6*	*0.8*
315.1	0.1	8.6	9.0	0.5	0.5	8.0	*315.0*	*11.1*	*4.4*

Figure S2: NICI-GC-MS Spectra of TMS-d6-sucralose

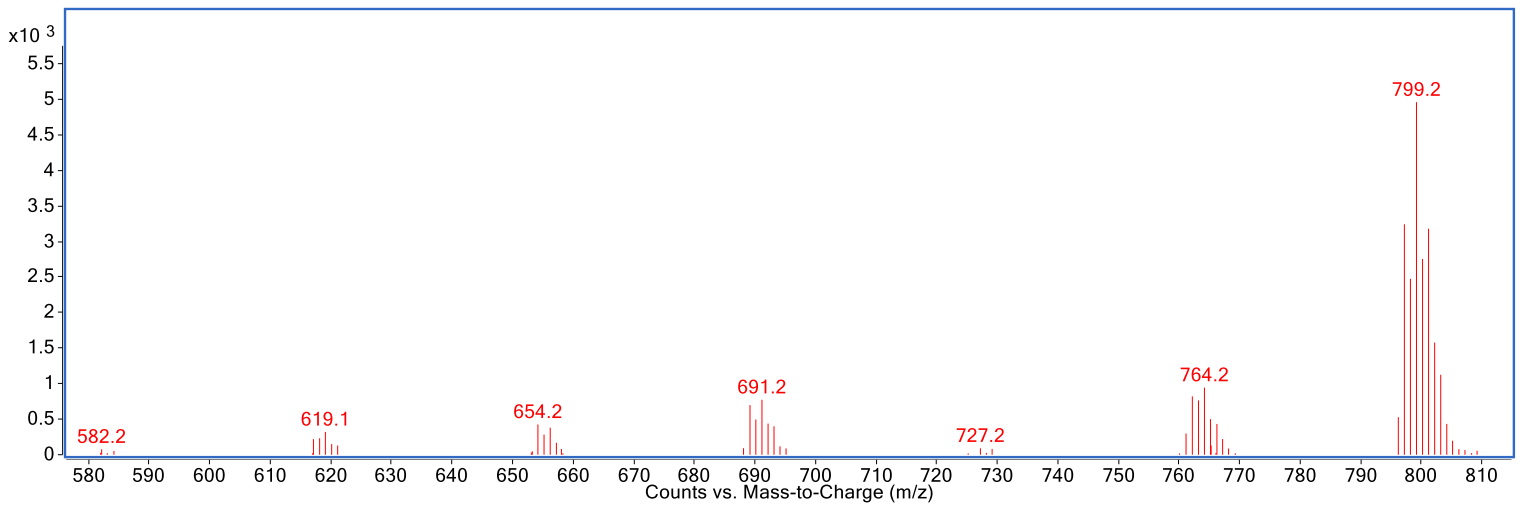


Table S2: Tabulated theoretical and observed abundances for major m/z clusters observed in the NICI-GC-MS spectra of TMS-*d6*-sucralose

5 TMS-*d6*-sucralose (M+e^-) ($C_{27}H_{53}D_6Cl_3O_8Si_5$)				
	Theoretical		Observed	
m/z	Percent Abundance	m/z	Percent Abundance	RSD (%) of Percent Abundance
NA	NA	760.2	2.0	155.4
NA	NA	761.2	28.6	10.5
762.3	77.5	762.2	84.0	3.4
763.3	43.0	763.2	75.0	6.4
764.2	100.0	764.2	100.0	0.0
765.2	50.5	765.2	56.2	10.4
766.2	51.4	766.2	46.6	23.6
767.2	22.8	767.2	21.8	5.9
768.2	13.5	768.2	10.4	9.3
5 TMS-d6-Sucralose + Cl ($C_{27}H_{53}D_6Cl_4O_8Si_5$)				
	Theoretical		Observed	
m/z	Percent Abundance	m/z	Percent Abundance	RSD (%) of Percent Abundance
NA	NA	795.2	0.4	156.0
NA	NA	796.2	11.5	4.8
797.2	62.1	797.2	65.2	2.4
798.2	34.5	798.2	48.9	2.8
799.2	100.0	799.2	100.0	0.0
800.2	51.5	800.2	57.7	3.4
801.2	66.9	801.2	65.5	3.5
802.2	31.2	802.2	31.8	2.7
803.2	24.0	803.2	22.7	3.1
804.2	9.9	804.2	9.1	6.3
805.2	5.0	805.2	4.4	10.7
806.2	1.8	806.2	1.4	32.0
807.2	0.6	807.2	1.0	24.8
808.2	0.2	808.2	0.5	109.6
809.2	0.0	809.2	1.0	28.0
5 TMS-d6-Sucralose -1TMS ($C_{24}H_{44}D_6Cl_3O_8Si_4$)				
	Theoretical		Observed	
m/z	Percent Abundance	m/z	Percent Abundance	RSD (%) of Percent Abundance
NA	NA	688.1	12.9	22.6
689.2	82.3	689.2	84.1	6.4
690.2	38.8	690.2	57.5	10.6
691.2	100.0	691.2	100.0	0.0
692.2	43.7	692.2	50.4	19.8
693.2	47.3	693.2	46.5	7.1
694.2	18.5	694.1	18.1	10.1
695.2	11.1	695.2	8.0	35.9

Table S3: Sample ID, water volume, instrumental concentration, calculated mass of sucralose per sample, calculated environmental concentration and reported concentration of entire dataset.

Table S3

Data release ID	Vol (mL)	Instrument Concentration (ppb)	Mass per vial (ng)	Concentration in water (ng/L)	Reported Concentration (ng/L)
GWNPB1	500	42.87	10.72	21.43	-
GWNPB2	530	21.76	5.44	10.26	-
GWNPB3	485	20.33	5.08	10.48	-
GWNPB4	520	18.52	4.63	8.90	-
GWNPB4r	520	19.42	4.86	9.34	-
GWNPB5	481	14.64	3.66	7.61	-
GWNPB6	433	4.19	1.05	2.42	-
GWNPB7	500	8.44	2.11	4.22	-
GWNPB8	465	9.46	2.37	5.09	-
GWNPB8r	465	13.37	3.34	7.19	-
GWNPB9	510	13.02	3.25	6.38	-
GWNPB10	540	7.96	1.99	3.68	-
GWNPB12	540	14.48	3.62	6.71	-
GWNPB16	510	13.42	3.35	6.58	-
GWNPB17	NA	NA	NA	NA	-
GWNPB19	520	0.00	0.00	0.00	-
GWNPB20	550	7.27	1.82	3.30	-
GWNPB21	550	7.85	1.96	3.57	-
PC1	1000	716.31	179.08	179.08	-
MSPC0808170900	1000	5464.91	1366.23	1366.23	-
MSPC080920171300	1000	736.02	184.01	184.01	-
MSPC0810170920	1000	602.40	150.60	150.60	-
MSPC0821171300	1000	615.90	153.98	153.98	-
MSPC0822171400	1000	717.96	179.49	179.49	-
MSPC0823171200	1000	637.79	159.45	159.45	-
MSPC0911170900	1000	749.80	187.45	187.45	-
MSPC0912171400	1000	709.48	177.37	177.37	-
MSPC0913170930	1000	784.14	196.03	196.03	-
MSPC0914170900	1000	721.19	180.30	180.30	-
MSPC1002171300r	1000	705.47	176.37	176.37	-
MSPC1002171300	1000	726.97	181.74	181.74	-
MSPC1003171300	1000	708.25	177.06	177.06	-

Table S3

Data release ID	Vol (mL)	Instrument Concentration (ppb)	Mass per vial (ng)	Concentration in water (ng/L)	Reported Concentration (ng/L)
MSPC1004171230	1000	689.99	172.50	172.50	-
MSPC1005171230	1000	677.47	169.37	169.37	-
MSPC1016170940	1000	792.74	198.18	198.18	-
MSPC1017170920	1000	807.01	201.75	201.75	-
PCr1	1000	751.21	187.80	187.80	-
MSPC1018171040	1000	760.38	190.10	190.10	-
MSPC1019170920	1000	787.05	196.76	196.76	-
PC101917	1000	698.79	174.70	174.70	-
PC2	1000	807.56	201.89	201.89	-
MSPC1017190920r	1000	718.94	179.73	179.73	-
PC3	1000	756.46	189.12	189.12	-
PC4	1000	822.69	205.67	205.67	-
PC5	1000	737.87	184.47	184.47	-
PC6	1000	819.00	204.75	204.75	-
PC7	1000	842.74	210.68	210.68	-
0824171320	1035	13.25	3.31	3.20	ND
0809170840	985	23.61	5.90	5.99	ND
0823171200	965	27.99	7.00	7.25	ND
0807170830	980	10.75	2.69	2.74	ND
0823170850	1030	14.68	3.67	3.56	ND
0824170940	980	9.14	2.29	2.33	ND
0822171400	950	18.75	4.69	4.93	ND
0808170900	535	26.99	6.75	12.61	ND
0821170900	1037	10.44	2.61	2.52	ND
0809171300	555	7.17	1.79	3.23	ND
0810170920	580	24.54	6.13	10.58	ND
0808171200	965	12.54	3.14	3.25	ND
0822170840	890	11.91	2.98	3.35	ND
0807171200	485	1405.98	351.50	724.73	>
0810171240	975	99.06	24.76	25.40	25.40
0823170850r	1030	18.00	4.50	4.37	ND
0809171300r	555	9.31	2.33	4.20	ND
0911171500	982	5.75	1.44	1.46	ND
0912170900	957	46.31	11.58	12.10	ND
0912171400	980	4.97	1.24	1.27	ND
0913170930	956	5.57	1.39	1.46	ND

Table S3

Data release ID	Vol (mL)	Instrument Concentration (ppb)	Mass per vial (ng)	Concentration in water (ng/L)	Reported Concentration (ng/L)
0913171330	1032	6.35	1.59	1.54	ND
0914170900	968	251.39	62.85	64.92	64.92
1002170930	1070	13.98	3.49	3.27	ND
1002171300	1036	9.03	2.26	2.18	ND
1003170930	885	6.86	1.72	1.94	ND
1004170930	930	10.25	2.56	2.75	ND
1005171230	1064	34.72	8.68	8.16	ND
1005171230r	1064	45.38	11.35	10.66	ND
1002171300r	1036	7.59	1.90	1.83	ND
0914170900r	968	238.29	59.57	61.54	61.54
1004171230	NA	NA	NA	NA	NA
1005170930	NA	NA	NA	NA	NA
1019170920	940	10.90	2.73	2.90	ND
103171300	990	573.83	143.46	144.91	144.91
1114171000	860	11.44	2.86	3.33	ND
1113171000	890	110.45	27.61	31.02	31.02
1016171230	NA	NA	NA	NA	NA
1102171130	NA	NA	NA	NA	NA
1031170900	NA	NA	NA	NA	NA
MS0822171400r	490	525.76	131.44	268.24	268.24
MS0822171400	490	625.94	156.48	319.35	319.35
MS0823171200	500	666.21	166.55	333.10	333.10
MS0911170900	448	681.41	170.35	380.25	380.25
MS0914170900	489	794.47	198.62	406.17	406.17
MS1002171300	500	724.23	181.06	362.11	362.11
MS1004171230	555	746.17	186.54	336.11	336.11
MS1005171230	530	671.28	167.82	316.64	316.64
MS1016170940	585	1126.07	281.52	481.22	481.22
MS1019170920	495	822.55	205.64	415.43	415.43
MS1031171300	505	705.12	176.28	349.07	349.07
MS1101171230	490	594.33	148.58	303.23	303.23
MS1113171230	470	783.26	195.81	416.63	416.63
MS1114171000r	460	865.84	216.46	470.56	470.56
MS1114171000	460	854.57	213.64	464.44	464.44
MS0821171300	NA	NA	NA	NA	NA

Footnotes to Table S1: Sample IDs are unique numerical codes derived from sample time and date. QC samples are coded as follows: GWNPB# = blank, PC# = positive control, MSPC# = matrix spike paired positive control, MS# = matrix spike. The reported concentration is ng/L in water, with ND referring to below the detection limit of 21.8 ng/L, NA referring to "not analyzed", and > referring to detection above the calibration range.

Table S4. Matrix spike samples with instrumental concentrations, matched positive control spiked masses, and calculated accuracies and recoveries.

Matrix Spike ID	Sucralose Mass (ng)	% Accuracy	PC mass	% Recovery
MS0822171400	141.5	69%	179.5	79%
MS0823171200	162.9	79%	159.4	102%
MS0911170900	147.8	72%	187.5	79%
MS0914170900	166.9	81%	180.3	93%
MS1002171300	180.0	88%	181.7	99%
MS1004171230	186.5	91%	172.5	108%
MS1005171230	163.5	80%	169.4	97%
MS1019170920	204.2	99%	196.8	104%
MS1031171300	103.1	50%	177.1	58%
MS1101171230	135.5	66%	184.5	73%
MS1113171230	183.8	89%	204.7	90%
MS1114171000	201.9	98%	210.7	96%

Quantification of Sucralose in Groundwater Well Drinking Water by Silylation Derivatization and Gas Chromatography-Mass Spectrometry
Voss, et al
Supplemental Information

References

1. USGS-GAMA. *California Water Quality Groundwater Ambient Monitoring & Assessment (GAMA)*. 2019; Available from: https://ca.water.usgs.gov/gama/.

2. California State Water Resources Control Board. *Groundwater Ambient Monitoring and Assessment Program (GAMA)*. 2019; Available from: https://www.waterboards.ca.gov/gama/.

3. Bennett, G.L., V, Shelton, J.L., and Jasper, M., *Groundwater-quality data in the Sacramento Metropolitan Shallow Aquifer Study Unit, 2017: Results from the California GAMA Priority Basin Project.* U.S. Geological Survey data release, 2019.

4. McLafferty, F.W.T., Frantisek, *Interpretation of Mass Spectra, 4th ed. (McLafferty, Fred W.; Turecek, Frantisek).*