A Review of the Environmental Fate and Effects of Acesulfame-Potassium

Kerry Belton, † Edward Schaefer, ‡ and Patrick D Guiney* §
† Grocery Manufacturers Association, Arlington, Virginia, USA
‡ Eurofins-EAG Agroscience, Easton, Maryland, USA
§ ECOTOX-Guiney Consulting, LLC, Stoughton, Wisconsin, USA

ABSTRACT
The use of low and no calorie sweeteners (LNCSs) has increased substantially the past several decades. Their high solubility in water, low absorption to soils, and reliable analytical methods facilitate their detection in wastewater and surface waters. Low and no calorie sweeteners are widely used in food and beverage products around the world, have been approved as food additives, and are considered safe for human consumption by the United States Food and Drug Administration (USFDA) and other regulatory authorities. Concerns have been raised, however, regarding their growing presence and potential aquatic toxicity. Recent studies have provided new empirical environmental monitoring, environmental fate, and ecotoxicity on acesulfame potassium (ACE-K). Acesulfame potassium is an important high-production LNCS, widely detected in the environment and generally reported to be environmentally persistent. Acesulfame-potassium was selected for this environmental fate and effects review to determine its comparative risk to aquatic organisms. The biodegradation of ACE-K is predicted to be low, based on available quantitative structure–activity relationship (QSAR) models, and this has been confirmed by several investigations, mostly published prior to 2014. More recently, there appears to be an interesting paradigm shift with several reports of the enhanced ability of wastewater treatment plants to biodegrade ACE-K. Some studies report that ACE-K can be photodegraded into potentially toxic breakdown products, whereas other data indicate that this may not be the case. A robust set of acute and chronic ecotoxicity studies in fish, invertebrates, and freshwater plants provided critical data on ACE-K’s aquatic toxicity. Acesulfame-potassium concentrations in wastewater and surface water are generally in the lower parts per billion (ppb) range, whereas concentrations in sludge and groundwater are much lower (parts per trillion [ppt]). This preliminary environmental risk assessment establishes that ACE-K has high margins of safety (MOSs) and presents a negligible risk to the aquatic environment based on a collation of extensive ACE-K environmental monitoring, conservative predicted environmental concentration (PEC) and predicted no-effect concentration (PNEC) estimates, and prudent probabilistic exposure modeling. Integr Environ Assess Manag 2020;16:421–437. © 2020 The Authors. Integrated Environmental Assessment and Management published by Wiley Periodicals, Inc. on behalf of Society of Environmental Toxicology & Chemistry (SETAC).

Keywords: Acesulfame potassium (CAS No. 55589-62-3) Artificial sweeteners Ecological risk assessment Probabilistic exposure modeling

INTRODUCTION
Over the last several decades, the use of low and no calorie sweeteners (LNCSs) has increased substantially. As such, and due to their high solubility in water, low absorption to soils, and reliable analytical methods, some LNCSs, including acesulfame-potassium (ACE-K) have been routinely detected in wastewater influents, effluents, and surface waters (see Table 1, as well as Supplemental Data Tables S3–S6 for a detailed collation of ACE-K’s environmental monitoring studies). The major route of entry for ACE-K into the environment is as unchanged human excretion product that flows down the drain and is ultimately discharged from wastewater treatment plants (WWTPs), which were not originally designed to remove these compounds (Petrovic et al. 2004). Early research has indicated that many LNCSs, including ACE-K, are not completely removed by WWTPs, and thus there is a growing concern that ACE-K and its potential transformation products (TPs) may have an impact on the environment (Lange et al. 2012). Acesulfame-potassium is a common LNCS food and beverage additive, with its uses expected to increase (Sylvestsky and Rother 2016); therefore...
Acesulfame potassium (chemical formula C4H4KNO4S; CAS registry number 55589-62-3) is a sulfamate ester that is 1,2,3-dioxime substituted by a methyl group at position 6. It was discovered in 1967 by Karl Clauss, a German researcher. A 3% solution is approximately 2000 times as sweet as sucrose (Lipinski and Hanger 2001; Magnuson et al. 2016). Acesulfame potassium was approved for use in a variety of foods and beverages by the United States Food and Drug Administration (USFDA) in 1988 (Chattopadhyay et al. 2014), and is often blended with other LNCSs (e.g., aspartame, sucralose). Acesulfame-potassium is sold directly to consumers as Sweet One and Sunett. It has been reported to be one of the LNCSs more resistant to efficient removal in WWTPs (Scheurer et al. 2009). Due to its frequent occurrence and persistence in the environment, ACE-K has been proposed as a tracer of anthropogenic contaminant activity in wastewater (Scheurer et al. 2011). The suitability of ACE-K as an environmental tracer apparently arises from its unequivocal production for and consumption by human activity. Acesulfame-potassium analytical methods are well established, providing an easily achieved and accurate chemical marker for other more difficult to identify hydrophilic contaminants. Although the human health safety profile of ACE-K has been well established, there has been a paucity of ecological effects data reported in the literature until more recently. Given the high likelihood of environmental exposure to ACE-K, it is important to critically review the state of the science with respect to its environmental fate and effects. An initial evaluation of the environmental fate of ACE-K was established by considering its predicted and measured physical-chemical properties (Supplemental Data Figure S1).

### Table 1. Summary of concentration of ACE-K observed in wastewater and environmental compartments

| Compartment                   | Nr observations | Weighted average | Range          | Locations                                                                                     | References                                                                 |
|-------------------------------|-----------------|------------------|----------------|----------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Wastewater influents to WWTPs | 192             | 22.9 µg/L        | <LOQ to 81 µg/L | Multiple Germany; multiple Switzerland; Albany, NY, USA; Tianjin, China; Singapore               | Buerge et al. (2009), Scheurer et al. (2011), Gan et al. (2013), Subedi and Kannan (2014), Tran et al. (2015), Castronovo et al. (2017), Seitz and Winzenbacher (2017), Kahl et al. (2018) |
| Wastewater effluents from WWTPs| 314             | 29.9 µg/L        | <LOQ to 2500 µg/L| Multiple EU unspecified; multiple Germany; multiple Switzerland; Albany, NY, USA; Tianjin, China; Singapore | Buerge et al. (2009), Berzet and Ochsenbein (2012), Gan et al. (2013), Loos et al. (2013), Subedi and Kannan (2014), Tran et al. (2015), Castronovo et al. (2017), Seitz and Winzenbacher (2017), Kahl et al. (2018) |
| Surface water                 | 1008            | 2.9 µg/L         | <LOQ to 53.7 µg/L| Tianjin, China; multiple Switzerland; multiple Germany; multiple Finland; Barbados; NW Spain; multiple Canada | Buerge et al. (2009), Scheurer et al. (2009), Müller et al. (2011), Van Stempvoort et al. (2011), Berzet and Ochsenbein (2012), Ordóñez et al. (2012), Gan et al. (2013), Moschet et al. (2013), Perkola and Sainio (2014), Ruff et al. (2015), Edwards et al. (2017), Seitz and Winzenbacher (2017), Kahl et al. (2018) |
| Groundwater                   | 323             | 0.653 µg/L       | <LOQ to 9.7 µg/L | Multiple Switzerland; multiple Canada; Cape Cod, MA, USA; Germany; Tianjin, China               | Buerge et al. (2009), Van Stempvoort et al. (2011), Berzet and Ochsenbein (2012), Gan et al. (2013), Wu et al. (2014), Schaider et al. (2016), Seitz and Winzenbacher (2017) |
| WWTP sludge                   | 65              | 120.7 ng/g       | <LOQ to 190 ng/g | Albany, NY, USA; multiple Spain; Zurich, Switzerland; Singapore                              | Buerge et al. (2011), Ordoñez et al. (2013), Subedi and Kannan (2014), Arbeláez et al. (2015), Tran et al. (2015) |

ACE-K = acesulfame-potassium; EU = European Union; LOQ = limit of quantification; WWTP = wastewater treatment plant.
These data were not used directly for risk assessment purposes but rather were used to prioritize the most appropriate environmental compartment for risk assessment. Acesulfame-potassium is very water soluble, with a measured water solubility of 237 g/L at 20 °C, and a low measured log K_{ow} value of −2.35 at 23 °C. These 2 characteristics generally indicate that ACE-K will reside in the aquatic compartment and will not bioconcentrate or bioaccumulate in aquatic organisms. The measured K_{ow} values for ACE-K of 10.1 to 43.7 L/kg (Tran et al. 2015) further indicate a low sorption of this chemical to solids (i.e., sludge), which would suggest a low potential for exposure in soil and sediment compartments. The United States Environmental Protection Agency (USEPA 2012) modeled estimates (EPI Suite v4.11) clearly show that ACE-K will not be appreciably removed by WWTPs, and this has been confirmed by a number of early researchers (Buerge et al. 2009; Scheurer et al. 2009; Lange et al. 2012). However, more recently there is an interesting and emerging trend of demonstrating successful biodegradation of ACE-K by WWTPs (Castronovo et al. 2017; Kahl et al. 2018), a finding that has great significance for its potential future aquatic risk. Regarding environmental effects data, ACE-K is now receiving a considerable amount of attention in the peer-reviewed literature with respect to its ecotoxicological characteristics. Although in-depth analysis of the environmental fate, ecotoxicity properties, and environmental risks of other LNCSSs, such as sucralose, have been published (Tollefsen et al. 2012), such an assessment on ACE-K has not been performed. Based on the available information, the purpose of the present paper was to 1) critically review the ecotoxicology and environmental fate state-of-the-science for ACE-K, 2) develop an environmental risk assessment for ACE-K using both a conservative deterministic quotient approach of the ratio of the predicted environmental concentration (PEC) to the predicted no-effect concentration (PNEC) for aquatic organisms as well as a more advanced probabilistic risk assessment, and 3) provide a data-gap assessment for future work to more fully address the potential ecotoxicological risks from ACE-K.

OVERVIEW OF HUMAN HEALTH AND MAMMALIAN TOXICOLOGY STUDIES

Internationally, for the past 25 y, ACE-K has been accepted for use in a variety of food products as a sweetener and flavor enhancer. Several regulatory bodies and national food authorities, including the Scientific Committee on Food (SCF) of the European Commission, European Food Safety Authority (EFSA), USFDA, and the Joint Food and Agriculture Organization–World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) have established an acceptable daily intake (ADI) for the safe use of ACE-K. These ADIs are calculated on the basis of the results of extensive animal toxicology studies (WHO 1980, 1983; USFDA 1988; JECFA 1991; EC 2000; EFSA 2016). The USFDA (1988) set an ADI of 15 mg·kg^{-1}·d^{-1} of body weight (bw) in the United States; concomitantly, in Europe, the ADI was set at 9 mg·kg^{-1}·d^{-1} of bw (EC 2000). The JECFA set an ADI of 9 mg/kg of bw, which was later increased to 15 mg/kg (JECFA 1991). All approvals were based on long-term rat or dog studies that concurred that neither rats nor dogs exhibited adverse effects when fed diets containing up to 3% ACE-K for up to 2 y. Based on body weights, these exposures corresponded to no observed adverse effect levels (NOAELs) of 1500 mg·kg^{-1}·d^{-1} and 900 mg·kg^{-1}·d^{-1} of bw for rats and dogs, respectively (Magnuson et al. 2016).

The initial approval by JECFA established an ADI of 9 mg·kg^{-1}·d^{-1} based on the 2-y dog study (WHO 1983). The JECFA later reevaluated available data and revised the ADI to 15 mg·kg^{-1}·d^{-1} based on the 2-y study in rats, concluding that the rat study better represented humans because exposures in the rat study began in utero and because chronic exposure for 2 y represented a greater portion of the rats’ lifespan compared with the same exposure duration in dogs (JECFA 1991).

At this time, ACE-K is approved for use in more than 100 countries, and more than 90 studies support its safety. Additionally, recent safety reevaluations conducted by the European Food Safety Authority (EFSA) and the USFDA have reaffirmed the safe use of ACE-K as a nonnutritive sweetener with the same previously established ADIs (USFDA 1988; EU 2000; EFSA 2016).

ENVIRONMENTAL FATE AND EXPOSURE ASSESSMENT CHARACTERIZATION

Variations in removal efficiencies of ACE-K in activated sludge wastewater treatment plants

Acesulfame-potassium is excreted from the human body via the urine and enters WWTPs essentially unchanged (Renwick 1986). Acesulfame-potassium may be discharged into surface waters from domestic sewage via WWTP effluents if incomplete degradation and limited retention times are observed during wastewater treatment. It is well recognized that WWTP removal efficiencies for any chemical are driven by 2 independent sets of variables. The first relates to the inherent physical–chemical properties of the chemical, and the second is associated with the specific operational parameters of the activated sludge WWTP being investigated (Douziech et al. 2018). The inherent properties of the chemical dictate the extent to which the key processes, including biodegradation, hydrolysis, sorption to sludge solids, volatilization, and photodegradation, will occur and influence the rate of removal. The WWTP operating parameters critical for understanding removal efficiencies include sludge and hydraulic retention times, activated sludge concentrations and metabolic capabilities, pH, and operating temperature. The inherent physical–chemical properties of ACE-K (see Supplemental Data Figure S1) essentially exclude the potential for abiotic removal mechanisms to significantly contribute to its removal efficiency in WWTPs (Buerge et al. 2009; Tran et al. 2014, 2015). Hydrolysis of ACE-K was not a significant WWTP removal mechanism based on its stability in wastewater treatment and surface waters and similarity of ACE-K concentrations in those
environmental compartments (Buerge et al. 2009). Sorption of ACE-K is expected to be low, based on its measured $K_d$ values (Supplemental Data Figure S1). This was confirmed by Subedi and Kannan (2014), who measured a $K_d$ sorption coefficient for ACE-K of 289 L/kg and less than 2.0% removal from 2 WWTPs in Albany, New York, USA using activated biological sludge treatment. Tran et al. (2015) reported comparably low ACE-K sludge $K_d$ sorption coefficients of 10.1 L/kg to 34.7 L/kg in lab batch experiments for conventional activated sludge and nitrifying activated sludge, respectively. The $K_d$ values <500 indicate a strong potential to migrate with water rather than attach to soil or sludge particles (Morais et al. 2013). In addition, the pK$_a$ of ACE-K is 2.0 (Supplemental Data Figure S1), which suggests that it will exist almost completely in the anion form in the environment. Anions commonly do not absorb tightly to soils high in organic C and clay when compared to their corresponding neutral chemicals. Confirming these conclusions, ACE-K has been detected in sewage sludge samples at low mean concentrations ranging from 32 to 163 ng/g (Supplemental Data Table S5). Interestingly in a previous study, (Tran et al. 2014) found 16% to 21% removal of ACE-K after 7 d of incubation in a nitrifying activated sludge and suggested that this removal of ACE-K was attributable to biodegradation and the presence of autotrophic and heterotrophic microorganisms in the nitrifying activated sludge as well as potential induction of nonspecific oxidative enzymes rather than any abiotic factors. Acesulfame-potassium is also not expected to volatilize appreciably during WWTP operations due to its very low estimated vapor pressure and Henry’s Law Constant (Supplemental Data Figure S1). Tran et al. (2014) also reported a very low vapor pressure for ACE-K of $9.6 \times 10^{-9}$ atm m$^3$/mol and concluded that this mechanism did not contribute to ACE-K removal in their batch experimental nitrifying activated sludge studies. Acesulfame-potassium has a half-life of 2.8 h in AOPWin v1.92 (USEPA 2012) using updated measured ACE-K input values rather than defaults, which is less than the 2 d assigned to substances that exhibit potential for long-range atmospheric transport. Acesulfame-potassium does not absorb well at wavelengths >290 nm (the cutoff of solar irradiation at the earth’s surface) so therefore it might not be expected to be susceptible to direct photolysis by sunlight at neutral pH (Perkola et al. 2016). Although there may be some indirect photolysis of ACE-K in surface waters exposed to sunlight, the extent to which it might be photodegraded in a typical WWTP would be limited to only the exposed top layers of wastewater treatment waters, and the typical high turbidity of those wastewaters can be assumed to block most sunlight.

Given the above discussion of the insignificance of abiotic factors influencing the removal of ACE-K in a typical activated biological sludge treatment plant, we can conclude that when removal of ACE-K from WWTPs is observed, the rate of removal is likely associated with one form of biodegradation or another. The scientific literature suggests a changing profile of ACE-K’s removal efficiency by WWTPs over time (Supplemental Data Table S1). Early pioneering laboratory studies on the biodegradation potential of ACE-K indicated that aerobic degradation of ACE-K was negligible after 90 d in activated sludge (Buerge et al. 2009) and surface waters (Scheurer et al. 2009, 2010; Lange et al. 2012). Buerge et al. (2011), however, also studied the biodegradation of ACE-K in 6 different Swiss soil samples under aerobic conditions and found >90% degradation of ACE-K in 5 of 6 soils and approximately 60% degradation in the 6th soil sample. Although these findings were in contrast to their previous reports of persistence of ACE-K in WWTPs, they rationalized these findings of fairly rapid biodegradation in soils by the presence of divergent populations of microorganisms present in the soil versus the activated sludge. No biodegradation of ACE-K was observed in fixed-bed reactors (aqueous compost and soil) over a 56-d period, whereas ACE-K was completely biodegraded within 28 d in diluted wastewater effluent (Storck et al. 2016).

Beginning around 2014, studies began showing up in the literature suggesting that biodegradation of ACE-K could in fact occur under specific environmental conditions. The degradation of ACE-K (16%–21% in 7 d) by an enriched nitrifying culture enhanced with ammonium was reported by Tran et al. (2014). In 2016, a group of Swiss and German scientists observed 80% removal of ACE-K in aerobic sequential batch reactors (SBRs) but no removal in anaerobic SBRs (Falás et al. 2016). The following year, Castronovo et al. (2017) published a follow-up paper confirming this trend of increasing removal as well as documented biodegradation of ACE-K when they reported between 59% and 97% ACE-K removal in 13 German and Swiss WWTPs using conventional activated sludge treatment with both denitrification and nitrification. They also reported that ACE-K was quantitatively transformed to sulfamic acid and complete removal of C originating from ACE-K. They reported that sulfamic acid was the only relevant final TP in the presumed degradation pathway of ACE-K and, based on similar measured concentrations of sulfamic acid in the WWTP influents and effluents (maximum concentrations of up to 2.3 mg/L), that the biodegradation of ACE-K added only insignificantly to the typical total load of sulfamic acid discharged into surface waters consistent with its widespread use in commercial acid cleaning products. The authors ultimately concluded that the biodegradation of ACE-K in municipal WWTPs was not as rare as had been previously reported, and removal was attributed to biologically mediated degradation processes.

Subsequently, Kahl et al. (2018) sought to identify whether the removal of ACE-K in WWTPs was now an even more general trend. These authors confirmed that ACE-K removal and biodegradation was significant in 9 additional German WWTPs, although the removal efficiency exhibited some seasonal fluctuation. Monthly median removal efficiencies exceeded 95% from July to October/November. However, during the colder winter season (January through April), less than 20% ACE-K was removed from smaller WWTPs (plants B, D, and H). Acesulfame-potassium biodegradation activity was restored by increasing the
temperature of the original cold (winter) WWTP sludge matrix, indicating that ACE-transforming microorganisms were present but simply inactive during the cold season. Thus, the seasonality differences observed were much more pronounced for the 3 smaller WWTPs, whereas in the larger WWTPs (i.e., those with capacities of >200 000 population equivalents; plants A and E–G), ACE-K removal was actually very efficient (>80%) throughout the entire year. In these larger WWTPs, there was good removal even at temperatures of approximately 16 to 22 °C. They also observed that ACE-K removal was enhanced by low carbonaceous biochemical O demand (CBOD5) and high O availability, values which typically go hand in hand with longer retention times. Thus, it appears that while recovery of ACE-K removal and biodegradation was temperature driven (mesophilic temperature ranges being optimal), it was also well correlated with longer retention times typically employed at larger WWTPs. The ACE-K biodegrading microbes appeared to be slow growers, which might suggest that longer retention times may be an additional enhancing factor for ACE-K biodegradation. Another important finding from Kahl et al. (2018) was the documented generation of sulfamic acid as the major biodegradation product formed from ACE-K, which confirmed the previous findings of Castronovo et al. (2017). This developing trend for observing increased removal and biodegradation of ACE-K in German WWTPs was also reflected in reductions in mass loading of ACE-K in 2 large rivers in Germany over the study period of 2013 to 2016 (Kahl et al. 2018). During this 4-y period, they observed reductions in ACE-K loading of around 80% in the Elbe River and 70% in the Mulde River. Measured influent ACE-K concentrations in their study (10–70 μg/L) were not significantly different from influent concentrations reported previously in other German and Swiss surface waters (Buerg et al. 2009, 12–434 μg/L; Scheurer et al. 2011, 8.2–37 μg/L; and Castronovo et al. 2017, 20–81 μg/L; see also Supplemental Data Table S3). During this same time period, Kahl et al. (2018) further suggested that wastewater treatment processes and techniques had not changed significantly in Germany. These facts suggest that the observed enhanced rates of ACE-K removal and biodegradation in Germany had a positive influence (reduction) on the reported surface-water concentrations of ACE-K over the 4-y period (2013–2016) of the study.

There now also seems to be an emergence of confirmed ACE-K biodegradation in other areas of the world, including China (Yang et al. 2017) where >90% ACE-K removal was reported with a common biological treatment process (oxidation ditch and membrane bioreactors). Huang et al. (2019) also recently isolated an ACE-K degrading Chelatococcus sp. from WWTP activated sludge cultures in China. A similar oxidation ditch process combined with typical bioreactors in conventional 3-step WWTP process (primary, activated biological sludge, and chlorination) in Australia (Cardenas et al. 2016) resulted in 92% ACE-K removal efficiencies in September 2012. Average air temperatures in Queensland, Australia in September are 21.1 °C. It appears that if the WWTP operating conditions are suitable, including mesophilic temperature ranges and perhaps longer retention times, there may be an emerging trend for the biodegradation of ACE-K in WWTPs worldwide. Following up on these emerging findings, Kleinsteuber et al. (2019) have recently provided additional preliminary mechanistic evidence that ACE-K can be mineralized in a catabolic process and used as the sole C source by pure strains of bacteria isolated from activated sludge. Three slow-growing strains of ACE-K biodegraders were isolated and degraded 1 g/L ACE-K within 8 to 9 d.

Laboratory-based physical–chemical simulation approaches for predicting the removal of ACE-K

The high detection frequency of ACE-K in various environmental compartments combined with the early reported absence of significant biodegradation led to attention being focused on developing various potential alternate physical–chemical strategies that might more effectively remove it from the waste stream and receiving waters. A number of laboratory-based studies have been pursued with mixed results (see Supplemental Data Table S2, which summarizes representative examples of physical–chemical degradation processes that have been explored). For the most part, these approaches have focused on processes with the greatest relevance to domestic wastewater, which include chlorination, UV radiation, and ozonation. Degradation of ACE-K by chlorination (21%) and ozonation (100%) was reported by Soh et al. (2011), and Scheurer et al. (2010) confirmed 100% removal efficiency of ACE-K using a similar ozone method. Permanganate oxidation resulted in 43% to 80% ACE-K removal efficiency with higher removal rates associated with lower dissolved organic matter concentrations (Yin et al. 2017). Finally, UV light exposure combined with advanced oxidation catalysts has been investigated by several research groups with varying results ranging from 19% up to 100% removal efficiencies (Scheurer et al. 2014; Kattel et al. 2017; Fu et al. 2019). Another research group (Sang et al. 2014) investigated a more novel laboratory-based approach of combining UV treatment in the presence of a TiO2 catalyst and reported >84% removal of ACE-K after 30 min and complete removal after 2 h. Although the reported photocatalytically enhanced degradation of ACE-K in this study (Sang et al. 2014) is interesting, the practical application of such an approach has not been implemented in any full-scale WWTPs due to a number of limitations (Dong et al. 2015). In addition, Sang et al. (2014) also reported the generation of several potentially unwanted ACE-K TPs as a result of these experiments, which were determined to exhibit enhanced toxicity as measured by Microtox (an in vitro screening test system using bioluminescent bacteria for the detection of relative toxicity in water; Microbics Corporation 1992). ACE-K does not absorb at wavelengths >290 nm and therefore should not be susceptible to direct photolysis by natural sunlight. Scheurer et al. (2014), however, successfully demonstrated the degradation of ACE-K by direct photolysis during...
short-wavelength ultraviolet C light (UVC) disinfection of water as a function of pH, concentration, and water matrix in laboratory and full-scale waterworks. The chemical structure of ACE-K does contain an olefin bond and olefins are known to react with photooxidants in natural waters (i.e., hydroxyl, peroxo, and singlet O) when exposed to sunlight with a half-life on the order of about 25 d (Mill 2000). Indirect photolysis reactions of dissolved chemicals can occur as the result of chemical or electronic excitation transfer from light-absorbing humic acid species in the natural water. In contrast to direct photolysis, this photoreaction is governed initially by the spectroscopic properties of the natural water (USEPA Test Guidelines OPPTS 835.5270 Indirect Photolysis Screening Test [USEPA 1998]). Thus, natural photolysis of ACE-K (a combination of direct and indirect photodegradation) is expected to contribute to its removal in the environment, but understanding the extent of this photodegradation and the identification of potential TPs are important issues that deserve further research.

Lange et al. (2012) reviewed the literature that reported qualitative efficiencies of different physical–chemical water treatment processes for the removal of a variety of artificial sweeteners, including ACE-K. They deduced that ozonation was the only process investigated which consistently demonstrated medium to high removal efficiencies for ACE-K. Buerge et al. (2009), however, reported that ACE-K was only partly removed during ozonation of groundwater in a WWTP. These results were confirmed by Scheurer et al. (2010) in a systematic field study in which ACE-K was oxidized quickly with a half-life of approximately 15 min at an ozone concentration of 0.5 mg/L, but after a contact time of 30 to 40 min (typical of WWTPs in Germany), 30% residual concentrations of ACE-K remained in the test solution. Care, however, must be given when interpreting ozonation treatment results because some unexpected kinetic reactions can occur, depending on the combination of what is and is not present in the treatment water. Thus, oxidation of ACE-K appears to show the greatest promise for a single treatment option for advanced WWTPs where biodegradation of ACE-K may not have been demonstrated. It may also be that a combination of advanced oxidation combined with UVC light may hold the greatest non-biological indication for success if it could be implemented cost effectively at full-scale WWTPs along with carefully adjusted treatment process conditions. In this case, the combination of UVC light plus an oxidizer such as hydrogen peroxide or ozone would absorb the UVC light and produce hydroxyl radicals that could enhance the oxidation of contaminants such as ACE-K.

Bioaccumulation potential

The low measured octanol–water partitioning coefficient ($K_{ow}$) of $-2.35$ at $23 \, ^\circ C$ for ACE-K (Supplemental Data Figure S1) suggests a bioaccumulation potential well below any regulatory trigger (ECHA 2017). Using this log $K_{ow}$ and the Anot-Gobas method from the USEPA’s EPI Suite v. 4.11 (USEPA 2012), a bioconcentration factor (BCF) in fish of 0.8931 can be calculated for ACE-K. Federico (2017) studied the bioconcentration of ACE-K in bivalves over a 10-d period. At an exposure concentration of 100 µg/L, the measured BCF value was approximately 7 L/kg. This low experimental BCF value approaches the predicted BCF value from the USEPA EPI Suite model (USEPA 2012). According to established classification schemes (Franke et al. 1994), this BCF suggests that the potential for bioconcentration in aquatic organisms is low.

Wide-ranging survey of monitored ACE-K concentrations in various environmental matrices

Acesulfame-potassium has been detected in wastewater influents, effluents, and sludge, as well as surface water and groundwater across the world. The results of our detailed collation of all the ACE-K environmental monitoring data are presented in the Supplemental Data of the present paper (see Table S3 influents, Table S4 effluents, Table S5 sludge, Table S6 surface waters, and Table S7 groundwater). Table 1 presents an overall summary of this worldwide ACE-K environmental monitoring data. Wastewater influent and effluent ACE-K concentrations were not meaningfully different. Acesulfame-potassium concentrations in influents had a sample-weighted average of 22.9 µg/L with a range of less than the limit of quantification (<LOQ) to 81 µg/L whereas the weighted average for effluents was 29.9 µg/L with a weighted average of <LOQ to 2500 µg/L. As anticipated based on the physical–chemical properties of ACE-K (Supplemental Data Figure S1), it was detected in wastewater sludge at much lower concentrations (sample-weighted average of 120.7 ng/L with a range of <LOQ to 190 ng/L). Surface waters had a sample-weighted average ACE-K concentration of 2.9 µg/L with a range of <LOQ to 53.7 µg/L. It is interesting to note that the average surface-water concentration of ACE-K appears to be decreasing since about 2014. Literature studies from 2009 to 2013, summarized here, reported an average surface concentration ACE-K of 4.65 µg/L, whereas the average surface concentration of ACE-K reported in studies published between 2014 and 2018 was significantly lower at 1.1 µg/L. Finally, groundwater monitoring studies revealed the lowest sample-weighted ACE-K water concentration average of 0.653 µg/L with a range of <LOQ to 9.7 µg/L. The ACE-K monitoring data presented in this wide-ranging survey of the literature along with the physical–chemical environmental fate properties of ACE-K (Supplemental Data Figure S1) confirm that surface water is the pathway most relevant for environmental risk assessment.

Acesulfame-potassium might also be expected to have some potential to end up in the terrestrial environment via wastewater when used for supplying water to land to help plants grow, the application of digested sewage sludge as fertilizers, or in septic tank leach fields. Given the typical ACE-K sludge concentration (Supplemental Data Table S5), it would not be expected to be a major source. Acesulfame-potassium was reported to have minimal soil sorption, matching the observations reported with sewage sludge.
The $K_d$ and $pK_d$ of ACE-K (Supplemental Data Figure S1) indicate that it will have high mobility in soil and low absorption, suggesting potential migration to groundwater and surface water. Acesulfame-potassium is not expected to volatilize from dry soils based on its low vapor pressure. Interestingly, Buerge et al. (2011) found that when ACE-K is added to soils it was readily degraded, with half-lives ranging from 3 to 49 d, which should reduce this migration.

**EFFECTS ASSESSMENT**

**Effects on aquatic organisms**

An excellent set of laboratory-generated ecotoxicological data are available for ACE-K (Supplemental Data Table S8), which include acute and chronic toxicity in fish and invertebrates, fish embryo toxicity, toxicity to freshwater plants, and toxicity to domestic sludge microorganisms. A significant proportion of the ecotoxicity studies conducted on ACE-K were provided by data compiled in a European Union Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) registration dossier, prepared and submitted electronically by the supplier to the European Chemicals Agency (ECHA) through their chemical software program, International Uniform Chemical Information Database (IUCLID; ECHA 2017). The ACE-K ecotoxicity data were extracted from robust study summaries that included objectives, methods, results, and conclusions of the full study reports. This detailed information allowed for the accurate determination of the relevance of these studies. The fact that the vast majority of these studies were conducted under Organisation for Economic Co-operation and Development (OECD) guidelines (many using good laboratory practices [GLP]) ensured the quality, credibility, and traceability of the data submitted (ECHA 2018).

Fish acute toxicity is represented by 3 static studies, two equivalent or essentially similar to OECD 203 methods (OECD 2019) and one according to OECD 203 under full GLP conditions. The first two of these studies were conducted in zebrafish and golden orfe fish and generated 96-h LC50 values $>1000$ mg/L. The third study, also in zebrafish, reported an LC50 value of $>1800$ to $<2500$ mg/L. Long-term toxicity to zebrafish was also studied in an OECD 210 (OECD 2013) fish early life stage (ELS) test conducted under GLP. The 30-d no observed effect concentration (NOEC) based on mortality, weight, and length changes versus controls was calculated to be 22 mg/L. This OECD 210 ELS NOEC was the lowest chronic NOEC reported for ACE-K and thus served as the basis for calculating its PNEC.

Li et al. (2016) studied the embryo toxicity of ACE-K and 6 phototransformation products of ACE-K in zebrafish. They established NOECs of 10 000 mg/L for sensitive sub-lethal endpoints of tail detachment, edema, and heart rate and slightly lower NOECs ranging from 5000 to 10 000 mg/L for the ACE-K phototransformation products based on these same endpoints. In another study, common carp were exposed to ACE-K for 96 h at concentrations of 0.05 and 149 $\mu$g/L (Cruz-Rojas et al. 2019). Measures of oxidative stress (e.g., superoxide dismutase) were evaluated and found to be elevated in the gill, brain, and muscle. Ren et al. (2016) also exposed carp to $\leq 10$ mg/L ACE-K for 7 d and reported no differences in oxidative stress markers in exposed fish relative to controls. However, following UV irradiation, oxidative stress markers were significantly induced. These data also highlight the potential for phototransformation and enhanced ecotoxicity associated with ACE-K. Although the changes in a nonstandard endpoint such as oxidative stress are interesting, survival, growth, and reproduction in standardized ecotoxicity data were unaffected at similar exposure levels.

Short-term toxicity to Daphnia magna were determined to have a 48-h NOEC of 1000 mg/L (Stolte et al. 2013) and a 24-h EC50 of $>1000$ mg/L based on an OECD 202 test (OECD 2004). Long-term toxicity to D. magna based on an OECD 211 study (OECD 2012) and reproduction as the measured endpoint resulted in a 21-d NOEC of $>100$ mg/L.

Stolte et al. (2013) investigated the short-term ecotoxicity of ACE-K in aquatic plants in a 72-h algal test (growth as endpoint) and a 7-d Lemma minor plant test (growth as an endpoint). No statistically significant effects were observed at concentrations up to 1000 mg/L. Another study on green algae conducted according to OECD 221 methods (OECD 2006) resulted in an NOEC of 100 mg/L. A variety of studies have also examined the potential toxicity of ACE-K to domestic sludge microorganisms (Stolte et al. 2013; ECHA 2018). An OECD 209 (OECD 2010) activated sludge respiration inhibition test generated a 3-h NOEC of 1000 mg/L (Stolte et al. 2013), whereas other methods conducted for REACH measured NOEC ranging from $>140$ mg/L to $>2500$ mg/L based on a lack of any measurable toxicity.

**Consideration of ACE-K degradation products under conventional WWTP process as well as simulated laboratory experimental conditions**

Potential ACE-K degradation products and their latent aquatic toxicity are generally poorly characterized, but a number of studies have suggested that potential physical-chemical TPs may represent a higher level of aquatic toxicity than the parent ACE-K (Sang et al. 2014; Li et al. 2016; Ren et al. 2016; Yin et al. 2017). The transformation that ACE-K undergoes in the natural environment is considered to be complex and may very well involve a combination of various physical, chemical, and biological processes (see Supplemental Data Tables S1 and S2). However, the ultimate degradation of ACE-K in the real world will depend upon the specific degradation process involved (i.e., biodegradation vs physical–chemical degradation) and the specific conditions under which TPs are formed (i.e., full-scale WWTP vs simulated or laboratory generated). Regarding degradation products formed from biodegradation processes, many of these studies have been based on full-scale operating WWTPs (Supplemental Data Table S1). Cardenas et al. (2016) studied a large full-scale WWTP in South East Asia.
Queensland, Australia equipped with a conventional 3-step treatment process, which included primary treatment, an activated biological process that integrated 4 oxidation ditches with an aerobic and anaerobic zone followed by clarifiers, and disinfection by chlorination. They reported 92% removal of ACE-K and attributed this reduction to biodegradation. Although they did not investigate possible biodegradation products, they did suggest a need to further investigate the possible biodegraders and compare them to those previously reported to be formed by physical–chemical processes (Sang et al. 2014; Scheurer et al. 2014, 2010). Castronovo et al. (2017) investigated the removal and biodegradation of ACE-K during activated sludge processes at 13 WWTPs and reported 59% to 97% removal depending on the different WWTPs with denitrifying conditions maximizing this biodegradation. Furthermore, they used high-resolution mass spectrometry to identify sulfamic acid as the predominant and only relevant TP. A subsample of 3 WWTPs revealed that concentrations of sulfamic acid in the influent and effluent were similar and therefore the degradation of ACE-K added insignificantly to the normal loading of sulfamic acid discharged into surface waters (typical for municipal WWTPs receiving high loads of sulfamic acid cleaning products). Kahl et al. (2018) confirmed the mineralization of ACE-K to stoichiometric amounts of sulfamic acid in their study of ACE-K removal and biodegradation in an aerated horizontal flow treatment wetland and an adjacent municipal WWTP using activated sludge. Finally, in follow-up studies conducted by Kleinsteuber et al. (2019), ACE-K has been shown to be mineralized in a catabolic process and used as the sole C source by bacterial pure strains isolated from WWTP activated sludge identified as Bosea sp. and Chelatococcus sp. These authors observed the detection of acetoacetamide-N-sulfonic acid (ANSA) and the subsequent stoichiometric generation of the same sulfamic acid biodegradation compound. Although further experiments are required to elucidate the entire ACE-K biodegradation pathway, it now seems clear that where biodegradation of ACE-K has been reported from conventional WWTPs, it most likely results in the generation of relatively nontoxic concentrations of sulfamic acid.

Several researchers have found that ACE-K can be transformed by various physical–chemical methods although the majority of these studies have been at the laboratory simulation level (Supplemental Data Table S2). For example, a number of studies have shown that ACE-K can be phototransformed to multiple degradation products via potential interaction with dissolved organic matter (Scheurer et al. 2012; Gan et al. 2013; Li et al. 2016; Perkola et al. 2016; Minella et al. 2017). Some of this reported photodegradation of ACE-K in the environment may also be due to photooxidation or catalytic photolysis. Nevertheless, phototransformation is an important finding because it suggests that ACE-K has an additional depletion mechanism beyond the emerging reports of significant biodegradation once released into the environment. In one of the more reliable studies in this area, hydroxylated acesulfame and iso-acesulfame were the primary degradation products of UV irradiation (Scheurer et al. 2014). In another well-designed study by Scheurer et al. (2010), the structural elucidation of the main ozonation products of ACE-K were identified. The main products of these ACE-K oxidation studies were acetic acid and dihydroxyacetyl sulfamate, both of which showed good removal in activated C filters downstream of the ozonation unit in a full-scale WWTP. Some studies have suggested that although the parent ACE-K chemical represents minimal or no significant adverse impact on aquatic organisms, various TPs (TPs) of ACE-K may exhibit increased aquatic toxicity. Sang et al. (2014) studied the feasibility of using a nonconventional TiO2 catalyst-enhanced photodegradation of ACE-K and compared the resulting toxicity of the TPs to the parent ACE-K. They employed the Microtox screening bioassay using the marine bioluminescent bacteria Vibrio fischeri for this purpose. The EC50 of ACE-K alone was 72.190 mg/L whereas the EC50 in the phototreatment group was amplified at 125 mg/L. Although the results of this study (Sang et al. 2014) are of course interesting because the transformation of ACE-K in these laboratory-based simulations opens the possibility for treatment options, the levels of TiO2 used to trigger these catalytic reactions do not appear to be currently cost effective or scalable to fully operating WWTPs. Based on the doses used in this study, 10000- to 50 000-gallon bioreactor would require 380 kg to 1900 kg per biotreater just for a single dose. In a related study, Yin et al. (2017) also used Microtox to screen the potential toxicity of ACE-K oxidation TPs by permanganate (Mn[III]) and found that ACE-K elicited a 16% inhibition rate without oxidation, which was increased by 29% in the presence of Mn[III]. Measured EC50 values for ACE-K were not reported in the Yin et al. (2017) paper and could not be derived from the data presented; however, if we assume DOC as a surrogate for ACE-K, we can extrapolate a starting ACE-K test concentration of about 21 mg/L, which would represent an extremely high environmental concentration of ACE-K. The bioluminescent bacteria V. fischeri used in Microtox is a marine species, so the chemistry of the test substance is not what it will be in fresh water. Nevertheless, Microtox has been widely used as a screening tool to estimate the acute toxicity of water samples and chemical substances. Although Microtox can be used to identify potentially toxic substances for further testing, it cannot serve as a substitute for traditional tiered acute and sublethal hazard assessments. It has typically been used as an initial part of a battery of tests or to supplement data obtained in other more established ecotoxicology testing. The Microtox results obtained by Sang et al. (2014) and Yin et al. (2017) provide important information but were conducted at unrealistically high concentrations of ACE-K under simulated laboratory conditions. Given the inherent disadvantages of the Microtox screening method and the exploratory conditions under which these ACE-K degradates were engineered, the environmental relevance of this work is quite low, and these studies should be
reviewed as unreliable for the purpose of risk assessment. They should, however, be considered preliminary findings that require further confirmation at environmentally relevant concentrations of ACE-K and with higher tiered ecotoxicity guideline studies, should these methods ever become a viable cost-effective full-scale treatment option.

Li et al. (2016) investigated the photocatalytic transformation of ACE-K and the embryotoxicity of the TPs to zebrafish. They identified 6 TPs more polar than ACE-K. Very high NOECs (10 000 mg/L) based on tail detachment, edema, coagulation, and heart rate for ACE-K were compared with augmented embryotoxicity (NOECs 5000–10 000 mg/L) for the TPs. These findings suggest that developmental toxicity of ACE-K to zebrafish represents a low risk. Although the observed enhanced developmental toxicity of these photocatalytically formed TPs is a noteworthy finding, the environmental relevance of these TP NOECs as evaluated in the context of this ACE-K aquatic risk assessment (refer to Figure 1) is minimal.

Ren et al. (2016) exposed carp to ≤10 mg/L ACE-K for 7 d and reported no differences in oxidative stress markers in exposed fish relative to controls. Following UV irradiation, oxidative stress markers (i.e., hydroxyl radicals and malondialdehyde content) were significantly induced in the 0.1 and 10 mg/L ACE-K UV groups; however, these ACE-K exposure concentrations were not environmentally relevant and the study design also appeared flawed given that the control group (0 mg/L ACE-K) did not appear to be UV irradiated as were the other comparative test concentrations. A study by Saucedo-Vence et al. (2017), conducted with the LNCS sucralose, found oxidative stress biomarkers in carp gill, brain, and muscle following exposure to ≤155 µg/L sucralose. As a follow-up study, the same research group exposed common carp to ACE-K for 96 h at more environmentally realistic concentrations of 0.05 and 149 µg/L (Cruz-Rojas et al. 2019). Measures of oxidative stress (e.g., superoxide dismutase) were evaluated and found to be elevated in the gill, brain, and muscle. These data highlight the potential for enhanced ecotoxicity of phototransformation of ACE-K based on changes in a sensitive biomarker of exposure. It is important to point out that although the changes in a nonstandard assessment endpoint such as oxidative stress are interesting, survival, growth, and reproduction in standardized ecotoxicity data were unaffected at similar exposure levels. One way to enhance the value of a sensitive biomarkers of exposure like the reported oxidative stress caused by ACE-K in these studies (Ren et al. 2016; Cruz-Rojas et al. 2019) and make it more relevant for environmental risk assessment would be to develop an adverse outcome pathway (AOP) that incorporates these data. Adverse outcome pathways are structured frameworks that link measurable biological changes (key events) from a molecular initiating event (MIE) to a specific adverse outcome (AO) at the individual or population level. Efforts are currently underway to develop the AOP knowledge base and make them more quantitative for use in regulatory decision making (Conolly et al. 2017; Carusi et al. 2018; Perkins et al. 2019).

![Figure 1. Ecotoxicity benchmarks compared to river concentrations calculated by iSTREEM at mean flow, assuming low percent removal (15%) by WWTPs and higher removal (80%) more representative of recent observations. ACE-K = acesulfame-potassium; iSTREEM = in-STREam Exposure Model; NOEC = no observed effect concentration; WWTP = wastewater treatment plant.](image-url)
MODELING OF ACE-K STREAM CONCENTRATIONS

Model descriptions

Two different models were used to predict ACE-K concentrations in rivers and streams throughout the United States:

- The in-STREam Exposure Model (iSTREEM Version 2.2; ACI 2020) to best evaluate geographical patterns of exposure for the United States, and
- The Exposure and Fate Assessment Screening Tool model (E-FAST; USEPA 2014) to obtain some useful statistics about the probability of observing ACE-K at specific levels in US rivers and streams under varying flow conditions.

The iSTREEM model is designed to evaluate down-the-drain products. The iSTREEM model was initially developed by the University of Cincinnati, Ohio, USA and Procter & Gamble, based on USEPA databases and building on algorithms developed by USEPA. The iSTREEM model was acquired by the American Cleaning Institute (ACI, formerly the Soap and Detergent Association) in 2008. The ACI makes the modeling system freely available to the public, and they have sponsored further development and updating (Kapo et al. 2016). Version 2.2 incorporates the most current information available on wastewater treatment facilities, and updates to the national river network to allow development of the most up-to-date national-scale surface-water exposure estimates.

Wastewater treatment facility information in iSTREEM 2.2 was updated using the 2012 Clean Watershed Needs Survey from USEPA (USEPA 2016), increasing the number of facilities in the model. All WWTPs treating municipal wastewater and discharging to rivers or streams were included, with information describing specific location, treatment processes, wastewater flow, and population served. The iSTREEM model now uses an underlying river network with improved spatial resolution derived from the National Hydrography Dataset Plus (NHDPlus) version 2, jointly developed by the US Geological Survey and USEPA (McKay et al. 2012). The iSTREEM model predicts concentrations in 228,000 river segments that represent more than 243,000 river miles resulting from discharges from 13,245 WWTPs across the continental United States. The stream system examined in the model includes all perennial streams and rivers with WWTPs either on the segment or upstream. The database contains mean annual stream flow, an estimate of the lowest weekly flow expected to occur only once in a 10-year period (“7Q10” low flow), and an estimate of the time of travel and velocity for the full length of each reach. The model predicts the concentration of a chemical in each segment, accumulating inputs of water and chemical in effluents of WWTPs and from all upstream reaches. Cumulative distributions of a chemical are produced for river segments, WWTP effluents, and at the intake of municipal drinking-water treatment facilities as a result of upstream WWTP discharges. Removal by WWTPs is accounted for by the model, and other instream removal processes are represented by a simple first-order decay model. The decay constant can be used to represent the net effect of biodegradation and any other significant depletion processes. Key assumptions are described here; for detailed discussion of the model’s background, algorithms, and testing see Kapo et al. (2016) and ACI (2019).

Model assumptions

The following are the key conservative scientific assumptions made for running the iSTREEM model for ACE-K:

- Acesulfame-potassium in the stream and river system is assumed to decline at a minimal rate of 5% per day. Adsorption is not specifically represented but is not a dominant process for ACE-K.
- Acesulfame-potassium use rate reported for 2019 was 999 metric tons per year for the United States, and this includes all known uses of ACE-K: food, drink, and nonfood. The total market volume was obtained as a net figure considering sales, imports, and exports from the Euromonitor International Passport Database. Euromonitor Passport is a proprietary database, but anyone who has subscription access to the database can do their own searches and can generate the same data. We have made available the comprehensive results of our search as a data table for the ACE-K total volume of food/nonfood/other application available and it can be accessed in the Supplemental Data (Table S11). The total US ACE-K loading was applied to an estimated total population of 330 million (rounded to two significant figures) people (US Census 2020), and divided by 365 d/y. The resulting per capita daily load equals 0.00829 g/(person · day) = 8.29 mg/(person · day).
- Dietary ACE-K is assumed to pass completely unchanged to wastewater.
- The calculated per capita load is assumed to be disposed 100% to surface waters. In reality some will be disposed elsewhere (e.g., to the earth via septic systems, or to solid waste facilities).
- Current removal by treatment plants is assumed to be 80%, except no removal by primary plants was assumed. A second case was also examined with removal of 15%, more realistic for earlier years (when biodegradation of ACE-K was not commonly reported) or poorly functioning treatment plants.
- Calculated percentiles of river segments were plotted versus concentrations, along with multiple ACE-K aquatic toxicity benchmarks (Supplemental Data Table S8). Note that most of these benchmarks represent concentrations for which effects were not observed in various toxicity tests. The most conservatively predicted aquatic species PNEC for ACE-K is 2.2 mg/L (derived as the 30-d zebrafish ELS NOEC divided by 10 as per the EC Technical Guidance Document [EC 2003]).
Results of the iSTREEM model are shown in Figure 1. The green curve on the left side of the figure represents the percent of river segments that exceed a given concentration of ACE-K for 80% removal by treatment plants, and the blue curve represents the percentiles if only 15% is removed. Comparison with several ecotoxicity benchmarks (indicated on the right side of the figure) shows that expected concentrations (with 80% removal) are more than 4 orders of magnitude lower than the lowest NOEC. If removal were only 15%, then the concentrations would still be more than 3.5 orders of magnitude below the lowest NOEC. Note that because of ACE-K’s low toxicity almost all of these benchmarks indicate lack of toxicity; the lowest concentrations that show clear toxicity in a standard ecotoxicity test are indicated by the orange line, showing acute mortality percentiles for zebrafish. The figure shows that river concentrations calculated by iSTREEM in all cases are more than 5 orders of magnitude lower than the observed toxicity.

The E-FAST model was then applied using essentially the same loading assumptions to investigate the effect of changing flows over time. The E-FAST model was developed for and made available by USEPA, Office of Pollution Prevention and Treatment, Exposure Assessment Branch (USEPA 2014). Similar to iSTREEM, E-FAST uses data developed by USEPA on locations, population served, and flows from publicly owned treatment works (POTWs) and river and stream segments across the United States. The E-FAST model contains a down-the-drain module that was used to track ACE-K loadings downstream of POTWs across the United States. Unlike iSTREEM, E-FAST does not account for any degradation or adsorption occurring in the river systems. The consumer exposure portions of E-FAST have been peer reviewed by experts outside USEPA, and USEPA has developed E-FAST 2014 using the external peer-review comments for the general population, down-the-drain, environmental exposure aspects of E-FAST. Figure 1 shows how E-FAST predictions for 2 lower flow conditions compare with the iSTREEM predictions for median river concentration at mean flow. The E-FAST values are for harmonic mean flow, and the lowest weekly flow expected in a 10-y period (7Q10 flow). The harmonic mean weights the prediction toward the lowest flows, thus toward the highest concentrations. The 7Q10 flow is quite extreme in that it is a rare event. Table 2 presents percentiles of river concentrations predicted by iSTREEM, and also the 50th percentile and 10th percentile predicted by E-FAST for the comparatively low flow conditions. Note that the lower 10th percentile of river flows and corresponding upper 10% of concentrations given by E-FAST can be considered a nearly upper-bound case because, at this and lower flows, the

### Table 2. Probabilistic ACE-K stream exposure modeling values and estimated margins of safety

| Model and flow         | Percent reaches exceeding | Percent below | 15% removal (µg/L) | 80% removal (µg/L) | 15% removal MOS vs NOEC | 80% removal MOS vs NOEC |
|------------------------|----------------------------|---------------|-------------------|-------------------|-------------------------|-------------------------|
| iSTREEM mean flow      | 90                         | 10            | 0.005             | 0.001             | 4.6E+06                 | 1.98E+07                |
|                        | 75                         | 25            | 0.017             | 0.004             | 1.33E+06               | 5.63E+06                |
|                        | 50                         | 50            | 0.056             | 0.013             | 3.9E+05                | 1.68E+06                |
|                        | 25                         | 75            | 0.175             | 0.041             | 1.25E+05               | 5.33E+05                |
|                        | 10                         | 90            | 0.609             | 0.143             | 3.61E+04               | 1.54E+05                |
|                        | 5                          | 95            | 1.307             | 0.307             | 1.68E+04               | 7.15E+04                |
| iSTREEM low flow       | 90                         | 10            | 0.035             | 0.008             | 6.37E+05               | 2.71E+06                |
|                        | 75                         | 25            | 0.171             | 0.040             | 1.28E+05               | 5.46E+05                |
|                        | 50                         | 50            | 0.757             | 0.178             | 2.91E+04               | 1.24E+05                |
|                        | 25                         | 75            | 2.872             | 0.676             | 7.66E+03               | 3.26E+04                |
|                        | 10                         | 90            | 8.250             | 1.941             | 2.67E+03               | 1.13E+04                |
|                        | 5                          | 95            | 13.132            | 3.090             | 1.68E+03               | 7.12E+03                |
| E-FAST harmonic mean flow | 50                        | 50            | 0.150             | 0.036             | 1.47E+05               | 6.11E+05                |
|                        | 10                         | 90            | 2.590             | 0.610             | 8.49E+03               | 3.61E+04                |
| E-FAST 10-y low flow   | 50                         | 50            | 0.850             | 0.200             | 2.59E+04               | 1.10E+05                |
|                        | 10                         | 90            | 20.620            | 4.850             | 1.07E+03               | 4.54E+03                |

ACE-K = acesulfame-potassium; E-FAST = Exposure and Fate Assessment Screening Tool model; iSTREEM = in-STREam Exposure Model; MOS = margin of safety; NOEC = no observed effect concentration.
dilution factor for effluent mixing with river water is estimated to be 1.0 by the E-FAST program, representing no significant dilution.

Predicted ACE-K concentrations in rivers for each percentile examined for each case are compared in Table 2 as a ratio (the margin of safety [MOS]) to the lowest NOEC of 22 mg/L. The MOSs show that there is greater than a factor of 1000 between the predicted concentrations and possible toxic effects even in the most extreme cases. Note here also that the PNEC is based on observations of no effect; all of the predicted concentrations, even the most extreme, are greater than 105x lower than clearly observed toxic effects (fish mortality).

Regional comparison

The detailed exposure assessment for the United States forms the primary basis for the risk assessment of ACE-K because 1) it is the country with the largest total use of ACE-K, and close to the highest use among regions in terms of mass per capita per day; and 2) detailed data are available on water use and disposal, treatment plant types and locations, river system characteristics (especially flows), and geography; and 3) software tools have been developed and tested that are appropriate for estimating exposure to chemicals that occur in consumer products disposed down the drain. Other regions were compared to ascertain if the conclusions of the risk assessment (considering modeling and available monitoring) for the United States are expected to be applicable to other regions where ACE-K is used.

Acesulfame-potassium is not used in all countries, so for purposes of this comparison only countries with known ACE-K consumption are considered. It should be noted that this is based on actual total ACE-K use, considering sales, imports, and exports for all uses (food, drink, and nonfood). These countries were then sorted into regions, and the weighted average consumption was calculated for each region in terms of grams per capita per day. A second major factor controlling the concentrations of ACE-K that can be expected in the environment is water use. Water use by consumers and disposed via collection systems is most relevant for this purpose, and it was based on statistics for Municipal Water Withdrawal in billions of cubic meters per year, listed in the Food and Agriculture Organization of the United Nations AQUASTAT Database (FAO 2016). This source was used as a basis for comparison, although not all aspects of use and disposal are represented. This measure is available for all of the relevant countries, whereas several other measures describing more specific aspects were available only for some countries. The water use was then divided by population (Worldometers 2019; based on UN statistics) to give the average in liters per person per day.

An index representing the relative potential for ACE-K exposure for regions in comparison with North America (NA) was then calculated as

\[
\text{ACE-K Exposure Index (country i)} = \frac{\text{ACE-K use (country i) g/person/d}}{\text{Water use (country i) L/person/d}} \times \frac{\text{Water use (NA) L/person/d}}{\text{ACE-K use (NA) g/person/d}}
\]

Table 3 presents a summary of these regional ACE-K exposure indices relative to North America (refer to Supplemental Data Table S11 ACE-K global metric tonnage data for all food and nonfood applications extracted from the Euromonitor Passport database for details on which countries with actual ACE-K market sales are included in each region). The significance of these calculated regional ACE-K exposure indexes is that most are very close to the estimated North American exposure framework, with no countries exceeding more than 1.39x that of North America. This suggests that the ACE-K aquatic risk assessment presented in the present paper can be reasonably extrapolated worldwide.

### Table 3. Regional ACE-K exposure index representing relative potential for ACE-K exposure for regions in comparison with North America

| Region              | ACE-K metric tons | 10⁹ m³/y Sum of municipal water | ACE-K countries | Average g/person/d | Average L/person/d | Exposure index Relative to NA |
|---------------------|-------------------|--------------------------------|-----------------|--------------------|-------------------|-----------------------------|
| Asia Pacific        | 1646.9            | 146.75                         | 2394055354      | 0.00188            | 167.9             | 0.64                        |
| Australasia         | 106.9             | 4.723                          | 30322117        | 0.00966            | 426.7             | 1.30                        |
| Eastern Europe      | 393.8             | 19.937                         | 203018764       | 0.00531            | 269.0             | 1.13                        |
| Latin America       | 1013.6            | 41.998                         | 507301944       | 0.00547            | 226.8             | 1.39                        |
| Middle East and Africa | 348.1          | 25.595                         | 510971118       | 0.00187            | 137.2             | 0.78                        |
| North America       | 1104.6            | 63.411                         | 368744805       | 0.00821            | 471.1             | 1.00                        |
| Western Europe      | 99.9              | 5.839                          | 84339067        | 0.00325            | 189.7             | 0.98                        |
| Grand total         | 4713.8            | 308.253                        | 4098753169      | 0.00315            | 206.0             | 0.88                        |

ACE-K = acesulfame-potassium; NA = North America.
RISK CHARACTERIZATION

Risk estimation

Studies reporting widespread ACE-K occurrence in various aquatic matrices (Supplemental Data Tables S3–S7) suggest that a thorough environmental assessment accounting of various recent environmental information is needed, including the identification of any potential data gaps. In the case of ACE-K, the environmental risk was estimated on the basis of 2 separate methods. The first was a basic scientific deterministic approach of comparing the PEC and a PNEC. The PEC in this ACE-K environmental risk assessment was based conservatively on the weighted average concentrations of ACE-K reported in WWTP effluents. The dilution that occurs in surface waters where aquatic organisms live therefore provided an additional safety factor. The species sensitivity to ACE-K was expressed as a PNEC based on the most conservative chronic ACE-K aquatic toxicity reported. This generalized approach has been described in the Technical Guidance Document on Risk Assessment in support of European Commission regulations (EC 2003) and has become widely accepted by ecotoxicologists worldwide. The ACE-K PEC and PNEC were then used in a simplistic risk quotient approach. If the PEC-to-PNEC ratio is lower than 1, the substance is generally not considered to be of concern; if the PEC-to-PNEC ratio is higher than 1, further testing must be carried out to refine more accurately the determination of PEC or PNEC with a consequent adjustment of the PEC-to-PNEC ratio, or risk reduction measures need to be considered (EC 2003).

The weighted average concentration of ACE-K reported in effluents was 29.9 µg/L (Table 1), was considered a conservative PEC. Additionally, the weighted average ACE-K concentration reported in surface water was an order of magnitude lower at 2.9 µg/L with a maximum measured value of 53.7 µg/L, which could be considered an extreme worst case. No acute ecotoxicological effects were reported for ACE-K at concentrations ≥1000 ppm. In chronic ecotoxicological studies, no ecotoxicity was observed with the lowest NOEC of 22 mg/L reported for the fish ELS study (ECHA 2018). Given the robust ecotoxicological data set available for ACE-K (acute and chronic toxicity in fish and invertebrates, fish embryo toxicity, toxicity to freshwater plants, and toxicity to domestic sludge microorganisms), a conservative safety factor of 10x (i.e., based on 3 long-term NOECs from 3 trophic levels, fish daphnia, and algae) was appropriate leading to a PNEC of 2.2 mg/L. The resultant hazard for ACE-K (i.e., PEC-to-PNEC ratio) is 0.0299 mg/L/2.2 mg/L = 0.014. A worst-case PEC scenario utilizing the highest ACE-K concentration reported in surface water yields a PEC-to-PNEC ratio of 0.0537 mg/L/2.2 mg/L = 0.024. Thus, using a basic deterministic PEC-to-PNEC ratio approach for ACE-K indicates that ACE-K presents a negligible risk to the environment. Employing the more sophisticated probabilistic risk assessment approach based on US river PECs predicted using 2019 ACE-K total loading data and the iSTREEM and E-FAST models, the MOSs based on an NOEC of 22 mg/L ranged from more than 1000 to 4.7E+6 for the 15% removal scenario and even higher for the 80% removal scenario.

Uncertainty analysis

Uncertainty exists in any environmental risk assessment whenever environmental risk managers are asked to make decisions on the basis of incomplete or limited data. Uncertainty is often conservatively addressed by the use of safety or uncertainty factors applied to PNECs or the use of worst-case measured exposure scenarios for PECs, rather than using probabilistic exposure modeling based on a full data set of PECs and total loading to the environment.

In the case of ACE-K’s effects characterization, due to the extensive nature of the effects testing reported (Supplemental Data Table S8), uncertainties are not great for the ecotoxicity data set. Margins of safety are considered to be only minimal estimates when they are based on ecotoxicity studies in which there was no effect at the highest concentration tested (e.g., NOECs listed as greater than a given value). In these situations, the true MOSs may be greater, and perhaps much greater, than the minimal estimate.

Uncertainties related to the PEC for ACE-K are intimately linked to the estimated and measured amounts of ACE-K entering wastewater, removal efficiencies in WWTPs, and its eventual degradation. Regarding the reported measured ACE-K concentrations in various environmental matrices, there is often a bias (unrecognized or stated) that researchers will focus their sampling efforts on “hot spots” anticipated to be potential problem areas, in an effort to maximize the chances of finding the analyte of interest. In our review of the ACE-K environmental monitoring literature, we noted multiple references to sampling locations that were selected adjacent to high population catchment basins, WWTPs receiving high population loadings, and sites located directly downstream from WWTP effluents. With regard to the uncertainties associated with the iSTREEM and E-FAST models, very low flow conditions (both flow and contaminant concentrations) are inherently difficult to measure, evaluate statistically, and predict (mainly because they are rare, and very few streams are continuously monitored). The response to this by the developers of these models is to take a conservative approach, especially where uncertainty is greatest. For a more detailed discussion on the uncertainties associated with both iSTREEM and E-FAST models and how these models attempt to compensate for them, please refer to the information provided in the Supplemental Data of the present paper.

Although the use of ACE-K as an artificial sweetener is expected to continue to grow globally (Sylvestsky and Rother 2016), there are numerous processes (i.e., physical, chemical, and most recently biological) that have been reported to effectively degrade ACE-K with increasing elimination efficiencies. Now that microorganisms and conditions capable of biodegrading ACE-K have been identified, it remains to be clarified whether environmental concentrations may in fact begin to decrease (as has been
documented in Germany by Kahl et al. 2018), potentially offsetting the continuing input of this artificial sweetener. Future studies are needed to assess the ultimate impact of the spreading biodegradation capabilities of WWTP microorganisms on future ACE-K environmental concentration. If the efficient biodegradation of ACE-K by WWTPs continues to expand around the world as appears to be the developing case, will this transformation mechanism become the predominant route for eliminating ACE-K in the environment and reducing its persistence? If ACE-K biodegradation spreads to other regions and perhaps other environmental compartments, will the importance of secondary processes such as photolysis and oxidation reactions be reduced? Nevertheless, the present environmental risk assessment used a conservative estimate for the ACE-K PEC value based on weighted average concentration reported in effluents (and even an upper-bound maximum concentration reported in surface water) under both low and higher removal scenarios. In addition, application of the more sophisticated probabilistic exposure assessment using an accurate 2019 total ACE-K loading to the environment resulted in lower PEC-to-PNEC ratios and correspondingly higher MOSs.

A final uncertainty associated with the present ACE-K environmental risk assessment is related to the uncertainty surrounding the relevance of enhanced ecotoxicity that has been reported for various ACE-K TPs (Sang et al. 2014; Li et al. 2016; Ren et al. 2016; Yin et al. 2017). Sang et al. (2014) and Yin et al. (2017) used Microtox to study the toxicity of ACE-K photodegradates and oxidation degradates, respectively. Li et al. (2016) investigated the photocatalytic transformation of ACE-K and the embryotoxicity of the TPs to zebrafish while Ren et al. (2016) studied oxidative stress markers in carp and found elevated levels following UV irradiation of ACE-K. In contrast to these studies, a recent investigation used brine shrimp (Artemia salina) to examine the potential toxicity of ACE-K TPs following photocatalysis (TiO₂/UV-A) for 60 min (Zelinski et al. 2018). The EC50 values for both the parent ACE-K and its TPs was >1000 mg/L, and these authors concluded that the formation of toxic ACE-K TPs following photocatalysis probably does not occur. Clearly the question surrounding potentially toxic TPs formed from various ACE-K transformation processes remains unresolved, as does the potential to encounter these TPs under realistic WWTP operating conditions given the cost effectiveness of their implementation at full scale. Nevertheless, supplementary research may be needed to confirm or refute the ecotoxicity of putative ACE-K TPs using standard aquatic toxicity test species. Quantifiable data on reaction kinetics, realistic environmental concentrations that can be generated under viable WWTP operational conditions, and dose–response information for key events capable of feeding into a quantitative AOP are some of the lines of evidence that will help define whether there is a potential for an AO (at the individual or population level) and make this area more relevant to evaluating the environmental risk of ACE-K.

SUMMARY AND CONCLUSIONS

In summary, environmental fate and ecological effects data necessary to conduct an aquatic risk assessment for ACE-K have heretofore been inadequate. The present paper presents significant new data covering ACE-K’s ecotoxicity and environmental fate parameters which have not previously been published. These new data have been put into context with the existing ACE-K environmental data set to enhance its aquatic risk assessment. Based on extensive ACE-K environmental monitoring, conservative PEC and PNEC estimates, and circumspect probabilistic exposure modeling, safety margins indicate that ACE-K presents a low risk to the aquatic environment. Acesulfame-potassium does not bioaccumulate, and concentrations in the environment are predicted to be well below any toxic effect in a variety of representative aquatic species.

A couple of data gaps exist, which if addressed might help refine our understanding of the potential risk ACE-K poses to the environment, if any. The first relates to a couple of different photolysis studies that have been conducted under varying environmental conditions and methods. Some of these studies indicate that ACE-K can be phototransformed into potentially more toxic TPs while at least one recent study indicates that toxic TPs are not formed after photolysis. It is unknown what percentage of the metabolites may be formed relative to the parent. Much of the existing published studies on ACE-K photolysis were conducted using nonguideline methods, and many study details are lacking. Although these data do point to ACE-K being at least partially degraded by light, a standard guideline study (e.g., OPPTS 835.5270, USEPA 1998) investigating indirect and direct photolysis is needed. Identification and quantification of metabolites relative to parent would help determine the need for further studies. For instance, if hydroxylated ACE-K is one of the major phototransformation products, it might be assumed that its ecotoxicological profile is similar to that of ACE-K, but confirmation of the potential toxicity of any TPs formed using classical ecotoxicity surrogate test species is warranted. The second gap is the biotransformation of ACE-K in WWTP activated sludge. As identified in the present paper, conflicting data are available with respect to sludge biotransformation. If recent reports of successful biodegradation of ACE-K by evolving WWTP microorganisms is true, it would be ideal to confirm this using a standard guideline study (e.g., OECD 314B) focused on the potential for mineralization and biodegradation (OECD 2008). A key factor in designing such a study would be to attempt to obtain appropriate microbial strains capable of degrading ACE-K from WWTPs with demonstrated success of efficient removal rates. Regardless, the low environmental concentrations of ACE-K (ppb to ppt) indicate that even lower concentrations of breakdown products are of limited concern.

In conclusion, the available data on ACE-K indicate that it is safe for use and compatible with the aquatic environment at current usage levels. Although ACE-K usage is expected
SUPPLEMENTAL DATA

Figure S1. e-fate parameters for ACE-K.
Table S1. ACE-K removal by WWTPs
Table S2. Representative ACE-K tertiary treatment option
Table S3. Mean ACE-K concentrations in influents
Table S4. Mean ACE-K concentrations in effluents
Table S5. Mean ACE-K concentrations in sludge
Table S6. Mean ACE-K concentrations in surface water
Table S7. Mean ACE-K concentrations in groundwater
Table S8. Summary of ACE-K ecotoxicity test
Table S9. Predicted ACE-K US river concentrations from iSTREEM and E-FAST
Table S10. Comparison of selected iSTREEM model concentrations and corresponding USGS-NWIS monitoring data for DEET from Kapo et al. (2016)
Table S11. Active Excel spreadsheet containing the total 2019 market sales data for ACE-K as extracted from the Euromonitor International Passport database.

REFERENCES

[ACI] American Cleaning Institute. 2019. iSTREEM™ descriptions, training materials, and references. Washington (DC). [accessed 2020 Jan 21]. https://www.cleaninginstitute.org/industry-priorities/science/istreem
[ACI] American Cleaning Institute. 2020. iSTREEM™ version 2.2. Washington (DC). [accessed 2020 Jan 21]. http://www.istreem.org
Arbeláez P, Borrull F, Marcé RM, Pocurull E. 2015. Trace-level determination of sweeteners in sewage sludge using selective pressurized liquid extraction and liquid chromatography-tandem mass spectrometry. J Chromatogr A 1408:15–21.
Berset J-D, Ochsenbein N. 2012. Stability considerations of aspartame in the direct analysis of artificial sweeteners in water samples using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Chemosphere 88:563–569.
Buerge IU, Buser HR, Kahle M, Müller MD, Poiger T. 2009. Ubiquitous occurrence of the artificial sweetener acesulfame in the aquatic environment: An ideal chemical marker of domestic wastewater in groundwater. Environ Sci Technol 43:4381–4385.
Buerge IU, Keller M, Buser HR, Müller MD, Poiger T. 2011. Saccharin and other artificial sweeteners in soils: Estimated inputs from agriculture and households, degradation and leaching to groundwater. Environ Sci Technol 45:615–621.
Cardenas MAR, Ali I, Lai FY, Dawes L, Thier R, Rajapakse J. 2016. Removal of micropollutants through a biological wastewater treatment plant in a subtropical climate, Queensland-Australia. J Environ Health Sci Eng 14:14. https://doi.org/10.1186/s40201-016-0257-8
Carusi A, Davies MR, De Grandis G, Escher BI, Hodges G, Leung KMY, Whelan M, Willett C, Ankley GT. 2018. Harvesting the promise of AOPs: An assessment and recommendations. Sci Total Environ 628–629:1542–1556.
Castronovo S, Wick A, Scheurer M, Nodler K, Schultz M. 2017. Bio-degradation of the artificial sweetener acesulfame in biological wastewater treatment and sandfilters. Water Res 110:342–353.
Chattopadhyay S, Raychaudhuri U, Chakraborty R. 2014. Artificial sweeteners—A review. J Food Sci Technol 51:611–621. https://doi.org/10.1007/s13197-011-0571-1
Conolly RB, Ankley GT, Cheng WY, Mayo ML, Miller DH, Perkins EJ, Ville-neuve DL, Watanabe KH. 2017. Quantitative adverse outcome pathways and their application to predictive toxicology. Environ Sci Technol 51: 4661–4672.
Cruz-Rojas C, SanJaun-Reyes N, Fuentes-Benites MPAG, Dublan-García O, Galar-Martinez M, Islas-Flores H, Gómez-Oliván LM. 2019. Acesulfame potassium: Its ecotoxicity measured through oxidative stress biomarkers in common carp (Cyprinus carpio). Sci Total Environ 647:772–784.
Dong H, Zeng G, Tang L, Fan C, Zhang C, He X, He Y. 2015. An overview on limitations of TiO₂-based particles for photocatalytic degradation of organic pollutants and the corresponding countermeasures. Water Res 79:128–146.

Dousiech M, Rosique Conesa I, Benítez-López A, Franco A, Huijbregts M, van Zelm R. 2018. Quantifying variability in removal efficiencies of chemicals in activated sludge wastewater treatment plants—A meta-analytical approach. Environ Sci: Processes Impacts 20:171–182.

[EC] European Commission. 2000. European Commission Scientific Committee for Food, Opinion: Re-evaluation of acesulfame K with reference to the previous SCF opinion of 1991. Brussels (BE). SCF/CS/ADD/EDU/194 final. p 1–8.

[EC] European Commission. 2003. Technical guidance document on risk assessment in support of Commission Directive 93/67/EEC on Risk Assesment for new notified substances Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances Directive 98/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. European Commission Joint Research Centre. Brussels (BE). EUR 20418 EN/2. 337 p.

[ECHA] European Chemicals Agency. 2017. Guidance on information requirements and safety assessments. Chapter R.7c: Endpoint specific guidance Version 3.0. Established under EC Regulation No 1907/2006 of the European Parliament and of the Council concerning Registration, Evaluation, Authorization and Restriction of Chemicals (REACH). Helsinki (FI). 609 p.

[ECHA] European Chemicals Agency. 2018. International Uniform Chemical Information Database (IUCLID). Acesulfame potassium, CAS No. 55589-14-8. EINECS No. 259-715-3. Helsinki (FI). [accessed 2019 Jul 9]. https://echa.europa.eu/registration-dossier/registered-dossier/10775/18

Edwards QA, Kulikov SM, Gamer-O’Neale LD, Mtecafi CD, Sultana T. 2017. Contaminants of emerging concern in surface waters in Barbados, West Indies. Environ Monit Assess 189:636–639. 13 p.

[EFS] European Food Safety Authority Panel on Food Additives and Nutrient Sources added to Food (ANS). 2016. Safety of the proposed extension of use of acesulfame K (E 950) in foods for special medical purposes in young children. EFS A / J 14.4.4:437.

Euromonitor International Passport Database. [accessed 2019 Nov 15]. http://www.euromonitor.com/

Falás P, Wick A, Castronovo S, Habermacher J. 2016. Tracing micropollutant removal in biological wastewater treatment. Water Res 95:240–249.

[FAO] Food and Agriculture Organization of the United Nations. 2016. AQUASTAT Main Database. [accessed 2020 Jan 7]. http://www.fao.org/aquastat/en/databases/

Federico T. 2017. Bioconcentration of selected personal care products in Rudatypes philippinarum (Manila clam) [master’s degree thesis]. Bologna (IT): Univ Bologna, Study Course in Environmental Analysis and Management. https://ams.laurea.unibo.it/id/eprint/13084

Franke C, Studinger G, Berger G, Bohlking S, Bruckmann U, Cohors-Fresenborn D, Johncke U. 1994. The assessment of bioaccumulation. Chemosphere 29:1501–1514.

Fu Y, Wu G, Geng J, Li J, Li S, Ren H. 2019. Kinetics and modeling of artificial sweetener degradation in wastewater by the UV/persulfate process. Water Res 150:12–20.

Gan Z, Sun H, Feng B, Wang R, Zhang Y. 2013. Occurrence of seven artificial sweeteners in the aquatic environment and precipitation of Tianjin, China. Water Res 47:4928–4937.

Huang Y, Deng Y, Zhang T. 2019. Bacterial populations responsible for acesulfame degradation. TransCon2019, 2019 Apr 28–3 May, Ascona, Switzerland. https://www.transcon2019.ch/images/docs/TransCon2019_ Program_Current.pdf

[JECA] Joint FAO/WHO Expert Committee on Food Additives. 1991. Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives (JECA). Geneva (CH). WHO Technical Report Series No 806. 20 p.

Kahl S, Kleinsteuber S, Nivala J, van Affercen M. 2018. Emerging biodegradation of previously persistent acesulfame in biological wastewater treatment. Environ Sci Technol 52:2717–2725.

Kapo KE, DeLeo PC, Vamshi R, Holmes CM, Ferrer D, Dyer SD, Wang X, White-Hull C. 2016. iSTREEM®. An approach for broad-scale in-stream exposure assessment of “down-the-drain” chemicals. Integr Environ Assess Manag 12:782–792. https://doi.org/10.1002/ieam.1793

Kattel E, Trapido M, Dulova N. 2017. Oxidative degradation of emerging micropollutant acesulfame in aqueous matrices by UVA-induced H₂O₂/Fe³⁺ and SO₂⁻/Fe³⁺ processes. Chemosphere 171:528–536.

Kleinsteuber S, Rohwerder T, Lohse U, Seiwert B, Reemtsma T. 2019. Sated by a zero-calorie sweetener: Wastewater bacteria can feed on acesulfame. Front Microbiol 10:2606. https://doi.org/10.3389/fmicb.2019.02606.

Lange FT, Scheurer M, Brauch HJ. 2012. Artificial sweeteners—A recently recognized class of emerging environmental contaminants: A review. Anal Bioanal Chem 403:2503–2518.

Li AJ, Schmitz OJ, Stephan S,Lenzen C, Ying-Yue P, Li K, Li H, Leung S-Y. 2016. Photocatalytic transformation of acesulfame: Transformation products identification and embryotoxicity study. Water Res 89:68–75.

Lipinski G, Hanger LY. 2001. Acesulfame K. In: Nabors L, editor. Alternative sweeteners. 3rd ed. New York (NY): Marcel Dekker. p 13–30.

Loos R, Carvalho R, António DC, Comoro S, Locoro G, Tavazzi S, Paracchini B, Michela Ghiani M, Lettieri T, Blaha L et al. 2013. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water Res 47:6475–6487.

Magnusson BA, Carakostas MC, Moore NH, Poulos SP, Renwick AG. 2016. Biological fate of low-calorie sweeteners. Nutr Rev 74:670–687.

Mckay L, Bondelid T, Dewald T, Johnston J, Moore R, Rea A. 2012. NHDPlus version 2. User guide. p 1–182. https://nhdplusplus.nhdplus.com/NHDPlusV2_home.php

Microtox Corporation. 1992. Microtox manual. Vol I to V. Carlsbad (CA). 715 p.

Mill T. 2000. Photoreactions in surface waters. In: Boehling RS, Mackay D, editors. Handbook of property estimation methods for chemicals. Boca Raton (FL): Lewis. 368 p.

Minella M, Giannakis S, Mazzavillani A, Maurino V, Minero C, Vione D. 2017. Phototransformation of acesulfame K in surface waters; Comparison of two techniques for the measurement of the second-order rate constants of indirect photodegradation, and modelling of photoreaction kinetics. Chemosphere 186:185–192.

Morais SA, Delerue-Matos C, Garbarrell X. 2013. Accounting for the disassociating properties of organic chemicals in LCIA: An uncertainty analysis applied to micropollutants in the assessment of freshwater ecotoxicity. J Hazard Mater 248–249:461–468.

Moschet C, Gütz C, Longrie P, Hollender J, Singer H. 2013. Multi-level approach for the integrated assessment of polar organic micropollutants in an international lake catchment: The example of Lake Constance. Environ Sci Technol 47:7028–7036.

Müller CE, Gerecke AC, Alder AC, Scheringer M, Hungerbühler K. 2011. Identification of perfluorooalkyl acid sources in Swiss surface waters with the help of the artificial sweetener acesulfame. Environ Pollut 159:1419–1426.

[OECD] Organisation for Economic Cooperation and Development. 2004. Test No. 202. Daphnia sp. acute immobilisation test. OECD Guidelines for the Testing of Chemicals, Section 2. Paris (FR): OECD Publishing. https://doi.org/10.1787/9789264069947-en

[OECD] Organisation for Economic Cooperation and Development. 2006. Test No. 221. Lemma sp. growth inhibition test. OECD Guidelines for the Testing of Chemicals, Section 2. Paris (FR): OECD Publishing. https://doi.org/10.1787/9789264016194-en

[OECD] Organisation for Economic Cooperation and Development. 2008. Test No. 314. Simulation tests to assess the biodegradability of chemicals discharged in wastewater. OECD Guidelines for the Testing of Chemicals, Section 3. Paris (FR): OECD Publishing. https://doi.org/10.1787/9789264067493-en

[OECD] Organisation for Economic Cooperation and Development. 2010. Test No. 209. Activated sludge, respiration inhibition test (carbon and ammonium oxidation). OECD Guidelines for the Testing of Chemicals, Section 2. Paris (FR): OECD Publishing. https://doi.org/10.1787/9789264067493-en

[OECD] Organisation for Economic Cooperation and Development. 2012. Test No. 211. Daphnia magna reproduction test. OECD Guidelines for the Testing of Chemicals, Section 2. Paris (FR): OECD Publishing. https://doi.org/10.1787/9789264185203-en

[OECD] Organisation for Economic Cooperation and Development. 2013. Test No. 210. Fish, early-life stage toxicity test. OECD Guidelines for the
Testing of Chemicals, Section 2. Paris (FR): OECD Publishing. https://doi.org/10.1787/9789264023785-en

[OECD] Organisation for Economic Cooperation and Development. 2019. Test No. 203: Fish, acute toxicity test. OECD Guidelines for the Testing of Chemicals, Section 2. Paris (FR): OECD Publishing. https://doi.org/10.1787/9789264059961-en

Ordóñez EY, Quintana JB, Rosario Rodil R, Cela R. 2012. Determination of artificial sweeteners in water samples by solid-phase extraction and liquid chromatography–tandem mass spectrometry. J Chromat A 1256:197–205.

Ordóñez EY, Quintana JB, Rodil R, Cela R. 2013. Determination of artificial sweeteners in sewage sludge samples using pressurized liquid extraction and liquid chromatography tandem mass spectrometry. J Chromatogr A 1320:10–16.

Perkins EJ, Ashauer R, Lyle Burgloon L, Conolly R, Landesmann B, Mackay C, Murphy CA, Pollesch N, Wheeler JR, Zupanic A et al. 2019. Building and applying quantitative adverse outcome pathway models for chemical hazard and risk assessment. Environ Toxicol Chem 38(9):1850–1865. https://doi.org/10.1002/etc.4505

Perkola N, Sainio P. 2014. Quantification of four artificial sweeteners in Finnish surface waters with isotope-dilution mass spectrometry. Environ Pollut 184:391–396.

Perkola N, Vaaliguruma S, Jernberg J, Vähätalo AV. 2016. Degradation of artificial sweeteners via direct and indirect photochemical reactions. Environ Sci Pollut Res Int 23:13288–13297.

Petrovic M, Eljarrat E, Lopez De Alda MJ, Barcelo D. 2004. Endocrine disrupting compounds and other emerging contaminants in the environment: A survey of new monitoring strategies and occurrence data. Anal Bioanal Chem 378:549–562.

Ren Y, Geng J, Li F, Ren H, Ding L, Xu K. 2016. The oxidative stress in the liver of Carassius auratus exposed to ascesulfame and its UV irradiation products. Sci Total Environ 571:755–762.

Renwick AG. 1986. The metabolism of intense sweeteners. Xenobiotica 16:1057–1071.

Ruff M, Mueller MS, Loos M, Singer HP. 2015. Quantitative target and systemic non-target analysis of polar organic micro-pollutants along the river Rhine using high-resolution mass spectrometry—Identification of unknown sources and compounds. Water Res 87:145–154.

Sang Z, Jiang Y, Tsoi Y-K, Leung KS-Y. 2014. Evaluation of the environmental impact of artificial sweeteners: A study of their distributions, photo-degradation and toxicities. Water Res 52:260–274.

Saucedo-Vence K, Elizalde-Velázquez A, Dublan-Garcia O, Galar-Martinez M, Islas-Flores H, Sanjuán-Reyes N, García-Medina S, Hernández-Navarro MD, Gómez-Oliván LM. 2017. Toxicological hazard induced by sucralose to environmentally relevant concentrations in common carp (Cyprinus carpio). Sci Total Environ 575:347–357.

Schaefer LA, Ackerman JM, Rudel RA. 2016. Septic systems as sources of organic wastewater compounds in domestic drinking water wells in a shallow sand and gravel aquifer. Sci Total Environ 547:470–481. https://doi.org/10.1016/j.scitotenv.2015.12.081

Scheurer M, Brauch HJ, Lange FT. 2009. Analysis and occurrence of seven artificial sweeteners in German waste water and surface water and in soil aquifer treatment (SAT). Anal Bioanal Chem 394:1585–1594.

Scheurer M, Godejohann M, Wick A, Happel O, Temes TA, Brauch HJ, Ruck WKl, Lange FT. 2012. Structural elucidation of main ozonation products of the artificial sweeteners cyclamate and ascesulfame. Environ Sci Pollut Res Int 19:1107–1119.

Scheurer M, Schmutz B, Happel O, Brauch H-J, Walser R, Storck FR. 2014. Transformation of the artificial sweetener ascesulfame by UV light. Sci Total Environ 481:425–432.

Scheurer M, Storck FR, Brauch HJ, Lange FT. 2010. Performance of conventional multi-barrier drinking water plants for the removal of four artificial sweeteners. Water Res 44:3573–3584.

Scheurer M, Storck FR, Graf C, Brauch HJ, Ruck W, Lev O, Lange FT. 2011. Correlation of six anthropogenic markers in wastewater, surface water, bank filtrate, and soil aquifer treatment. J Environ Monit 13: 966–973.

Setz W, Winzenbacher R. 2017. A survey of trace organic chemicals in a German water protection area and the proposal of relevant indicators for anthropogenic influences. Environ Monit Assess 189:244. 17 p.

Soh L, Connors KA, Brooks BW, Zimmerman J. 2011. Fate of sucralose through environmental and water treatment processes and impact on plant indicator species. Environ Sci Technol 45:1363–1369.

Stolle S, Steudte S, Scheded NH, Willenberg I, Stepanovski P. 2013. Ecotoxicity of artificial sweeteners and stevioside. Environ Int 60:123–127.

Storck FR, Skark C, Remmler F, Brauch H-J. 2016. Environmental fate and behavior of ascesulfame in laboratory experiments. Water Sci Technol 74:2832–2842.

Subedi B, Kannan K. 2014. Fate of artificial sweeteners in wastewater treatment plants in New York State, USA. Environ Sci Technol 48:13668–13674.

Sylvestry AC, Rother KI. 2016. Trends in the consumption of low-calorie sweeteners. Physiol Behav 164:446–450.

Tollefsen KE, Nizzetto L, Huggett DB. 2012. Presence, fate and effects of the intense sweetener sucralose in the aquatic environment. Sci Total Environ 438:510–518.

Tran NH, Gan J, Nguyen VT, Chen H, You L, Duarah A, Zhang L, Yew-Hoong Gin K. 2015. Sorption and biodegradation of artificial sweeteners in activated sludge processes. Bioreasour Technol 197:329–338.

Tran NH, Nguyen VT, Urase T, Ngo HH. 2014. Role of nitrification in the biodegradation of selected artificial sweetening agents in biological wastewater treatment process. Bioreasour Technol 161:40–46.

[USCB] United States Census Bureau. 2020. Website. [accessed 2020 Mar 16]. https://www.census.gov/popclock/

[USEPA] United States Environmental Protection Agency. 1998. Test guide lines OPPTS 835.5270 indirect photolysis screening test. Washington (DC). EPA 712-C-98-099. p 1–2.

[USEPA] United States Environmental Protection Agency. 2012. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11. Washington (DC).

[USEPA] United States Environmental Protection Agency. 2014. Exposure and fate assessment screening tool (E-FAST) Version 2014, Documentation manual. Washington (DC): Exposure Assessment Branch, Office of Pollution Prevention and Treatment. [accessed 2019 Dec 20]. https://www.epa.gov/tscsa-screening-tools/e-fast-exposure-and-fate-assessment-screening-tool-version-2014

[USEPA] United States Environmental Protection Agency. 2016. Clean watersheds needs survey 2012. Report to Congress. EPA-830-R-15005. 36 p.

[USFDA] United States Food and Drug Administration. 1988. Food additives permitted for direct addition to food for human consumption; ascesulfame potassium. Fed Regist 53(145):28379.

Van Stempvoort DR, Roy JW, Brown SJ, Bickerton G. 2011. Artificial sweeteners as potential tracers in groundwater in urban environments. J Hydrolo 410:126–133.

[WHO] World Health Organization. 1980. Toxicological evaluation of certain food additives. WHO Food Additives Series, No 496. 16.11.

[WHO] World Health Organization. 1983. Evaluation of certain food additives and contaminants. Geneva (CH): Technical Report Series 696. 21 p.

Worldometers. 2019. Elaboration of data by United Nations, Department of Economic and Social Affairs, Population Division. World population prospects: The 2019 revision. [accessed 2020 Jan 21]. www.Worldometers.info

Wu M, Qian Y, Boyd JM, Hurede SE, Le XC, Li X-F. 2014. Direct large volume injection ultra high performance liquid chromatography tandem mass spectrometry determination of artificial sweeteners sucralose and ascesulfame in well water. J Chromatogr A 1359:156–161.

Yang Y-Y, Liu W-R, Liu Y-S, Zhao J-L, Zhang Q-Q, Zhang M, Zhang J-N, Jiang Y-X, Zhang L-J, Ying G-G. 2017. Suitability of pharmaceuticals and personal care products (PPCPs) and artificial sweeteners (ASs) as wastewater indicators in the Pearl River Delta, South China. Sci Total Environ 590:611–619.

Yin K, Li F, Wang Y, He Q, Deng Y, Chen S. 2017. Oxidative transformation of artificial sweetener ascesulfame by permanganate, reaction kinetics, transformation products and pathways, and ecotoxicity. J Hazard Mater 330:52–60.

Zelinski DW, dos Santos TPM, Takashina TA, Leifeld V, Igarashi-Mafra L. 2018. Photocatalytic degradation of emerging contaminants: Artificial sweeteners. Water Air Soil Pollut 229:207. https://doi.org/10.1007/s11270-018-3856-4