IS THE LOW-CALORIE SWEETENER SUCRALOSE AN ENVIRONMETAL PROBLEM?

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Abstract
The low-calorie sweetener sucralose was recently introduced in Scandinavia. The compound is very recalcitrant and excreted almost entirely unchanged. It is also very hydrophilic and will be partitioned into the water phase in the environment. Registration of a food additive does not require any environmental risk assessment. Because of the lack of data we measured sucralose in ingoing and outgoing sewage water at a number of Norwegian sewage treatment plants, and also in two recipients. We then measured the sucralose content of some foodstuffs and performed a semi-quantitative calculation of how much of the sucralose that reaches the consumers that can be found the sewage treatment plants. There was no retention of sucralose in the sewage treatment plants, and within the error limits, all sucralose that reaches the consumer is also found in outgoing water at concentrations up to 6.7 μg/L. Sucralose was also found in all water samples from the recipients, up to 69 ng/L in seawater from Oslofjorden. Available data suggests that the half-life of sucralose is several years in water, and it will be necessary to investigate if sucralose affects the aquatic ecosystem in unexpected ways, e.g., disturbing functions where sucrose normally plays a role.

Introduction
Sucralose (1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside, Fig. 1), produced by Tate & Lyle (Decatur, IL, USA), is a low-calorie sweetener that in recent years has gained increased interest from the food industry. Registered as food additive in Canada 1991 and in the USA in 1998, other countries have followed suite and it was registered in Norway in 2005. Today, some 80 countries have registered sucralose.

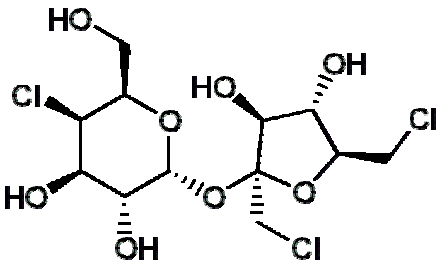

Figure 1. The structure of sucralose (trichlorogalactosucrose).

Sucralose has many benefits compared to other sweeteners; its taste is reported to be more sugar-like than other sweeteners, it is 600 times sweeter than sucrose, i.e., sweeter than other sweeteners giving a favorable relationship between sweetness and advisable daily intake, and its chemical and physical properties are such that there are many technical benefits for the food industry. One such benefit is its chemical stability at low pH making it useful in acidic low-calorie foods.

Although sucralose has undergone a number of toxicity tests[1] (mainly mortality tests) that all seem to show that there is little need for concern about the toxicity of sucralose, it is a matter of fact that a full environmental risk assessment is not required for a food additive. The risk assessment of a food additive is left entirely with food safety agencies, agencies that have as their foremost obligation to protect human health, not to do far-reaching evaluations of possible risks to the environment. Mortality tests have been performed on aquatic organisms to ensure that sucralose does not render sewage water toxic to aquatic organisms. However, the half-life in water is very long. The only (short-time) study published so far indicates a half-life of sucralose in surface water of substantially more than one year at 25 °C,[2] and data from the producer indicates a half-life of more than three years at pH 3 and 7 (25 °C), and of more than one year at pH 9 (25 °C). The presence of a potentially recalcitrant compound mimicking sucrose but with a higher efficacy gives cause for concern about unexpected ecosystem effects that are not covered by mortality tests. Sucrose has many eco-physiological functions besides being sweet to the mammalian palate, and there is little or no information on whether or not sucralose may interfere with any of these functions. As such functions are not of relevance to food safety

agencies that handle the registration of food additives there has been neither any requirement nor any drive to investigate potentially problematic ecosystem effects. Consequently, very little has been published about sucralose in the scientific literature that is of relevance to environmental issues, and this lack of information is a problem for the evaluation of any environmental effects.

As a first step in evaluating the possible environmental effects of sucralose, we performed a screening of sewage water in a number of Norwegian sewage treatment plants (STPs). We also measured sucralose in some samples from the recipient waters and in some food items to calculate how much of the sucralose that reaches the consumers that actually reaches the sewage plants.

Materials and Methods

Water samples (200-400 mL) were acidified to pH 3 after sampling and stored in glass bottles in dark at 4 °C until analysis. Solid-phase extraction (SPE) cartridges HBL-plus preconditioned by sequential washing with acetone, methanol, and water (5 mL each) after which the samples were extracted and the sorbent rinsed and desalted with water (20 mL). Sucralose was eluted from the column with acetone:methanol (5:1, 7 mL), and the eluate further cleaned by passing through a ion-exchange (Isolute-MM, IST, Mid Glamorgan, UK). Sucralose retained in the ion-exchange bed was washed out with additional acetone:methanol (5:1, 3 mL), which were pooled with the extract, and the solvent volume reduced to 0.5 mL on a Zymark TurboVap 500 (Bergman, Lillestrøm, Norway) evaporating apparatus.

Separation was performed with an Agilent 1100 liquid chromatography system (Agilent Technologies, Waldbronn, Germany), with an autosampler, a quaternary pump, an on-line degassing system and a diode array detector (UV). Quantification was performed with a Micromass LCT orthogonal-acceleration time-of-flight (TOF) mass spectrometer (MS) equipped with a Z-spray MUX dual inlet electrospray ion source and a 4 GHz time to digital converter (TDC) (Micromass Ltd., Manchester, UK).

Chromatography was performed with a reversed phase C_{18} column (Atlantis dC18, 2.1 mm ID x 150 mm length, 3 μm, Waters, Milford, PA, USA). A stainless steel inlet filter (Supelco, 0.8 μm) was used in front of a pre-column with the same stationary phase as the separation column. Gradient elution was performed with water as solvent **A** and acetonitrile as solvent **B**. The binary linear gradient had a flow rate of 0.2 mL min^{-1}, started with 95% **A** kept isocratic for 0.1 minute. After 10 min the eluent contained 90% of solvent **B**, kept isocratic until 16 min, after which the content of solvent **B** was again increase to reach 100% at 16.5 min, kept isocratic until 19.5 min. The eluent was then shifted to 95% **A** and the flow rate increased to 0.4 mL min^{-1} kept isocratic till 29.5 min where the flow rate was reduced to 0.2 mL min^{-1}. The total runtime was 30 min including washing and equilibration.

The mass spectrometer was operated in negative mode with a resolution of 6000 and the electrospray source parameters were optimized to the following values: sample cone 20 V, capillary voltage 2.8 kV, extraction cone 3 V, source temperature 125 °C, desolvation temperature 350 °C and desolvation gas flow 600 L h^{-1}. The pusher frequency was operated in automatic mode. Leucine enkephaline was used as internal reference compound for the TOF calibration. Data processing and instrument (HPLC/HRMS) control were with the MassLynx software; quantification was performed with signal extraction of a centroided peak width of 90 amu (typical) of the [M-H] isotope cluster at m/z 395.

Due to the lack of a suitable surrogate standard, quantification was done with a standard addition procedure. The limit of detection (LOD) routinely attained (signal/noise = 3/1) was 13 pg, corresponding to 3 ng L^{-1} in a 200 mL sample. For the sewage water samples a sample volume of 200 mL was usually adequate for quantification. For surface water samples this volume was not always sufficient, but the method LOD can easily be lowered using larger samples. The precision was better than 20%.

Results and Discussion

Sucralose was present in all samples analyzed (Table 1). The concentrations in the effluent are essentially the same as in the influent, why there seems to be little capacity in the sewage treatment plants to remove sucralose.

And as almost all sucralose is excreted unchanged from humans with very little metabolism,[3] most sucralose that reaches the consumers will probably reach the environment. To get preliminary data on how much sucralose that may reach the sewage treatment plants, we determined the sucralose content in some foodstuffs and used sales data to estimate the amount of sucralose sold, and, although there is much uncertainty in the sales data, this seems to confirm that there is very little retention anywhere in the life-cycle of sucralose and that all sucralose sold will reach the environment. Both Oslofjorden and Lake Mjøsa are large bodies of water and it is interesting that already a year after the introduction of sucralose on the Norwegian market it can be found in these recipients. The higher concentrations of sucralose in Oslofjorden than in Lake Mjøsa may simply reflect the number of inhabitants serviced by the sewage treatment plants from which effluent reaches the respective water. However, as the number of samples so far is low and no systematic monitoring of surface water has been performed we cannot as yet draw any far-reaching conclusions on the relevance or environmental consequences of this.

Table 1. Concentration of sucralose in

Sample	n	Mean µg/L	Minimum µg/L	Maximum µg/L
Sewage treatment plants				
Breiskallen, influent	2	0.35	0.16	0.55
Breiskallen, effluent	2	0.62	0.58	0.68
HIAS, influent	4	1.1	0.21	2.1
HIAS, effluent	2	0.21	0.025	0.40
Lillehammer, influent	3	0.42	0.12	0.85
Lillehammer, effluent	5	0.69	0.37	1.4
Nes, influent	3	0.81	0.18	1.8
Nes, effluent	3	1.1	0.23	2.1
Rambekk, influent	5	0.42	0.35	0.50
Rambekk, effluent	6	0.74	0.17	2.5
VEAS, influent	7	4.0	2.6	5.2
VEAS, effluent	7	4.4	2.4	6.7
Hospital effluents				
Rikshospitalet	12	4.3	2.5	5.5
Ullevål hospital	12	3.1	2.2	4.5
Seawater		ng/L		
Oslofjorden, surface water (2 m)	1	8		
Oslofjorden, 21 m	1	47		
Oslofjorden, 35 m	1	69		
Lake water				
Lake Mjøsa, surface water (2 m)	1	0.5		
Lake Mjøsa, 40 m	1	<0.1		

Although sucralose seems to degrade relatively easily in soil and sediment,[4] this is not the case in water. The only published data indicates a half-life in surface water of well over a year,[2] while data from the producer indicates an even longer half-life, three years or longer at 25 °C. No half-life data exists that are relevant for cold climates, but the half-life in cold climates may be substantially longer than half-lives reported at 25 °C. Combined with the long half-life in water, sucralose is also very hydrophilic with a log K_{ow} around –0.8 it will

essentially be entirely partitioned into the water phase with little sorption to particles. Partitioning towards water is confirmed by analysis of sewage sludge in some of the sewage treatment plants where the sucralose content is attributable to the interstitial water.

Although all requirements for registration of sucralose have been adequately fulfilled, there is a conspicuous lack of environmental investigations other than traditional toxicity tests.[1] However, it must be noted that sucralose is specifically synthesized to mimic sucrose and to have a higher physiological activity than sucrose. We argue that for a compound as recalcitrant as sucralose in the environmental compartment into which it is partitioned we have to study effects on the ecophysiological functions of sucrose in aquatic ecosystems. Sucrose in not noted to be a potent toxin, but it does have many other important functions in the environment.

The only study of interest that we have found in the scientific literature shows that sucralose inhibits sucrose transport in sugar cane.[5] Sucrose transport is a key function in vascular plants, and although the sucralose concentrations reached in the environment may never be sufficiently high to entirely inhibit sucrose transport in the field, even a small reduction of the transport efficiency may reduce the plants capacity to utilize its photosynthesis products. Other physiological functions in which sucrose plays a role is in the regulation of gene expression in plants.[6] Among genes regulated are those for the photosynthetic apparatus. Sucrose also plays a role as infochemical (pheromones, kairomones, allelochemicals etc.) in many systems. Examples where sucrose plays a key role are the regulation of symbiotic interactions (e.g., between zoosymbiont and phytosymbiont in corals), and a function as feeding-cue for zoo-plankton. These are just a few examples.

Although it at present is not possible to foresee if sucralose may affect any other functions than sucrose transport in plants, it must be noted that the combination of a very efficacious and recalcitrant sucrose mimic may wreak havoc in any system where sucrose plays a role. Testing of such problems are not covered in the regulation of food-additives, and food safety agencies are generally neither required nor well equipped to do evaluations of problems not related to human health.

The situation described here indicates that the registration process of food-additives is not sufficient to identify possible environmental problems. The process seems to be based on a belief that if there are no problems for humans there cannot be any problems for the environment. Adding to the complexity is that as sucralose is very hydrophilic, and environmental agencies will not directly identify any potential risks with hydrophilic compounds, as the well-known problematic persistent organic pollutants are hydrophobic and bioaccumulating. In this case, the persistence means that most of the ingested sucralose passes unaltered through the body and may, therefore, be regarded as a positive trait in the evaluation of human risks.[3] However, persistence was also a positive trait for the technical use of polychlorinated biphenyls and other know problematic pollutants.

It is our opinion that persistence automatically must lead to questions about environmental risks. More generally, for compounds that are designed to have a physiological effect, whatever that physiological effect may be, we must investigate if any related physiological functions in the environment may be affected.

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