Artificial sweeteners

Studies of their environmental fate, drinking water relevance, use as anthropogenic markers, and ozonation products

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Summary

The world wide population growth and the increasing water scarcity endanger more and more the human society. Water saving measures alone will not be sufficient to solve all associated problems. Therefore, people in arid countries might come back to any kind of water available. In this context the way people regard wastewater must change in terms that it has to be recognized as a water resource. The reuse of wastewater, treated and untreated, for irrigation purposes in agriculture is already established in some semi-arid and arid countries. Countries with absolute water scarcity like Israel might not only be forced to reduce their water consumption, but even to transfer reused water to other sectors. Concerns of authorities and the general public about potential health risks are completely understandable. The health risks of wastewater are mainly originating from pathogens which are negatively correlated with its treatment. Therefore, the quality of a wastewater effluent derived from mechanical-biological treatment can be further improved by additional treatment steps like soil aquifer treatment (SAT). This process is adopted at the Israeli Shafdan facility in the south of Tel Aviv. Conventionally treated wastewater is applied on surface basins from where it percolates into the coastal plain aquifer which supplies approximately one quarter of Israel´s drinking water. After a certain residence time in the subsurface the water is recovered by wells surrounding the recharge area. Although the pumping regime creates a hydraulic barrier to the pristine groundwater, concerns exist that a contamination of the surrounding drinking water wells could occur. So far, little is known about the removal of organic trace pollutants during the SAT process in general and for the Shafdan site in particular.

Consequently, the need arose to study the purification power of the SAT process in terms of the removal of organic trace pollutants. For this purpose reliable wastewater tracers are essential to be able to differentiate between degradation and sorption processes on the one hand and dilution with pristine groundwater on the other hand. Based on their chemical properties, their worldwide usage in a variety of foodstuffs and beverages, and first data about the fate and occurrence of sucralose, artificial sweeteners came into the focus as promising tracer candidates.

Thus, in the present work an analytical method for the simultaneous determination of seven commonly used artificial sweeteners in different water matrices, like surface water and wastewater, was developed (see chapter 2). The method is based on the solid phase extraction (SPE) of the analytes by a styrene-divinylbenzene (SDB) copolymer material, and the analysis by liquid chromatography-electrospray ionization tandem mass-spectrometry (LC-ESI-MS/MS). The sensitivity in negative ionization mode was considerably enhanced by post-

column addition of the alkaline modifier tris(hydroxymethyl)aminomethane. In potable water, except for aspartame and neohesperidine dihydrochalchone, absolute recoveries >75 % were obtained for all analytes under investigation, but were considerably reduced due to matrix effects in treated wastewater. The widespread distribution of the artificial sweeteners acesulfame, saccharin, cyclamate, and sucralose in the aquatic environment was proven. Concentrations in two German wastewater treatment plant (WWTP) influents ranged up to 190 µg/L for cyclamate, several tens of µg/L for acesulfame and saccharin, and about 1 µg/L for sucralose. For saccharin and cyclamate removal rates >90 % during wastewater treatment were observed, whereas acesulfame and sucralose turned out to be very persistent. As a result of high influent concentrations and low removal rates in WWTPs, acesulfame was the dominant sweetener in German surface waters with concentrations up to 2.7 µg/L. The detection of acesulfame and sucralose in recovery wells in the Shafdan SAT site in Israel in the µg/L range was a promising sign for their possible use as anthropogenic markers.

As acesulfame and sucralose showed a pronounced stability in WWTPs and were detected in recovery wells of the SAT site in Israel it became worthwhile to assess their tracer suitability compared to other organic trace pollutants suggested as anthropogenic markers in the past (see chapter 3). Therefore, the prediction power of the two sweeteners was evaluated in comparison with the antiepileptic drug carbamazepine (CBZ), the X-ray contrast medium diatrizoic acid (DTA) and two benzotriazoles (1H-benzotriazole (BTZ) and its 4-methyl analogue (4TTri)). The concentrations of these compounds and their ratios were tracked from WWTPs with different treatment technologies, to recipient waters and further to river bank filtration (RBF) wells. Additionally, acesulfame and sucralose were compared with CBZ during advanced wastewater treatment by SAT in Israel. Only the persistent compounds acesulfame, sucralose, and CBZ showed stable ratios when comparing influent and effluent concentrations of four German WWTPs with conventional wastewater treatment. However, by the additional application of powdered activated carbon in a fifth WWTP CBZ, BTZ, and 4-TTri were selectively removed resulting in a pronounced shift of the concentration ratios towards the nearly unaffected sweeteners. Results of a seven months monitoring program along the rivers Rhine and Main showed an excellent correlation between CBZ and acesulfame concentrations ($r^2 = 0.94$), and still good values when correlating the concentrations with both benzotriazoles ($r^2 = 0.66 - 0.82$). In RBF wells acesulfame and CBZ were again the compounds with the best concentration correlation ($r^2 = 0.85$). A differentiated comparison of the individual sampling points enabled the identification of a CBZ point source at the river Rhine downstream of the city of Basel. A steady increase of the acesulfame/sucralose concentration ratio along the river Rhine was observed and can most likely be ascribed to a higher consumption of sucralose in Switzerland compared to Germany.

In the Israeli SAT field sucralose concentrations decreased by more than 80 % from the percolation basin to peripherical wells. However, the sweetener was still detected there with concentrations up to 3.5 µg/L, enabling the indication of a wastewater impact in a qualitative way. Acesulfame and CBZ showed a pronounced stability even at these longer residence times of more than one year in the subsurface of the SAT field, underlining their suitability as almost ideal anthropogenic markers.

From the tracer study it can be deduced, that concentration ratios of persistent organic trace pollutants should be evaluated carefully before transferring them from one sampling site to another for the prediction of a certain wastewater impact or for the estimation of the occurrence of other trace pollutants. In fact, ratio shifts can also indicate peculiarities like advanced wastewater treatment, enhanced application in the catchment area of WWTPs, country specific usage patterns, and point sources.

The persistence of some artificial sweeteners during wastewater treatment and their occurrence in surface waters, and river bank filtrate raised concerns that some of them might end up in raw waters used for drinking water production. Thus, the most important treatment steps applied in waterworks were investigated in laboratory experiments to assess their efficiency for the removal of artificial sweeteners. Additionally, the effectiveness of different treatment trains was studied in an extended sampling program in six full-scale waterworks which use surface water influenced raw waters (see chapter 4). Saccharin and cyclamate proved to have no relevance for drinking water production as they were effectively removed during biological active treatment steps, most important river bank filtration (RBF) and artificial groundwater recharge. Acesulfame and sucralose were found to be persistent during aerobic RBF which underlined again the possibility to use them as tracers for anthropogenic contaminations. Sucralose was also persistent against chemical treatment with ozone with removal rates of <20 % in both laboratory and field investigations, but the compound was completely removed by subsequent activated carbon filtration. For acesulfame the applied ozone doses and contact times in full-scale waterworks were not sufficient for the complete oxidation of the compound. Activated carbon filtration was only effective if the carbon-preload was below 30 m^3/kg. In this study acesulfame was the only sweetener with drinking water relevance. The compound was detected in about 90 % of the finished potable water samples with concentrations up to 0.76 µg/L.

The high mobility and persistence of acesulfame are beneficial for its use as a tracer compound. However, these properties also lead to its unwanted occurrence and subsequent oxidation in ozonation units of waterworks. To ensure that no undesired oxidation products (OPs) are formed, great importance was attached to the elucidation of the main OPs of acesulfame (see chapter 5). Additionally, cyclamate was included in the study, as it was identified as the sweetener with the highest influent concentrations in WWTPs, where ozonation might be applied as tertiary treatment. Batch experiments at two different pH values (3 and 7.5) with and without scavenging of OH radicals by *tert*-butanol were carried out to enable discrimination between oxidation by the ozone molecule itself or by OH radicals.

In the case of cyclamate, oxidation mainly occurred at the carbon atom, where the sulfonamide moiety is bound to the cyclohexyl ring. Consequently, amidosulfonic acid and cyclohexanone are formed as main OPs of cyclamate. When ozone attacked at a another carbon atom of the ring, a keto moiety was introduced into the cyclamate molecule yielding three isomers.

For acesulfame, acetic acid and dihydroxyacetyl sulfamate, a compound with an aldehyde hydrate moiety, were identified as the main OPs in all tests. The observed reaction products suggested an ozone reaction according to the Criegee mechanism due to the presence of a C=C double bond. Kinetic experiments for acesulfame confirmed that both OPs are formed during ozone contact times applied in waterworks. The aldehyde hydrate was also detected after the ozonation unit of a full-scale drinking water treatment plant which uses surface water influenced bank filtrate as raw water. However, it is not expected to cause any problems for drinking water suppliers, as primary findings suggest its further removal in biologically active layers of subsequent activated carbon filters.

It can be concluded that the ubiquitous occurrence of artificial sweeteners and their identification as emerging contaminants in the aquatic environment have been proven by this study. The main benefit for environmental scientists, public authorities, and waterworks is the use of acesulfame as a new tracer for anthropogenic contaminations with advantages in abundance compared to other persistent organic trace pollutants used in the past. Acesulfame was already successfully applied to prove the purification power of the SAT field in Israel. Stable concentrations in recovery wells with residence times of more than one year were observed, whereas e.g. pharmaceuticals (CBZ excluded) were reduced by more than 90 % compared to the applied wastewater. The multi-barrier concept in drinking water production is reliable (under certain boundary conditions) to remove traces of artificial sweeteners and their OPs from raw waters.

Zusammenfassung

Das weltweite Bevölkerungswachstum und die damit verbundene Wasserknappheit gefährden zunehmend die menschliche Gesellschaft. Lediglich den Wasserverbrauch zu reduzieren wird nicht ausreichen, um alle damit verbundenen Probleme zu lösen. Deshalb wird die Bevölkerung in ariden Ländern möglicherweise in Zukunft auf alle verfügbaren Wasserressourcen zurückgreifen müssen. In diesem Zusammenhang muss sich die Betrachtungsweise von Abwasser dahingehend ändern, dass die Bevölkerung solcher Länder dieses als Ressource erkennt. Die Wiederverwendung von unbehandeltem oder behandeltem Abwasser ist für Bewässerungszwecke bereits in einigen semi-ariden und ariden Ländern etabliert. Allerdings könnten Länder mit extremer Wasserknappheit, wie beispielsweise Israel, in Zukunft nicht nur dazu gezwungen sein ihren Wasserverbrauch zu reduzieren, sondern auch wiedergewonnenes Wasser in anderen Bereichen als der Landwirtschaft zu nutzen. Bedenken von Behörden und Öffentlichkeit bezüglich einer potentiellen Gesundheitsgefährdung sind dabei nur allzu verständlich. Die gesundheitlichen Risiken von Abwasser sind vor allem durch darin enthaltene Pathogene begründet, deren Anwesenheit negativ mit einer zunehmenden Abwasserbehandlung korreliert. Aus diesem Grund kann die Qualität eines Abwassers, das eine biologische Abwasserbehandlung durchlaufen hat, durch weitere Aufbereitungsschritte, wie das Versickern über eine Bodenpassage (engl. soil aquifer treatment, SAT), weiter verbessert werden. Im israelischen Shafdan-Gebiet südlich von Tel Aviv wird zu diesem Zwecke gereinigtes Abwasser auf Becken ausgebracht, von wo es in den Küstenaquifer versickert, der rund ein Viertel des israelischen Trinkwassers liefert. Nach einer gewissen Aufenthaltszeit im Untergrund wird das Wasser durch Förderbrunnen in der Peripherie des Versickerungsfeldes wiedergewonnen. Obwohl durch das Pumpregime eine hydraulische Barriere zum unbeeinflussten Grundwasser geschaffen werden soll, bestehen Bedenken, dass umliegende Trinkwasserbrunnen kontaminiert werden könnten. Insbesondere über die Entfernung von organischen Spurenstoffen während des SAT-Prozesses im Allgemeinen und für das Shafdan-Gebiet im Besonderen ist bislang wenig bekannt.

Für die Beurteilung der Reinigungsleistung des SAT-Prozesses hinsichtlich der Elimination organischer Spurenstoffe war es notwendig, zuverlässige Abwassermarkierungsstoffe (Tracer) zu identifizieren. Nur so war es möglich, zwischen einer Verdünnung mit landseitigem Grundwasser einerseits sowie biologischem Abbau und Sorption andererseits zu unterscheiden. Künstliche Süßstoffe erschienen auf Grund ihrer chemischen Eigenschaften, ihrer weltweiten Anwendung in einer Vielzahl von Lebensmitteln und Getränken sowie erster

Informationen über das Umweltverhalten von Sucralose aussichtsreiche Tracer-Kandidaten zu sein.

Daher wurde in dieser Arbeit eine Analysen-Methode zur simultanen Bestimmung von sieben allgemein gebräuchlichen künstlichen Süßstoffen aus verschiedenen wässrigen Matrizes, wie beispielsweise Oberflächen- und Abwasser, entwickelt (siehe Kapitel 2). Diese Methode basiert auf einer Festphasenextraktion der Analyten mit einem Polystyrol-Divinylbenzol-Copolymer und anschließender Analyse mittels Flüssigkeitschromatographie-Tandem-Massenspektrometrie mit Elektrospray-Ionisation (LC-ESI-MS/MS). Die Empfindlichkeit im negativen Ionisations-Modus konnte durch die Zugabe des basischen Puffers Tris(hydroxymethyl)aminomethan nach der chromatographischen Trennung deutlich verbessert werden. In Trinkwasser wurden für alle Zielsubstanzen, außer für Aspartam und Neohesperidin-Dihydrochalchon, absolute Wiederfindungen von >75 % erzielt. Diese nahmen allerdings in behandeltem Abwasser auf Grund von Matrixeffekten deutlich ab. Es konnte die ubiquitäre Verbreitung der künstlichen Süßstoffe Acesulfam, Saccharin, Cyclamat und Sucralose in der aquatischen Umwelt nachgewiesen werden. Die Zulaufkonzentrationen in zwei deutschen Kläranlagen betrugen für Cyclamat bis zu 190 µg/L, mehrere Zehn µg/L für Acesulfam und Saccharin und ca. 1 µg/L für Sucralose. Für Saccharin und Cyclamat konnte eine gute Elimination (>90 %) während der biologischen Abwasserreinigung beobachtet werden. Acesulfam und Sucralose erwiesen sich dagegen als sehr persistent. Als Folge hoher Zulaufkonzentrationen und geringer Entfernung während der Abwasserreinigung war Acesulfam, mit Konzentrationen von bis zu 2,7 µg/L, der vorherrschende Süßstoff in deutschen Fließgewässern. Der Nachweis von Acesulfam und Sucralose im µg/L-Bereich in Förderbrunnen des SAT-Feldes in Israel war ein vielversprechendes Anzeichen für deren möglichen Einsatz als Abwasser-Tracer.

Da Acesulfam und Sucralose eine ausgeprägte Persistenz während der Abwasserbehandlung aufwiesen und in Förderbrunnen des SAT-Feldes nachgewiesen wurden, erschien es lohnenswert, ihre Eignung als Abwassertracer gegenüber anderen organischen Spurenstoffen zu bewerten, die in der Vergangenheit ebenfalls als Tracer vorgeschlagen wurden (siehe Kapitel 3). Aus diesem Grund wurde die Fähigkeit der beiden Süßstoffe, einen Abwassereinfluss anzuzeigen, mit der des Antiepileptikums Carbamazepin (CBZ), des Röntgenkontrastmittels Amidotrizoesäure (DTA) und mit der zweier Benzotriazole (1H-Benzotriazol (BTZ) und seines 4-methylierten Analogons (4TTri)) verglichen. Ausgehend von Kläranlagen mit unterschiedlichen Behandlungstechniken wurden die Konzentrationen der Spurenstoffe und ihre Verhältnisse untereinander über Vorfluter bis hin zu

Uferfiltratbrunnen verfolgt. Außerdem wurde das Verhalten von Acesulfam, Sucralose und CBZ bei der weitergehenden Abwasserbehandlung mittels SAT in Israel miteinander verglichen.

In den Zu- und Abläufen von vier deutschen Kläranlagen mit konventioneller Abwasserreinigung waren nur die Konzentrationsverhätnisse der persistenten Spurenstoffe Acesulfam, Sucralose und CBZ untereinander konstant. Bei einem zusätzlichen Einsatz von Pulveraktivkohle in einer fünften Kläranlage wurden jedoch CBZ, BTZ und 4-TTri selektiv entfernt, was die Konzentrationsverhältnisse zu den kaum betroffenen Süßstoffen verschob. Die CBZ- und Acesulfam-Messwerte eines siebenmonatigen Untersuchungsprogramms an Rhein und Main korrelierten untereinander hervorragend (r^2 = 0,94) und ebenfalls noch gut mit den Konzentrationen beider Benzotriazole (r^2 = 0,66-0,82). Auch in den untersuchten Uferfiltratbrunnen war die Korrelation zwischen Acesulfam und CBZ am besten. Eine differenzierte Betrachtung der Substanzverhältnisse der einzelnen Probenahmestellen an Rhein und Main ermöglichte die Identifizierung einer Punktquelle für CBZ am Rhein flussabwärts von Basel. Im Rheinverlauf wurde zudem ein ansteigendes Acesulfam/Sucralose-Verhältnis beobachtet, was auf einen höheren Sucralose-Verbrauch in der Schweiz im Vergleich zu Deutschland schließen lässt.

Im israelischen SAT-Feld nahm die Sucralose-Konzentration auf dem Weg von den Versickerungsbecken zu den peripheren Brunnen um mehr als 80 % ab. Allerdings wurde der Süßstoff auch dort noch mit Konzentrationen von bis zu 3,5 µg/L detektiert und ist deshalb immer noch dazu geeignet, auch bei langen Aufenthaltszeiten einen Abwassereinfluss zumindest qualitativ anzuzeigen. Acesulfam und CBZ zeigten selbst bei langen Verweilzeiten im Aquifer von mehr als einem Jahr eine ausgeprägte Stabilität, was ihre Eignung als nahezu ideale Abwassertracer unterstreicht.

Die Ergebnisse dieser Tracer-Studie lassen den Schluss zu, dass Konzentrationsverhältnisse persistenter organischer Spurenstoffe nur nach genauer Prüfung von einer Probenahmestelle auf eine andere übertragen werden sollten, um den Abwassereinfluss oder das Vorkommen anderer Spurenstoffe abzuschätzen. Vielmehr können abweichende Konzentrations-Verhältnisse organischer Spurenstoffe Hinweise auf Unregelmäßigkeiten, wie z. B. eine weitergehende Abwasserreinigung, einen erhöhten Einsatz im Kläranlagen-Einzugsgebiet, international unterschiedliche Einsatzmuster oder Punktquellen geben.

Auf Grund der Persistenz einiger künstlicher Süßstoffe während der Abwasserreinigung sowie wegen ihres Vorkommen in Oberflächengewässern und Uferfiltratbrunnen erschien es wahrscheinlich, dass diese Stoffe auch in Rohwässern zur Trinkwassergewinnung vorhanden sein könnten. Deshalb wurden die wichtigsten in Wasserwerken angewendeten Aufbereitungsschritte im Labormaßstab untersucht, um ihre Eignung hinsichtlich der Entfernung von Süßstoffen beurteilen zu können. Zusätzlich wurde an sechs deutschen Wasserwerken, die Oberflächenwasser beeinflusstes Rohwasser nutzen, die Effektivität der Gesamtheit aller Aufbereitungsstufen in einem umfangreichen Untersuchungsprogramm überprüft (siehe Kapitel 4). Saccharin und Cyclamat erwiesen sich für die Trinkwassergewinnung als nicht relevant, da beide während biologisch aktiver Aufbereitungsschritte, wie der Uferfiltration oder der künstlichen Grundwasseranreicherung, effektiv entfernt wurden. Acesulfam und Sucralose waren dagegen während der aeroben Uferfiltration persistent, was die Möglichkeit, die Substanzen als Markierungsstoffe für eine anthropogene Verunreinigung zu nutzen, wiederholt unterstrich. Sucralose erwies sich auch bei der chemischen Behandlung mit Ozon mit Entfernungsraten <20 % in Labor- und Felduntersuchungen als persistent, wurde aber bei der nachfolgenden Aktivkohlefiltration effektiv aus dem Wasser entfernt. Für Acesulfam waren die in Wasserwerken angewandten Ozonkonzentrationen und -kontaktzeiten für eine vollständige Oxidation des Stoffes nicht ausreichend. Eine Aktivkohlefiltration war nur effektiv, wenn die Kohle eine Beladung von weniger als 30 m^3/kg aufwies. In diesem Untersuchungsprogramm war Acesulfam der einzige Süßstoff mit Trinkwasserelevanz. Die Verbindung wurde in ca. 90 % aller Proben am Ende der Trinkwasseraufbereitung mit Konzentrationen bis zu 0,76 µg/L nachgewiesen.

Die hohe Mobilität und Persistenz von Acesulfam sind nützlich, um diese Substanz als Tracer einzusetzen. Allerdings führen diese Eigenschaften auch zum unerwünschten Vorkommen und der anschließenden Oxidation bei der Ozonung in Wasserwerken. Um sicherzustellen, dass dabei keine unerwünschten Oxidationsprodukte entstehen, wurde ein weiterer Schwerpunkt der Arbeit auf die Aufklärung der Hauptozonungsprodukte von Acesulfam gelegt (siehe Kapitel 5). Zusätzlich wurde der Süßstoff Cyclamat in diese Untersuchung mit aufgenommen, da er mit den höchsten Konzentrationen in Kläranlagen nachgewiesen wurde und dort ebenfalls eine Ozonung während der weitergehenden Abwasserbehandlung stattfinden kann. Laborversuche zur Strukturaufklärung wurden bei zwei verschiedenen pH-Werten (3 und 7,5) mit und ohne den Radikalfänger *tert*-Butanol durchgeführt, um zwischen der Reaktion mit Ozon und der mit OH-Radikalen unterscheiden zu können.

Im Falle von Cyclamat erfolgte die Oxidation hauptsächlich an dem Kohlenstoffatom, an dem die Sulfonamid-Gruppe mit dem Cyclohexyl-Ring verbunden ist. Dies führte zu Amidosulfonsäure und Cyclohexanon als Hauptozonungsprodukte von Cyclamat. Beim Angriff von

Ozon an einem anderen Kohlenstoffatom des Cyclohexyl-Rings wurde eine Keto-Gruppe in das Cyclamat-Molekül eingeführt, was die Bildung von drei Strukturisomeren zur Folge hatte.

Für Acesulfam wurden Essigsäure und Dihydroxyacetylsulfamat, eine Substanz mit einer Aldehydhydrat-Gruppe, in allen Versuchen als Hauptozonungsprodukte identifiziert. Die beobachteten Reaktionsprodukte lassen auf eine Reaktion an der C=C Doppelbindung gemäß dem Criegee-Mechanismus schließen. Kinetik-Experimente für Acesulfam bestätigten, dass die Ozonungsprodukte direkt und nach kurzen Ozon-Kontaktzeiten, wie sie auch in Wasserwerken angewendet werden, entstehen. Das Aldehydhydrat wurde auch in einem Wasserwerk nach der Ozonung von Oberflächenwasser beeinflusstem Uferfiltrat detektiert. Es ist allerdings nicht davon auszugehen, dass dies ein Problem für Wasserversorger darstellt, da erste Untersuchungen darauf hindeuten, dass dieses Oxidationsprodukt in biologisch aktiven Schichten der nachfolgenden Aktivkohlefiltration entfernt wird.

Schlussfolgernd lässt sich sagen, dass mit der vorliegenden Arbeit die ubiquitäre Verbreitung künstlicher Süßstoffe in der aquatischen Umwelt nachgewiesen wurde. Der hauptsächliche Nutzen für Umweltwissenschaftler, Behörden und Wasserwerke liegt darin, dass Acesulfam als neuer Tracer für abwasserbürtige, anthropogene Verunreinigungen identifiziert werden konnte. Die Substanz bietet dabei hinsichtlich des Konzentrationsniveaus Vorteile gegenüber anderen persistenten Spurenstoffen. Acesulfam wurde bereits erfolgreich zum Nachweis der Reinigungsleistung eines SAT-Feldes eingesetzt. Selbst nach Aufenthaltszeiten im Aquifer von mehr als einem Jahr wurde Acesulfam dort noch in Förderbrunnen in vergleichbaren Konzentrationen, wie sie auch im versickerten Abwasser vorliegen, nachgewiesen. Dagegen nahm beispielsweise die Gesamtkonzentration der pharmazeutischen Wirkstoffe (ohne CBZ) um mehr als 90 % ab. Das in der Trinkwasseraufbereitung angewandte Multibarrierenprinzip ist (unter bestimmten Rahmenbedingungen) geeignet, um Spuren von künstlichen Süßstoffen und ihrer Oxidationsprodukte aus Rohwässern zu entfernen.

List of publications

The cumulative Ph.D. thesis “Artificial sweeteners - Studies of their environmental fate, drinking water relevance, use as anthropogenic markers, and ozonation products” is based on the following scientific publications:

Scheurer, M. Brauch, H.-J., Lange, F.T. (2009) Analysis and occurrence of seven artificial sweeteners in German waste water and in soil aquifer treatment (SAT), Anal Bioanal Chem 394(6), 1585-1594.

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Scheurer, M., Storck, F.R., Graf, C., Brauch, H.-J., Ruck, W., Lev, O., Lange F.T. (2011) Correlation of six anthropogenic markers in wastewater, surface water, bank filtrate, and soil aquifer treatment, J Environ Monit 13(4), 966-973.

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Scheurer, M., Storck, F.R., Brauch, H.-J., Lange, F.T. (2010) Performance of conventional multi-barrier drinking water treatment plants for the removal of four artificial sweeteners. Water Res 44(12), 3573-3584.

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General introduction

1.1 Water scarcity and wastewater reuse

Population growth and the associated increasing human use and pollution of water resources have come to a level, where the scarcity of clean water endangers ecosystem functions, biodiversity, food production and water supply (Jury and Vaux 2007). In many parts of the world water resources like rainwater, groundwater, surface water, and wetlands are very limited. In such regions it might not even be sufficient to merely follow water saving measures but also necessary to come back to any kind of water available to meet the demand. In this context the way we regard wastewater must change in terms that mankind has to recognize it as a resource rather than waste (United Nations, 2009). As a consequence it has to be proactively managed to make it available for urban landscaping, recreational uses, industrial applications, indirect potable reuse, and most important, for irrigation in agriculture as the major consumer (Angelakis et al., 1999; AQUAREC, 2006). According to the United Nations (2009) reused wastewater is currently accounting for only 2.4 % of the global water consumption but has a considerably higher potential.

Authorities as well as the general public are still concerned about the reuse of wastewater due to potential health risks. Especially when untreated wastewater is used for aquaculture or irrigation, as it is the case in countries like e.g. Cambodia, Vietnam, Mexico, Morocco, or Pakistan, these concerns are justified, because faecal pathogens may cause diseases to farmers, local communities and consumers of the respective products (Habbari et al., 1999; Cifuentes et al., 2000; Ensink et al., 2005; Anh et al., 2007; Trang et al., 2007). However, the health risks associated with the reuse of wastewater can be reduced by an appropriate wastewater treatment (Ensink et al., 2005; WHO, 2006), which might be further improved by tertiary treatment steps like chlorination or the application of ozone (de Velasquez et al., 2008; Nikaido et al., 2010). Besides microbial pathogens, concerns originate also from residual anthropogenic organic trace pollutants like endocrine disrupting compounds or pharmaceuticals, which are not completely removed during mechanical-biological wastewater treatment (Drewes et al., 2002; Yoo et al., 2006).

Only in countries, where the value and need for the reuse of treated wastewater have been recognized, regulations about the safety of reclaimed water have been implemented (Angelakis et al., 1999). However, strict water quality standards are always a matter of the related expenses. This is well illustrated by comparing quality guidelines of the World Health Organization (WHO) and the U.S. Environmental Protection Agency (EPA) or certain U.S. states. The latter ensure irrigation water for crops which meets quality standards comparable to drinking water, but requires cost intensive secondary treatment followed by disinfection (US EPA, 2004). However, the WHO notes, that in developing countries advanced wastewater treatment is not feasible. To reduce exposure to pathogens and harmful chemicals simple wastewater treatment with e.g. stabilization ponds in combination with consumer related measures like washing irrigated crops or thoroughly cooking them are recommended (WHO, 2006).

Therefore, one main objective of integrated wastewater applications should be a country-specific development from the uncontrolled use of raw sewage towards the controlled use of wastewater that has been treated to the most feasible extent.

1.2 Soil Aquifer Treatment (SAT) in Israel

The Mediterranean region including countries from the Near East and the Arabian Peninsula is among the most affected by water scarcity in the world (Bdour et al., 2009). The International Water Management Institute (IWMI) predicted the water demand and supply situation for 118 countries. Among the 16 countries, which were considered to suffer “absolute water scarcity” by 2025, 13 were assigned to that region (Seckler et al., 1999). Based on the annual water resources and withdrawals Israel is ranked eighth among these most affected ones. As the country is considered as an advanced economy (International Monetary Fund, 2010) it is in the position to pursue several main strategies to increase its water supply. One of them is the treatment of wastewater and its subsequent reuse for irrigation purposes to overcome the water shortage (Tal, 2006). Wastewater reuse has been put on the list of national priorities and Israel´s Water Law even defines wastewater as a water resource. As a consequence of the water stress, Israel´s water reuse is mature (Figure 1-1) and accounts for 70 % of the produced wastewater, which is among the highest rates in the world (State of Israel, 2011).

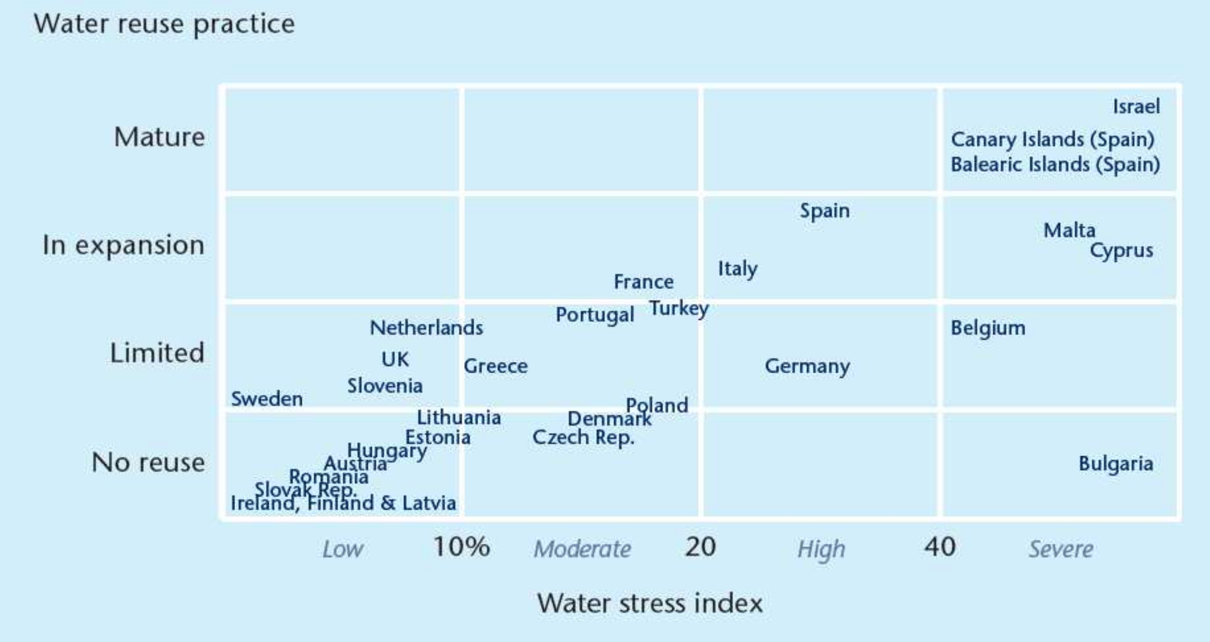


Figure 1-1 Water Stress index and reuse practice in Israel compared to European countries (adopted from United Nations, 2009; originally published by Bixio et al., 2006).

The wastewater which is reclaimed and used for unrestricted irrigation stems from secondary and chlorinated effluent or from a tertiary treatment by soil aquifer treatment (SAT) (Aharoni and Cikurel, 2006). SAT refers to the application of treated wastewater to surface basins and its percolation through the vadose zone as a method of aquifer recharge (Figure 1-2). Natural geopurification processes in the unsaturated zone as well as in the aquifer lead to an additional water quality improvement (Fox, 2001; Eusuff and Lansey, 2004).

Water purification in the vadose zone mainly takes place by filtration, adsorptive and chemical processes as well as by biological degradation and is followed by hydrodynamic dispersion and dilution in the aquifer (Nema et al., 2001). If microbial degradation processes take place, the dissolved oxygen is decreased during the seepage of the water. Therefore, the wetting and drying cycles are important for the redox conditions of the process, as they ensure the reaeration of the soil and the penetration of oxygen into the sub-surface (Fox, 2001).

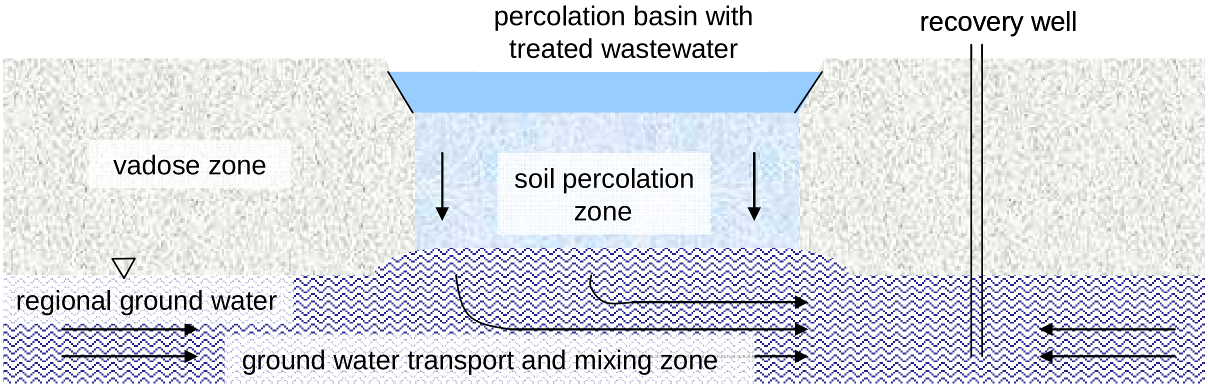


Figure 1-2 Scheme of soil aquifer treatment (Fox et al., 2005, modified)

The artificial groundwater recharge by the SAT process can help to protect coastal aquifers from seawater intrusion and enables the storage and conveyance of water for future use (Bouwer, 2002). According to Asano and Cotruvo (2004) the underground storage of water has several further advantages, as it i) might be cheaper than storage in surface water reservoirs, ii) can eventually act as a distribution system, iii) prevents from water evaporation and biological growth, iv) is suitable for sites where surface water reservoirs are not acceptable, and v) provides esthetic and psychological benefits. The transformation from treated wastewater to groundwater is considered as one major benefit of SAT as it breaks the “pipe-to-pipe” or “toilet-to-tap” connection in people´s minds which is important to overcome public resistance when the injected water is intended for reuse (Bouwer, 2002). The fact, that the recovered water can be considered as groundwater makes a reuse even possible for Islamic countries, where religious taboos against wastewater exist (Ishaq and Khan, 1997). The SAT technology is regarded as a low-technology and cost-effective process with a low degree of maintenance and is therefore also suitable for developing countries (Bouwer, 2000; Maeng et al., 2011). The direct injection of the treated wastewater into the aquifer is an alternative, where not enough land for percolation basins and/or no permeable soils are available or where the aquifers are too deep or confined. However, the direct injection requires a higher degree of pre-treatment, more maintenance and cannot benefit from the additional purification during the soil passage (Bouwer, 2002; Asano and Cotruvo, 2004).

An organic-rich layer which reduces permeability can be formed as a result of sedimentation, filtration, and biological growth. Therefore, the groundwater recharge by SAT is carried out by intermittent flooding and drying of the spreading basins to avoid the formation of such a clogging layer and to ensure high infiltration rates over a certain period of time. During the drying period of several days the clogging layer is drying up and cracking and thus becomes permeable again. Sometimes even the mechanical removal of the organic layer or its complete destruction by ploughing can become necessary. Taking the drying periods into account infiltration rates of 100 m to 400 m per year can be achieved (Bouwer, 2000; Bouwer, 2002).

1.2.1 The Shafdan reclamation project

About one quarter of Israel´s annual 500 million cubic meters of wastewater receive tertiary treatment by SAT in the Dan Region which corresponds to the metropolitan area of Tel Aviv including Jaffa and several neighboring municipalities (Icekson-Tal et al., 2003; State of Israel, 2011). The Shafdan SAT facility comprises the central municipal wastewater treatment plant (WWTP) including the percolation basins about 20 km south of Tel Aviv at the Mediterranean coastline. The SAT site is located in the coastal plain aquifer which supplies about 25 % of Israel´s drinking water (Oren et al., 2007; Gasser et al., 2010).

The WWTP serves about two million people living in the catchment area of Tel Aviv and treats some 130 million m^3/year of wastewater (Icekson-Tal et al., 2003; Tal et al., 2008). The treatment includes conventional mechanical treatment (preliminary bar screen and grit removal) and biological treatment (activated sludge with zones of nitrification and denitrification) (Shelef et al., 1994).

The recharge in the Shafdan site with WWTP effluent is based on a one to six days intermittent flooding and drying regime of the spreading basins (Figure 1-3), which maintains aerobic conditions in the subsurface and renews the percolation capacity. Additionally, the basins are ploughed every two to three months (Shelef et al., 1994; Icekson-Tal et al., 2003; Oren et al., 2007; Tal et al., 2010). The wastewater percolates through a variable unsaturated zone, with a few tens of meters in thickness. In the aquifer the water flows lateral and radial in all directions and is monitored by observation wells located in various distances around the recharge basins. The residence time in the aquifer is between six months and two years before the water is pumped by recovery wells surrounding the recharge area. This pumping regime creates a hydraulic barrier to pristine groundwater which is used for drinking water abstraction and forms a separated zone within the regional aquifer for effluent storage (Oren et al., 2007; Tal et al., 2010). Therefore, mixing with water from the nearby naturally charged aquifer on its way to the peripheral observation wells is expected to be very limited. In the vicinity of the recovery wells (when the treatment is practically finished) some dilution with aquifer water can be suspected. Finally, the recovered water is pumped to the south of Israel and used for unrestricted irrigation in the northern Negev Desert.

The reclaimed water is monitored in terms of physical, chemical, and biological parameters. A good removal (>70 %) for e.g. biological and chemical oxygen demand (BOD and COD), dissolved organic carbon (DOC), UV_{254} absorbance and ammonium during SAT is achieved. In the reclaimed water no coliforms, faecal streptococci, or enteroviruses were found in the past and the removal of bacteria was >99% (Icekson-Tal et al., 2003; Tal et al., 2010). However, little is known about the removal of organic trace pollutants during SAT in general and no published data at all were available for the Shafdan reclamation project.

For this reason a joint Israeli-German project was initiated to study the purification power of the SAT process in terms of the removal of organic trace pollutants. This is especially important as countries with absolute water scarcity like Israel will not only have to reduce the water used in agriculture, they even might be forced to transfer it to other sectors like drinking water supply, and import their food instead (Seckler et al., 1999).

Figure 1-3 Aerial view of several percolation basin within the Shafdan SAT site in different states of flooding (left, picture kindly provided by Prof. Lev, Hebrew University, Jerusalem, Israel) and flooding of a percolation basin with treated wastewater after ploughing (right, picture taken by the author).

1.3 Wastewater markers (tracers)

In any country the permission and acceptance to use reclaimed water for other purposes than irrigation will also depend on the removal of potentially harmful organic trace pollutants during SAT. If trace pollutants present in the treated wastewater are detected in reduced concentrations in the recovered water, the removal efficiency of the SAT process can only be assessed if a reliable differentiation between dilution with pristine groundwater and removal by microbial degradation and/or sorption is possible. This can be achieved by stable and reliable wastewater markers (tracers). If tracers are not present or have not reached a steady-state at the study site, they can also be used to determine the residence time of the water in the subsurface. This can be important as the travel time correlates with the removal of bacteria and viruses and is therefore used in guidelines to regulate the reuse of water originating from SAT or river bank filtration (RBF) (Massmann et al., 2008; Fox and Makam, 2009). Furthermore, the travel time is also considered to be an important aspect for the removal of trace pollutants. As the concentrations of most of the pollutants are too low to support microbial growth a co-metabolic degradation during SAT is assumed (Drewes and Jekel, 1998; Fox and Makam, 2009). Although previous studies have shown that a DOC removal of about 90 % in six months or less is possible, some organic trace pollutants are not completely removed even after longer residence times (Drewes et al., 2002; Yoo et al., 2006; Fox and Makam, 2009).

1.3.1 General requirements

It is not only questionable if an ideal tracer for all hydrological problems exits, but also site and case dependent. However, there are most essential prerequisites of tracers discussed in literature (Flury and Wai, 2003; Richardson et al., 2004; Kahle et al., 2009; Gasser et al., 2010) and the combination of several of the following aspects will consequently result in an increased prediction power:

1. The most important differentiation to microbial degradable trace pollutants is the *conservative behavior* of wastewater tracers. The compound has to be biorefractory and must not be affected by changing redox conditions in the subsurface.
2. As a mobility similar to water is desired a tracer should not tend to adsorb onto soil particles to ensure a *high mobility in the aqueous phase*.
3. If the main objective is to track wastewater like at SAT sites, the tracer should have an anthropogenic origin, a *high specifity to wastewater*, and should be unaffected by the treatment steps applied during wastewater treatment. Other sources like surface runoff or atmospheric deposition should not exist.
4. Tracers which are already present in the treated wastewater are preferred over the ones which have to be especially applied to the study area to minimize the chemical risk and burden to the environment. In any case and especially when conducting tracing experiments a *low toxicity* of the tracer is necessary to not disrupt the microbial population maintaining the treatment efficiency of the system.
5. *High concentrations* in the wastewater and

6. a *sensitive analytical method* enable an early detection of wasterwater plumes arriving at points which need special protection, e.g. drinking water wells and the identification of leakages in sewer systems.
7. Even if the persistence against microbial degradation is known a *constant input level* of the tracer is necessary to assess the effect of dilution with pristine groundwater correctly.
8. When calculating mixing ratios for certain points of interest *low background concentrations* of the tracer are necessary to achieve an acceptable uncertainty level.
9. This is closely related to a *reliable analytical method* which also influences the absolute errors associated with the prediction power of tracers.
10. The tracer´s fate and transport as well as the performance of the analytical method should be characterized by *low chemical interferences* such as matrix effects and changes in pH.

1.3.2 Examples and limitations of applied tracers

Even if the two most important properties of tracers, their persistence and mobility, are given, there is no compound or parameter known, which can fully meet all prerequisites described above. A careful evaluation of the study area in advance is essential to identify possible (case specific) tracers and to rule out others.

In water sciences microbial indicators play only a minor role as wastewater tracers. They can be useful if their growth potential in the aquatic environment is limited and if they show a certain resistance against disinfection measures compared to the pathogens they are intended to indicate (Elmund et al., 1999). But most microbial indicators are time consuming in analysis and lack specificity. This has been shown by Glassmeyer et al. (2005) who detected a bacterial burden in reference samples with minimal input of human wastewater in the same range as in wastewater influenced river water. This was attributed to other sources like livestock or wildlife in the study area. Furthermore, one of the most beneficial aspects of geopurification processes during RBF or SAT is the proven potential to remove pathogens, which is contradictory to their use as tracers in such cases.

Some inorganic tracers like chloride, bromide, or boron have been reported to meet the criteria of persistence and mobility to an almost ideal extent (Davis et al., 1998; Davis et al., 2004). Boron, for example, has been successfully used to trace recycled water in a brackish aquifer on a Hawaiian island and to assess nitrosamine precursors in U.S. surface waters (Babcock et al., 2006; Schreiber and Mitch, 2006). However, it was noted by Rabiet et al. (2005), that wastewater borne trace elements can not be considered as highly reliable tracers, because of their ubiquitous presence in water due to both natural and anthropogenic sources. But the combined information provided by contemplating several inorganic tracers or their ratios can become a valuable tool and was used in numerous studies to trace influences on aquifers (Vengosh and Pankratov, 1998; Rabiet et al., 2005; Alcala and Custodio, 2008). However, a saline environment like in the coastal plain aquifer in Israel with background concentrations of e.g. chloride of about 100 mg/L (Gasser et al., 2010) makes their use difficult. Less uncertainty should be associated with the use of gadolinium (Gd), a rare earth element (REE). Gd is occurring naturally together with other REEs due to similar chemical properties. As Gd is used as a magnetic resonance imaging (MRI) contrast agent, an enrichment of Gd in comparison to the other REEs was observed in urban areas, which is also referred as Gd anomaly (Bau and Dulski, 1996; Elbaz-Poulichet et al., 2002; Verplanck et al., 2005). However, in poor countries or in the catchment areas of small WWTPs with no MRI equipment in medical facilities, the Gd anomaly is not likely to be found.

In contrast to inorganic ions low background concentrations can be expected for most organic trace pollutants of anthropogenic origin. However, many of them, which have been suggested as wastewater tracers, lack biological or chemical stability, are not source specific, or have other drawbacks described only exemplarily below.

One of the most prominent compound groups used to track subsurface water movement are dyes due to their visualization potential and their easy quantification even on site (Flury and Wai, 2003). But most dyes are manufactured to color other materials, which requires sorption interactions to the target material and is opposed to the required mobility of a tracer compound. However, the strongest point against dyes is the additional introduction of a xenobiotic into the study area. This is supported by a toxicological and ecotoxicological assessment of water tracers, which classified some of the most popular rhodamine dyes as “not recommended” or even “ecotoxicologically unsafe” (Behrens et al., 2001).

A typical example for trace pollutants lacking specifity are plant protecting agents. After application on agricultural fields they or their metabolites might percolate into the aquifer or the surface runoff from fields can directly lead to nonpoint emissions into rivers and streams (e.g. Berenzen et al., 2005; Schriever et al., 2007).

Studies about benzotriazoles also showed a certain tracer potential, as their transformation in WWTPs and during RBF seems to be limited. High concentrations in comparison to most other trace pollutants of several tens of µg/L in WWTP effluents have been reported (Voutsa et al., 2006; Weiss et al., 2006; Reemtsma et al., 2010). Benzotriazoles are mainly used as corrosion inhibitors in household products such as dish washing liquids. Consequently, it can be assumed that these compounds are predominantly discharged by WWTPs. However, findings of benzotriazoles should be evaluated carefully, as they are also used as an additive in aircraft deicing fluids. Therefore, a seasonal variability by punctual inputs (besides WWTP effluents) by their application at airports in winter time can occur (Giger et al., 2006).

Besides the lack of wastewater specificity many organic contaminants are prone to biodegradation. A prominent example is caffeine, which is probably the most suggested wastewater tracer, but has drawbacks in terms of specificity and persistence. It has been reported that the application of compost and cesspool matter in agriculture might be additional sources for caffeine emissions (Schramm et al., 2006). Furthermore, as caffeine has been shown to be degraded with high efficiencies in WWTPs (Paxeus and Schröder, 1996; Buerge et al., 2003; Martínez Bueno et al., 2011) its application to track the fate of more persistent wastewater specific organic contaminants seems to be very limited. However, the detection of readily biodegradable compounds like caffeine or cotinine, a metabolite of nicotine, can be an important tool to discriminate between an influence of untreated and treated domestic wastewater (Buerge et al., 2003; Buerge et al., 2008).

To meet the criteria of specificity to domestic wastewater, pharmaceuticals (including their human metabolites) and personal care products (PPCP) gained increased attention in the last decade. Representatives of almost every compound class of pharmaceuticals like antiepileptics, anti-inflammatories, antibiotics, betablockers, and X-ray contrast media have been evaluated and proposed as chemical markers for domestic wastewater (e.g. Zwiener and Frimmel, 2004; Nakada et al., 2008; Kasprzyk-Hordern et al., 2009). The two most promising compounds among the numerous PPCPs seem to be the antiepileptic carbamazepine (CBZ) and the antipruritic drug crotamiton (CTM). Clara et al. (2004) reported about stable CBZ concentrations after infiltrating treated wastewater into groundwater. The persistence of CBZ in the subsurface was successfully used to calculate the wastewater burden of groundwater samples (Kahle et al., 2009), for evaluating leakages from a SAT system to nearby water wells (Gasser et al., 2010), and to detect and quantify sewer exfiltration (Ruzicka et al., 2011). In contrast to CBZ, there are only few scientific publications about CTM. All but one field studies originate from Japan, where CTM showed limited degradation during activated sludge treatment (Nakada et al., 2010) and was detected in concentrations up to several µg/L in treated wastewater (Nakada et al., 2007; Okuda et al., 2008). Due to its persistence, the mass flow of CTM in a Japanese stream correlated well with the cumulative inhabitants in the river basin (Nakada et al., 2007) and in another study the compound was successfully used to track the source of perfluorinated surfactants in rivers (Murakami et al., 2008). Although previous results for CBZ and CTM are promising, it should be considered that both occur in their neutral form at pH values expected for aqueous environmental matrixes and have octanol-water partition coefficients of approximately 2.5 to 3.1 (NLM, 2011; ChemAxon, 2011). Therefore, sorption processes in certain environmental compartments cannot completely ruled out. In soil column experiments (27 %) and batch tests with activated sludge (11 %) a low removal by sorption has been reported for CTM (Möhle et al., 1999; Shinohara et al., 2006). For CBZ most studies confirmed its possible use as a wastewater tracer (see above) and no limitations due to sorption processes occur. However, it was also shown that in a study area, where arable land is irrigated with treated wastewater for more than 45 years (a process very similar to SAT) its use is limited, due to a presumed combination of sorption and degradation in the subsurface (Ternes et al., 2007). A possible explanation and at the same time the strongest drawback of CBZ is, that its pronounced stability is only valid for aerobic conditions. A removal of up to 100 % has been observed for anoxic and anaerobic conditions during river bank filtration at the river Ruhr in Germany (Schmidt et al., 2007). Even though no studies about the behavior under different redox milieus exist for CTM, it seems to be inapplicable as a wastewater tracer for Europe in contrast to Japan. Results from Switzerland showed very low CTM concentrations in wastewater, a maximum of only 6 ng/L in lakes, and no positive findings in groundwater samples (Kahle et al., 2009).

1.4 Artificial sweeteners as potential tracer compounds

Nicotine and caffeine are the only ingredients of tobacco or (luxury) foodstuffs which have been evaluated as wastewater tracers. This is more or less surprising as ingredients of foodstuffs and beverages should be used in much higher quantities as e.g. pharmaceuticals. In 2008 the Swedish Environmental Research Institute brought the artificial sweetener sucralose into the focus of environmental scientists, when it reported high concentrations of up to 10.8 µg/L in WWTP effluents due to very low removal rates of the compound (<10 %) during wastewater treatment. Sucralose is a highly polar compound, it is very stable in water with a half life of several years, and hydrolysis occurs only at low rates. As a consequence an EU wide monitoring program was initiated and the compound was found in concentrations up to 1 µg/L mainly in surface waters in Western Europe and Scandinavia (Loos et al., 2009). Apart from that only two publications from the early 1990s dealt with the issue of sucralose in the environment. They reported about a rapid mineralization in soils but a slow degradation in lake water and aerobic wastewater (Labare and Alexander, 1993; Labare and Alexander, 1994).

Besides sucralose several other artificial sweeteners with similar properties are approved in the European Union (Table 1-1). Their application and maximum usable doses are regulated by the *Directive 94/35/EC of the European Parliament and of the Council on sweeteners in foodstuffs* (EU, 1994). However, due to health concerns associated with the consumption of some sweeteners the approval is heterogeneous on a world wide scale (for more detailed information see introduction of chapter 2). Furthermore, very little is known about the used quantities and the diversities in national usage patterns.

Artificial sweeteners are used in thousands of products all over the world. Their main advantages are that they provide no or only little calories and are therefore important ingredients in dietary and diabetic products. Some sweeteners develop characteristic aftertastes, but by blending two or more sweeteners this can be overcome and the sweetness can even be enhanced. Their application requires a certain stability as i) the sweetening power has to remain stable over the storage time of the products, ii) they are applied in rather acidic conditions (e. g. in carbonated soft drinks), and iii) they have to be heat resistant when used for baking or cooking. Besides ingredients of foodstuffs and beverages, artificial sweeteners are also used in sanitary products like toothpastes or mouthwashes and in pharmaceuticals. In any case, if excreted unchanged, a high wastewater specificity can be assumed for all artificial sweeteners. Surprisingly no studies about artificial sweeteners (besides the very few about sucralose mentioned above) were published at the beginning of this work, although they appeared to be promising candidates for an ideal tracer based on their chemical properties and their worldwide usage in a variety of products.

Table 1-1 Artificial sweeteners approved in Europe, including E-number, sweetness compared to saccharose and octanol-water distribution coefficient ($\log K_{OW}$).

Compound[a]	E-number	sweetness (x times saccharose)	$\log K_{OW}$
Acesulfame K	E 950	130-200	-1.49[e] (ionic species)
Aspartame	E 951	200	0.07[b]
Cyclamate (Cyclamic acid)	E 952	30-50	-1.61[b]
Saccharin	E 954	300-500	0.91[b]
Sucralose	E 955	600	-1.0[b], -0.51 ± 0.05[c], 0.3[d]
Neohesperidine DC	E 959	400-600	0.75[e]
Neotame	E 961	7,000-13,000	0.3[d]

[a]for chemical structures see Table 2-1 (page 32), [b](NLM, 2011) [c](Jenner and Smithson, 1989), [d](Grice and Goldsmith, 2000), [e]ChemAxon Ltd., logP calculator (ChemAxon, 2011)

1.5 Fate of tracer compounds in the aquatic environment

Like for other wastewater tracers used in the past several pathways for artificial sweeteners can be expected in the aquatic environment (Figure 1-4). Their occurrence in waterworks seems possible, if surface water or wastewater influenced river or groundwater is used as raw water for drinking water production and when sufficient persistence and mobility are given.

In developed countries with a high connection rate to the sewerage system wastewater specific trace pollutants from households (1) and industry (2) will end up in wastewater treatment plants after consumption or application. However, in some developing countries the use of untreated wastewater for irrigation is still common practice (3) (see above).

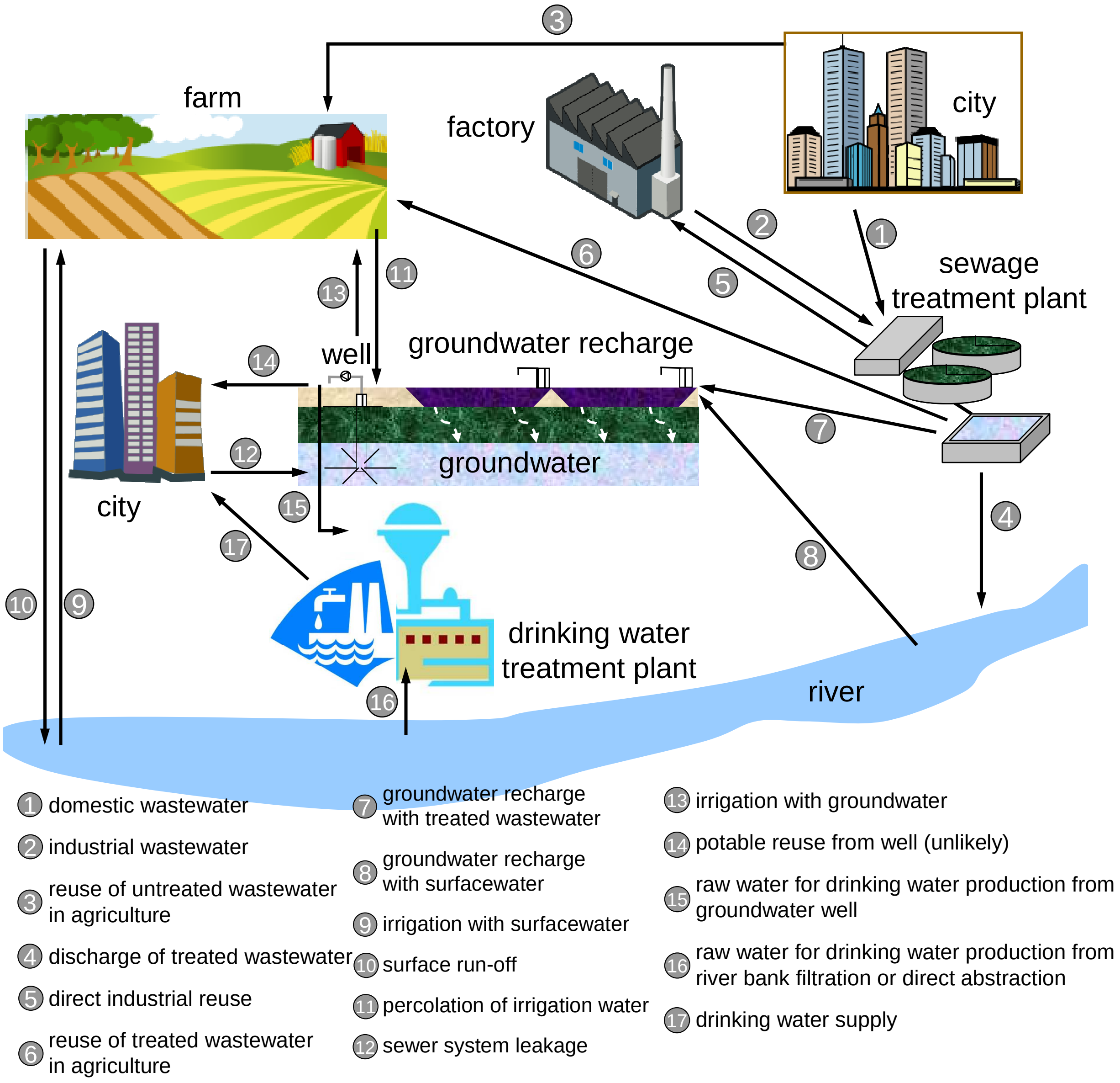


Figure 1-4 Possible pathways of wastewater borne organic trace pollutants (Angelakis and Durham, 2008, modified)

If persistent during mechanical-biological wastewater treatment, the discharge in recipient waters (4) is most likely, but the reuse of WWTP effluent for industrial purposes (5), irrigation (6) or groundwater recharge (7) is also possible. The latter two might lead to an indirect input into groundwater resources. Artificial groundwater recharge with river water (8) or when surface water is used for irrigation (9) can be other pathways into groundwater but due to dilution a lower basic load of trace pollutants can be expected. Conversely, trace pollutants applied with irrigation water might end up in surface water again by run-off (10) or percolate into the aquifer (11). But even the input of untreated wastewater into aquifers is possible by leaking sewers (12), e.g. even in Germany short or medium term rehabilitation measures are needed for about 17 % of the public sewer system (Berger et al., 2002).

Groundwater, contaminated or not, might be used for irrigation (13), for direct potable reuse (14), which is rather unlikely, and as raw water for drinking water production (15). River bank filtration (16) is the most likely way organic trace pollutants can enter drinking water treatment plants in Germany. As this pathway has a certain travel time in a biological active environment, only traces of pollutants, which meet the claims of persistence and mobility of an ideal anthropogenic marker, will be present in the raw water. However, such compounds pose quite a challenge for waterworks regarding their removal, as some treatment steps are likely to be ineffective for such small, polar, and recalcitrant molecules.

Most treatment steps nowadays applied for the removal of suspended or dissolved organic matter in drinking water treatment plants are implemented for many years. Mechanical procedures comprise sedimentation and filtration. Further physical, chemical and biological treatment steps can be integrated as pre- (e.g. river bank filtration, pre-ozonation, flocculation) or as subsequent (ozonation, activated carbon filtration) treatment steps (Sontheimer et al., 1980). The combination of treatment steps depends on the raw water quality and on the associated technical demands to achieve the required water quality. If residual organic trace pollutants can be expected, e.g. when surface influenced raw water is used, the combination of chemical and biological oxidation in combination with adsorption is widely applied in Germany. For this purpose ozonation is usually followed by an activated carbon filtration unit for the destruction of residual ozone, the removal of adsorbable compounds or for the microbial degradation in biological active layers of the activated carbon filter.

1.6 Formation of ozonation products

For polar and persistent compounds, which are present in raw waters a chemical oxidation with a strong oxidizing agent like ozone is one of the most important processes for their removal from water. Although only a few compounds have been identified in concentrations, which involve potential risks for the aquatic environment or humans only little is known about long term or synergistic effects of organic trace pollutants at low levels. Therefore, regulators and scientists support the implementation of additional treatment technologies already in WWTPs to protect recipient waters and drinking water resources (Dickenson et al., 2009). Tertiary treatment becomes necessary as conventional WWTPs are designed to remove dissolved organic matter and nutrients from the wastewater, but not especially for the elimination of trace pollutants. Switzerland, for example, is just about to change its Water Pollution Control Ordinance in this sense. A first draft schedules that 80 % of the trace pollutants present in the raw wstewater have to be removed during wastewater treatment, which can only be achieved by the installation of additional treatment technologies (Götz, 2011). The chemical treatment of wastewater has already been demonstrated to be a promising tool for the removal or transformation of wastewater derived compounds like pharmaceuticals, antibacterials, musk fragrances, and estrogens (Ternes et al., 2003; Dodd et al., 2006; Hollender et al., 2009). Additional benefits like disinfection, the lack of trihalomethane formation, and the fact that the process is considered as economically feasible (Richardson et al., 1999; Ternes et al., 2003; Hollender et al., 2009; Schaefer et al., 2009) will increase the use of ozone in wastewater treatment. As a consequence organic trace pollutants might face ozone treatment at two stages of their environmental fate: in the WWTPs and when surface water influenced raw water is treated with ozone for potable use (see above).

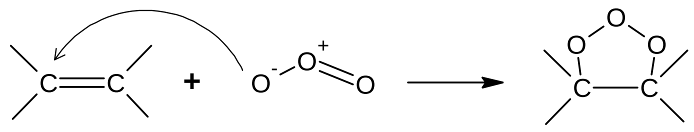
The ozone molecule itself is decomposing in water and its stability is a function of the water characteristics. Especially the pH of the water and the content of dissolved organic matter are important factors influencing the formation of a secondary oxidant, the OH radical. According to reaction (1) the ozone decomposition is accelerated with increasing pH. OH radicals can further react with ozone (reaction (3)) producing more radicals, which increase the decomposition of ozone in a chain reaction (Staehelin and Hoigne, 1982). Dissolved organic matter can strongly hinder reaction (3) as it scavenges OH radicals or it can directly react with the ozone molecule itself (von Gunten, 2003).

$$O_3 + OH^- \rightarrow HO_2^- + O_2 \qquad (1)$$

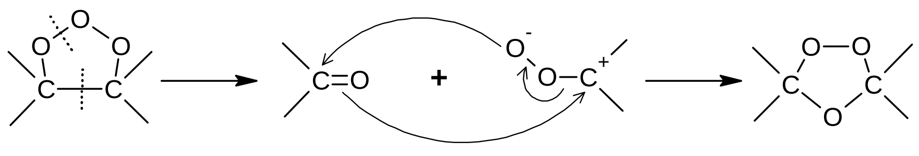
$$O_3 + HO_2^- \rightarrow {}^{\bullet}OH + O_2^{\bullet -} + O_2 \qquad (2)$$

$${}^{\bullet}OH + O_3 \rightarrow HO_2^{\bullet} + O_2 \qquad (3)$$

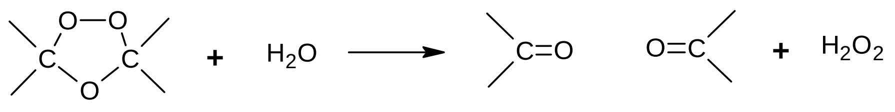
In any case when contemplating ozonation processes both ozone and OH radicals have to be taken into account. Measures have to be taken to be able to discriminate between both, as ozone is a very selective oxidizing agent, whereas OH radicals react faster and with a broad range of water constituents (von Gunten, 2003). The effectiveness of direct oxidation of trace pollutants by ozone is strongly related to the functional groups of the target molecule. High rate constants for the reaction of ozone with the target compounds can be expected for molecules with sites of high electron densities like unsaturated aromatic ring systems, olefinic groups and deprotonated amines (von Gunten, 2003). The most prominent mechanism for an ozone attack at double bonds is the Criegee mechanism (Criegee, 1975). This ozonolysis consists of three discrete steps. The first is the formation of a primary ozonide by a 1,3-dipolar cycloaddition (4). The primary ozonide decomposes into a carbonyl compound and a carbonyl oxide by the cleavage of the C-C and one O-O bond, while the stronger C-O bonds remain intact. The carbonyl oxide stabilizes by addition to an aldehyde or ketone forming a secondary ozonide (5) (Criegee, 1975). If the reaction takes place in aqueous solution, hydrolysis will decompose the secondary ozonide and two carbonyl compounds are formed (6). In this case hydrogen peroxide is formed, which can further oxidize the products.



(4)



(5)

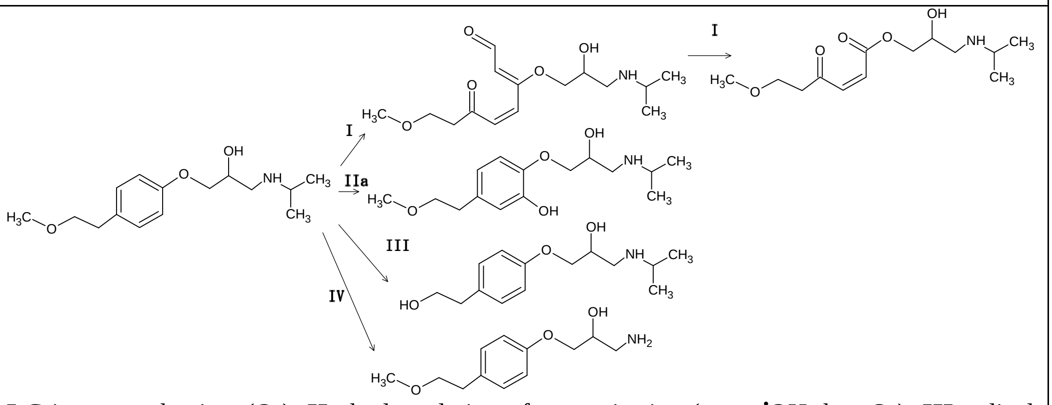


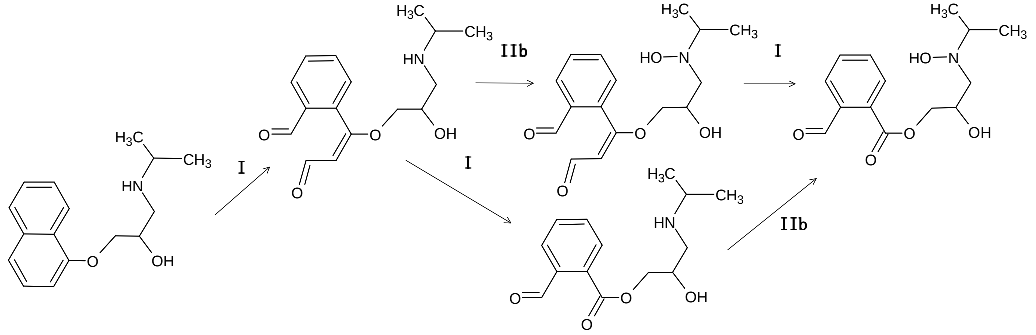
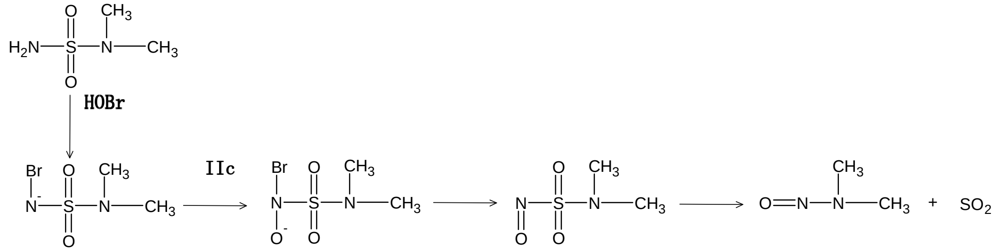
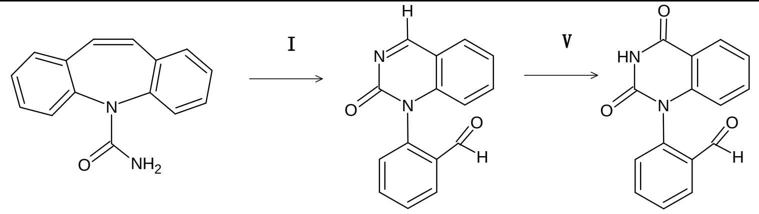
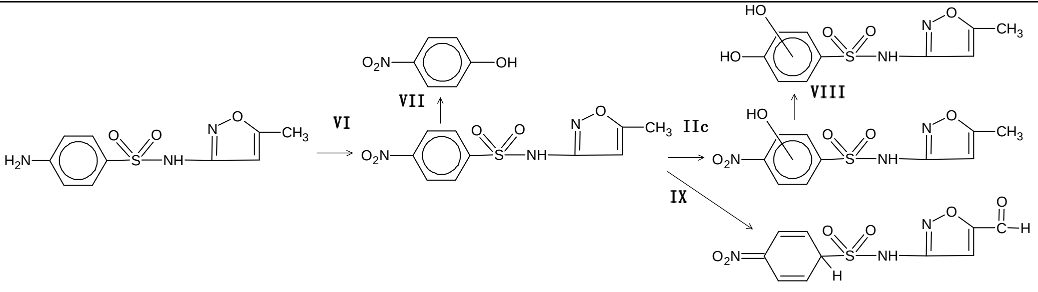
(6)

According to von Gunten (2003, and references therein) the reaction kinetics of hundreds of organic trace pollutants have been measured. However, very little is known about the oxidation products of these compounds. It has been shown that in general the oxidation of real matrices like surface and wastewater results in i) the transformation of natural organic matter into a more biodegradable form and ii) the formation of carboxylic acids, aldehydes and ketones as major ozonation products (Richardson et al., 1999; Huang et al., 2005; Wert et al., 2007). Often ozonation leads to the formation of oxidation products with a lower molecular weight and a higher polarity (Camel and Bermond, 1998), which decreases the removal by sorption in subsequent activated carbon filters.

The ozone doses applied in WWTPs or in drinking water treatment plants are not sufficient for complete mineralization of most organic trace pollutants and oxidation products (OPs) are likely to be formed. The molecular structure of the compound to be oxidized might allow a rough estimate of the transformation extent, but the structures of the formed OPs are comparatively difficult to predict. Table 1-2 shows examples of elucidated oxidation products of organic trace pollutants and the variety of oxidative reactions involved. The formation of N-nitrosodimethylamine after ozonation of N,N-dimethylsulfamide demonstrates that even harmful compounds might be generated from harmless precursors (Schmidt and Brauch, 2008). Thus, the structural elucidation of OPs can provide first information which compounds are formed during ozonation and are released into the aquatic environment or end up in the potable water. This is a prerequisite for subsequent toxicity tests to clarify if the formed ozonation products are harmful.

Table 1-2 Formation pathways of oxidation products after ozone application and proposed oxidation reactions of selected organic trace pollutants

Name (compound class)	**Oxidation product formation pathway and proposed oxidation reaction**
Metoprolol[1] (beta blocker)	 **I** Criegee mechanism (O_3); **IIa** hydroxylation of aromatic ring (more $^{\bullet}OH$ than O_3); **III** radical reaction at ether bond, demethylation ($^{\bullet}OH$); **IV** dealkylation at amine, cleavage of isopropyl moiety (O_3) Ozone attack can occur at several sites of the activated ring system forming constitutional isomers. Reaction IV occured mainly at pH 8 in $^{\bullet}OH$ scavenged samples.

Propranolol[2] (beta blocker)	 **I** Criegee mechanism (O_3); **IIb** hydroxylation of amine moiety ($^{\bullet}$OH and O_3) Initial ozone attack occurs at several sites of the naphthalene moiety forming constitutional isomers.
N,N-Dimethyl-sulfamide[3] (metabolite of the fungizide tolylfluanide)	 **IIc** hydroxylation of halogenated amine (O_3). Further reactions: loss of halide and intra-molecular rearrangement to N-nitrosodimethylamine (NDMA) Mechanism requires bromide for oxidation to HOBr. Reaction of protonated amine is slow. NDMA is also formed in the presence of HOCl.
Carbam-azepine[4] (anti-epileptic)	 **I** Criegee mechanism (O_3) with subsequent ring closure to quinazoline moiety by intramolcular attack of nitrogen on the formed aldehyde; **V** ketone formation ($^{\bullet}$OH and O_3) Low rate constants for reaction V with ozone but high with OH radicals.
Sulfa-methoxazole[5] (antibiotic)	 **IIc** hydroxylation of aniline ring; **VI** formation of nitro group by oxidation of amine moiety, **VII** C-S bond cleavage; **VIII** substation of nitro group by hydroxyl group; **IX** aldehyde formation by oxidation of methyl moiety Study identified more ozonation products than displayed. No main pathway is given here, as all tests were scavenged and products were attributed to oxidation by O_3.

[1]Benner and Ternes (2009a); [2]Benner and Ternes (2009b); [3]von Gunten et al., (2010); [4]McDowell et al., (2005); [5]Abellán et al., (2008)

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1.8 Objectives

The overall objective of this thesis was to clarify if artificial sweeteners can be used as markers for an anthropogenic contamination by wastewater and if they have to be considered as drinking water relevant compounds.

As an appropriate analytical method was lacking the first important task within this thesis was the development, validation, and application of a **multi-residue method** (chapter 2) for the simultaneous determination of artificial sweeteners in different aqueous matrices. The main focus was placed on sufficient separation of these polar analytes to keep the time of analysis as short as possible, and to achieve limits of detection down to the low ng/L range with minimum sample preparation. The method was applied to investigate the occurrence of the selected artificial sweeteners in WWTPs, rivers, and wells in an Israeli SAT field which has not been addressed before.

An important point to be clarified was to prove the suitability of artificial sweeteners as **wastewater markers** and to assess their prediction power compared to other persistent organic trace pollutants (chapter 3). Therefore, the ratios of six trace pollutants were followed in different environmental compartments. The research also aimed to outline the benefits and limitations of the wastewater tracers under investigation.

Another aim was to clarify the **drinking water relevance** of artificial sweeteners (chapter 4). The most important treatment steps applied in waterworks should be researched in well defined laboratory batch tests. At the same time an extended sampling campaign was initiated to elucidate the efficiency of full-scale treatment trains in waterworks to remove artificial sweeteners from surface water influenced raw waters used for drinking water production.

The final task was to **elucidate the ozonation products** of artificial sweeteners (chapter 5) formed during ozonation in WWTPs or drinking water treatment plants which is a prerequisite for future toxicity testing of those compounds.

1.9 Outline

The outline of this thesis is as follows:

Chapter 2 describes the development, validation, and application of an analytical method for the analysis of artificial sweeteners in drinking water, surface water, and wastewater. Data about the occurrence and behavior during wastewater treatment, soil aquifer treatment, and in German rivers are reported.

Chapter 3 studies the correlation of six anthropogenic markers including two artificial sweeteners in different aquatic environments to elucidate the prediction power and limitations of potential wastewater markers.

Chapter 4 deals with the behavior of four artificial sweeteners during drinking water treatment. Common treatment techniques were applied on a laboratory scale and the performance of six drinking water treatment plants for the removal of artificial sweeteners was investigated.

Chapter 5 reports about the elucidation of ozonation products of acesulfame and cyclamate by the application of several analytical techniques and proves the relevance for the full-scale applications of ozone in a drinking water treatment plant which uses surface influenced raw water for drinking water production.

Chapter 6 discusses the main research results of the studies mentioned above, and gives some final conclusions. In addition, suggestions for further research are addressed.

2

Analysis and occurrence of seven artificial sweeteners in German wastewater and surface water and in soil aquifer treatment (SAT)

A method for the simultaneous determination of seven commonly used artificial sweeteners in water is presented. The analytes were extracted by solid phase extraction using Bakerbond SDB 1 cartridges at pH 3 and analyzed by liquid chromatography electrospray ionization tandem mass spectrometry in negative ionization mode. Ionization was enhanced by post-column addition of the alkaline modifier tris(hydroxymethyl)amino methane. Except for aspartame and neohesperidin dihydrochalcone, recoveries were higher than 75 % in potable water with comparable results for surface water. Matrix effects due to reduced extraction yields in undiluted waste water were neglible for aspartame and neotame but considerable for the other compounds. The widespread distribution of acesulfame, saccharin, cyclamate, and sucralose in the aquatic environment could be proven. Concentrations in two influents of German sewage treatment plants (STPs) were up to 190 µg/L for cyclamate, about 40 µg/L for acesulfame and saccharin, and less than 1 µg/L for sucralose. Removal in the STPs was limited for acesulfame and sucralose and >94 % for saccharin and cyclamate. The persistence of some artificial sweeteners during soil aquifer treatment was demonstrated and confirmed their environmental relevance. The use of sucralose and acesulfame as tracers for anthropogenic contamination is conceivable. In German surface waters, acesulfame was the predominant artificial sweetener with concentrations exceeding 2 µg/L. Other sweeteners were detected up to several hundred ng/L in the order saccharin ≈ cyclamate > sucralose.

Scheurer, M., Brauch, H.-J., Lange, F.T. (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(6), 1585-1594.

2.1 Introduction

Artificial sweeteners (Table 2-1) are used as sugar substitutes in remarkable amounts in food and drinks, but also in drugs and sanitary products (Weihrauch and Diehl, 2004). They provide no or insignificant energy as they are not decomposed as carbohydrates. Insulin level is not affected by their consumption what makes them suitable for diabetics. They can help to reduce calorie-intake as their sweetness is much higher than that of table sugar. Consequently, these “high-intensity sweeteners” are used in comparably small amounts, making the calorie intake insignificant, even for those sweeteners that are metabolized (Kroger et al., 2006). Artificial sweeteners can prevent from potential dental caries as most of them are not metabolized like sugars or only fermented slightly by the mouth microflora (Imfeld, 1993; Strubig, 1988). They can develop an artificial, metallic, or licorice-like aftertaste. Therefore, they often can be found blended in food to overcome this disadvantage.

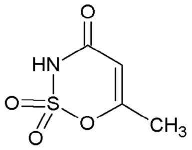
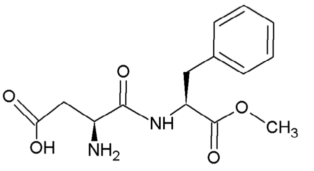
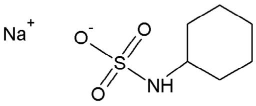
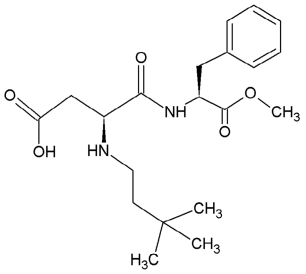
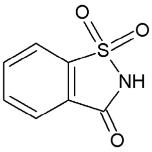
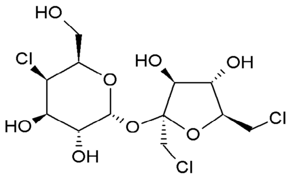
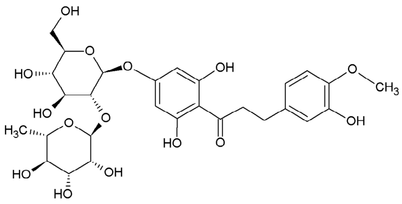
Since the beginning of use, there is an ongoing discussion about potential health risks of artificial sweeteners in gray literature as well as on a scientific base. Numerous internet forums, newspaper reports, and scientific publications deal with possible risks and other safety issues (Bandyopadhyay et al., 2008; Grice and Goldsmith, 2000; Kroger et al., 2006; Magnuson et al., 2007; Weihrauch and Diehl, 2004).

Five artificial sweeteners are approved by the US Food and Drug Administration (FDA) and are “generally recognized as safe” (GRAS) in the US: acesulfame-K, aspartame, neotame, saccharin, and sucralose (FDA, 2006). In the European Union, the use of neotame in foodstuffs is not allowed, but, contrary to the US, neohesperidin dihydrochalcone (NHDC) and cyclamate are additionally approved (EU, 1994; EU, 2003).

Cyclamate is banned in the US since 1970. Oser et al. (1975) accused cyclamate of causing bladder cancer in rats, which prompted the US Department of Health, Education and Welfare to remove cyclamate from the GRAS list. Further studies, however, did not show any relation between cyclamate and cancer. Cyclamate is still banned in the US but waiting for its reapproval by the FDA (CFSAN/Office of Food Additive Safety, 2009).

Acesulfame is commercially used as potassium salt and also known as acesulfame-K. It is 200 times sweeter than table sugar and provides the common benefits of artificial sweeteners mentioned above but has also a bitter aftertaste. Acesulfame-K is used in about 90 countries and, according to Kroger and co-authors (2006), no health problems associated with its consumption have been reported in scientific literature. However, in 2008, a study was published where DNA damage due to acesulfame exposure was reported. The authors suggested restricting the use of some artificial sweeteners (Bandyopadhyay et al., 2008). Acesulfame-K, cyclamate, and saccharin are excreted mainly unchanged through the renal system (Renwick, 1986).

Table 2-1 Compounds, CAS numbers, molecular weight and chemical structure of the artifical sweeteners investigated

Compound CAS No Molecular weight (MW)	Chemical structure	Compound CAS No Molecular weight (MW)	Chemical structure
Acesulfame CAS: 33665-90-6 MW: 163.2 g/mol		Aspartame CAS: 22839-47-0 MW: 294.3 g/mol	
(Sodium) Cyclamate CAS: 139-05-9 MW: (201.2) 178.2 g/mol		Neotame CAS: 165450-17-9 MW: 378.5 g/mol	
Saccharin CAS: 81-07-2 MW: 183.2 g/mol		Sucralose CAS: 56038-13-2 MW: 397.6 g/mol	
		Neohesperidin dihydrochalcone (NHDC) CAS: 20702-77-6 MW: 612.6 g/mol	

In the 1980s, studies showed an increasing risk for bladder cancer in rats when applying high doses of saccharin in the animals' diet (Squire, 1985; Taylor et al., 1980). Therefore, saccharin was prohibited in Canada. In the USA, products containing saccharin had to be labeled with a warning that saccharin "has to be determined to cause cancer in laboratory animals." In 2001, saccharin was removed from the list of potential carcinogens in the USA as the mechanism causing bladder cancer in rats is not relevant for humans (Cohen et al., 2008). In Canada, authorities have received a submission to reinstate saccharin as a food additive, and in the EU, an acceptable daily intake (ADI) of 0–5 mg/kg body weight is approved (Health Canada, 2007).

Aspartame provides, like sugar, 4 cal/g. Since it is about 180 times sweeter than sugar, only small amounts of aspartame are needed to sweeten food and drinks. Contrary to acesulfame, aspartame is not heat-stable and degrades in liquids when stored over a longer period of time. Aspartame is made up of phenylalanine, aspartic acid, and methanol. For people with a seldom genetic disorder, the generated phenylalanine does carry some risk as their body cannot metabolize the degradation product. As a consequence, all products containing aspartame have to be labeled to point out the presence of a phenylalanine source. In scientific literature, aspartame is the most controversially discussed artificial sweetener regarding health aspects. Numerous publications with contrary results about possible adverse effects of aspartame like neurological disturbances (Shaywitz et al., 1994; Simintzi et al., 2007; Tsakiris et al., 2006) or even cancer in rats (Soffritti et al., 2006; Soffritti et al., 2007) are available. Nevertheless, FDA and the European Union consider the compound as safe based on toxicological and clinical studies.

One of the latest outcomes of the research for new artificial sweeteners is neotame. Its structure is closely related to aspartame on which a branched hydrocarbon chain is attached. It is 7,000 to 13,000 times sweeter than sucrose, and like aspartame, it is metabolized, but phenylalanine release is insignificant. Products containing neotame are not required to be labeled as possible phenylalanine sources (Duffy and Sigman-Grant, 2004).

Neohesperidin dihydrochalcone (NHDC) is about 1,500 times sweeter than sugar but is also used as a flavor enhancer. It is produced by hydrogenation of a flavonoid found in citrus fruits. To overcome its licorice and menthol-like aftertaste, it is often found blended with other artificial sweeteners. NHDC is metabolized by intestinal microflora and excreted via urine (Varnam and Sutherland, 1994). Antioxidant properties have been proven (Choi et al., 2007).

Sucralose has a disaccharide structure where three hydroxyl groups are replaced by chlorine atoms. It is thermally stable and excreted unchanged with the feces (Roberts et al., 2000). More than hundred safety studies have been conducted on sucralose and proved its safety for human consumption (Grice and Goldsmith, 2000), but it is also discussed as a migraine trigger (Bigal and Krymchantowski, 2006; Patel et al., 2006). Due to its half-life in water of several years and a missing environmental review, its relevance in the aquatic environment is discussed. The compound is reported to pass sewage treatment plants (STPs) and was found up to several μg/L in STP influents and effluents and up to several hundred ng/L in surface waters in Sweden (Brorström-Lundén et al., 2008). In an EU wide monitoring program, concentrations up to 1 μg/L sucralose were found in European surface waters. The compound was mainly detected in samples from Western Europe and Scandinavia. In samples from Germany and Eastern Europe, minor concentrations were reported (Loos et al., 2009).

Findings of sucralose suggest its main distribution in Western Europe, likely based on the use of other artificial sweeteners in countries where sucralose is not predominant. If excreted unchanged and if artificial sweeteners should prove to be persistent during wastewater treatment, their ubiquitous distribution in the aquatic environment is likely. Robust analytical methods for clarifying their environmental fate are crucial. To the best of our knowledge, there is no scientific report on occurrence of artificial sweeteners in the aquatic environment other than for sucralose. This paper intends to provide first information on that topic.

2.2 Materials and methods

2.2.1 Chemicals

All reference compounds (Table 2-1) were of high purity (>98 %). Acesulfame potassium, saccharin, aspartame, and sucralose were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), sodium cyclamate from Supelco (Bellefonte, PA, USA), neotame from USP Reference Standards (Rockville, MD, USA), neohesperidin dihydrochalcone from European Pharmacopoeia Reference Standard (Strasbourg, France), and sucralose-d_6 from Campro Scientific GmbH (Berlin, Germany). Individual stock solutions were prepared by dissolving the compounds in methanol. Concentrations of the stock solutions were between 0.2 to 0.8 g/L. All stock solutions were stored at -18 °C. Standard mixtures containing all analytes were prepared by diluting the stock solutions with methanol to concentrations of 0.1 mg/L and 0.01 mg/L. A standard solution of sucralose-d_6 was prepared by diluting the stock solution with methanol to a concentration of 0.1 mg/L.

High-performance liquid chromatography (HPLC)-grade methanol and acetone as well as formic acid and hydrochloric acid (32 %) were supplied by Merck (Darmstadt, Germany). Purities of all organic solvents were higher than 99.8 %. Ammonium acetate (purity >98 %) was purchased from Sigma-Aldrich (Steinheim, Germany) and tris(hydroxymethyl)aminomethane (TRIS) from Carl Roth GmbH (Karlsruhe, Germany). Ultrapure water was provided by an Arium 611 laboratory water purification system (Sartorius AG, Göttingen, Germany).

The nitrogen used for drying the solid-phase cartridges and for evaporation of solvents was of 99.999 % purity and was purchased from Air Liquide (Düsseldorf, Germany).

2.2.2 Sampling sites and protocol

The STP in Eggenstein-Leopoldshafen (STP 1) is applying conventional, i.e., mechanical and biological treatment. It has a capacity of 20,000 population equivalents (PE) with about 15,000 inhabitants living in the catchment area and treats ca. 2,500 to 3,500 m^3 per day of domestic waste water. Hydraulic retention time is about 5 hours with an average sludge retention time of 20 days. The STP of the city of Karlsruhe (STP 2) treats 40 million cubic meters (capacity 875,000 PE) per year for about 350,000 inhabitants. It is applying mechanical treatment with additional phosphate precipitation, followed by biological treatment with a denitrification/nitrification unit, equipped with a trickling filter. Hydraulic retention time is about 1 day for dry weather conditions. Samples for both STPs were corresponding 24-h composite samples.

The soil aquifer treatment (SAT) site is located in a Mediterranean country and treats secondary effluent from a STP that processes over 100 million cubic meters waste water per year. Treatment includes primary mechanical treatment (bar screen and grit removal) followed by conventional activated sludge treatment including nitrification/denitrification and a limited biological phosphorous removal. The secondary STP effluent is spread in percolation basins, where infiltration through an unsaturated zone, up to 40 m in depth, takes place. The effluent flows lateral in the saturated zone to observation and recovery wells located in a circle of up to 1,000 m in the periphery of percolation basins. Influence of waste water was assessed using chloride as hydrogeological tracer. The residence time in the aquifer exceeds 1.5 years. The recharge operation is carried out by intermittent flooding up to 1 day and 2 days drying. Dilution with local groundwater is very limited. Samples from the SAT site were the STP effluent used for aquifer recharge, a sampling point located vertically below the percolation basin (well 1), two sampling points in the periphery (well 2 and well 3), and one from a private drinking water well supposed not to be influenced by waste water.

Grab samples from major German rivers were collected in 1 L brown glass bottles. If no immediate analysis was possible, samples were stored at 4 °C in the dark for a maximum of 3 days after sampling. No preservation agents were added. Filtration proved to be unnecessary in preliminary tests.

2.2.3 Solid-phase extraction

For solid-phase extraction (SPE), styrol-divinylbenzene cartridges were used (Bakerbond SDB 1, 200 mg / 6 mL from J.T. Baker, Deventer, The Netherlands). Other cartridges tested were Isolute ENV+ and C18 material (IST, Mid Glamorgan, UK), Varian Bond Elut PPL (Varian, Lake Forest, CA, USA), Strata X and Strata X-AW (Phenomenex, Aschaffenburg, Germany) and Oasis HLB, WAX, MAX and MCX (Waters, Milford, MA, USA). For detailed results for all cartridges tested see Table 2-4 in chapter 2.6.

A vacuum manifold set from IST equipped with 60 mL reservoirs from Supelco (Bellefonte, PA, USA) was used for SPE. Prior to SPE, the sample pH was adjusted with hydrochloric acid. Several sample pH values were tested to optimize the SPE procedure. For method optimization and validation, water samples (50 mL) were spiked with known amounts of each analyte. Cartridges were conditioned with 3 x 3 mL of methanol followed by 3 x 3 mL of ultra-pure water set to the pH of the corresponding sample. Subsequently, the water samples were passed through the cartridges, and the loaded sorbent materials were completely dried by a gentle nitrogen stream. If the sorbent had no anion or cation exchange capacity, the analytes were eluted with 3 x 3 mL of methanol. The strong cation exchanger material (MCX) and the weak anion exchanger materials (X-AW and WAX) were eluted with methanol/NH_4OH (98:2, *v/v*). The weak cation exchange material Strata X-CW was eluted with 2 % formic acid in methanol (*v/v*). SPE extracts were evaporated to dryness in a stream of nitrogen and reconstituted with 400 µL of solvent A and 100 µL of solvent B used for liquid chromatography.

2.2.4 Liquid chromatography electrospray tandem mass spectrometry (LC-ESI-MS/MS) analysis

Liquid chromatography (LC) analysis was carried out using a model 1200 SL HPLC system from Agilent Technologies (Waldbronn, Germany) equipped with a solvent cabinet, a micro vacuum degasser, a binary pump, a high performance autosampler with two 54 vial plates, and a temperature-controlled column compartment.

Several reversed phase (RP) and hydrophilic interaction chromatographic columns were tested. Chromatographic retention and separation was achieved using a Zorbax Eclipse XDB-C8 column (150 mm x 4.6 mm; 5 µm) from Agilent Technologies connected to a C18 guard column (4 x 2 mm) from Phenomenex. The extra-dense bonding (XDB) of organo-silane ligands and the double endcapping deactivates the column’s silica and makes it especially useful for retention of highly polar compounds in RP liquid chromatography. For separation a gradient consisting of (A) 20 mM ammonium acetate in water and (B) 20 mM ammonium acetate in methanol was used. Acetonitrile was also suitable for chromatographic separation, but with respect to the current worldwide shortage of acetonitrile, methanol was used for method development. The gradient program started with 98 % of eluent A, decreased to 25 % A in 13 min, kept isocratic for 4 min, and then returned to initial conditions within 1 min. Before each injection, the column was re-equilibrated for 7 min. Separation took place at 40 °C with a flow rate of 0.8 mL/min. This flow was chosen for optimum chromatographic performance during method development and kept constant thereafter. For the purpose of independent confirmation, a Hypercarb column (150 x 2.1 mm; 5 µm) from Thermo Fisher Scientific (Waltham, MA, USA) was used as a second chromatographic column.

Injection volume was 15 µL, and injector needle and injection port were automatically washed with methanol after each injection to avoid potential carryover. Instrumental and sample preparation contaminations were controlled by measuring injector and SPE blanks at regular intervals of every ten injections.

The HPLC system was connected to an API 4000 Q-Trap triple-quadrupole mass spectrometer (Applied Biosystems/MDS Sciex Instruments, Concord, ON, Canada) with an electrospray interface operated in negative ionization mode. Retention time (RT) windows were defined for every single compound in order to use dwell times which enabled optimized peak-to-noise ratios. All RT windows were set to the following mass spectrometer (MS) parameters: ion spray voltage, −4.5 kV; heater temperature, 550 °C; collision gas, medium; ion source gas ½, 60/75 psi, and curtain gas, 40 psi. Outside the RT windows, solvent flow was directed to waste to prevent the interface from any unnecessary contamination.

The two most intensive transitions between precursor ion and product ions were used for identification and quantification in multiple reaction monitoring (MRM) mode. For cyclamate, only one transition could be obtained. For sucralose, only the isotopic pattern obtained two transitions adequate as qualifier and quantifier. For the results presented, the average of the concentrations calculated for the two transitions (where possible) is reported. Declustering potential, collision energy, and cell exit potential were optimized for each ion transition. Results of this optimization procedure are summarized in Table 2-2. Analyst 1.4 software was used to record and evaluate the obtained chromatographic data.

Table 2-2 Precursor ions, products ions, and corresponding optimized MS parameters

	Precursor ion ***m/z* [M-H]$^-$**	**Product ions (P1/P2)** ***m/z***	**DP** **(V)**	**CE (P1/P2)** **(eV)**	**CXP (P1/P2)** **(V)**
Acesulfame	161.8	81.8/77.9	-35	-20/-42	-11/-1
Cyclamate	177.9	79.9	-35	-38	-1
Saccharin	181.8	41.9/105.9	-75	-48/-26	-5/-3
Aspartame	293.0	260.8/199.9	-55	-16/-20	-13/-9
Neotame	377.1	199.9/345.0	-90	-26/-18	-9/-9
Sucralose	394.7	358.8	-85	-16	-9
	396.8	360.8		-18	-9
NHDC	611.2	303.1/125.1	-150	-50/-64	-13/-7

DP declustering potential, *CE* collision energy, *CXP* cell exit potential

2.2.5 Quantification

For the quantification of sweetener levels, both environmental samples and fortified tap water samples for calibration were subjected to the entire analytical procedure. Surface water samples were analyzed as sampled. STP influent samples were diluted at least by a factor of 10 and STP effluent samples at least by a factor of 5 with Karlsruhe tap water to obtain a matrix, which approximately matches the calibration matrix. All sweeteners, except for sucralose, were quantified by external standard calibration of the entire analytical procedure. The results of the external standard evaluation were not corrected for recoveries deviating from 100 %. A similar approach was applied by Hernando et al. (2004) for the analysis of beta blockers and lipid lowering agents from waste water. These authors demonstrated that a dilution of 1:5 (*v/v*) and 1:10 (*v/v*) resulted in a complete elimination of ion suppression. If weak matrix effects could not have been avoided by sample dilution, the reported environmental levels in this study represent minimal values. Sucralose was quantified by internal standard calibration. For this purpose, the original or diluted water samples were spiked at a level of 200 ng/L of the internal standard (IS) sucralose-d_6 prior to SPE. By this means, matrix effects affecting the quantification of sucralose were corrected. The other analytes under investigation possess significantly different chemical structures compared to sucralose and elute at different retention times (eluent compositions) and, thus, under different ionization conditions. Therefore, sucralose-d_6 was not used as an IS for other sweeteners than sucralose.

2.2.6 Method Validation

An external calibration with directly injected standards ranging from 0.1 ng/mL to 1 µg/mL was set up to determine linearity of detection. An 11-point calibration curve from 1 to 1,500 ng/L was established with spiked tap water samples (50 mL, Karlsruhe tap water, free of any contamination with artificial sweeteners), which were subjected to the entire analytical protocol including SPE. For quantification in the lower range of the calibration, the highest calibration points were excluded. The limits of detection (LOD) and limits of quantification (LOQ) were calculated as three or six times the signal-to-noise ratio, respectively. If wastewater samples or other highly contaminated samples were diluted, the reduced sample volume was taken into account when calculating the LOQ.

Recoveries were determined for drinking water (Karlsruhe tap water), surface water (Rhine river at Karlsruhe), and waste water (municipal STP effluent of STP 1) at two different levels, one in the lower (200 ng/L) and one in the upper part (1 µg/L) of the linear calibration range. The recoveries for the entire sample preparation were calculated by comparing peak areas obtained from samples spiked prior to SPE to peak areas derived from a direct injected standard solution. External standards were prepared by evaporation and reconstitution of the same amount of analytes used for SPE. If native contaminations of artificial sweeteners were present in the original sample matrix, calculated recoveries were corrected for these background contaminations.

For method validation, the quantification of matrix effects for different sample matrices is crucial. Many publications do not differentiate to what extend a higher matrix burden effects the SPE yield or ionization in the interface of the MS or both. To determine if matrix impact on recoveries were due to signal suppression/enhancement or inappropriate SPE conditions, samples were (1) spiked prior to SPE and reconstituted as described above or (2) spiked after SPE by reconstituting the dry residue in a buffer solution containing the same absolute amount of analytes. Both final solutions for HPLC-electrospray tandem mass spectrometry (ESI-MS/MS) measurement contained the same matrix burden, but samples derived from (2) did not undergo changes in analyte concentration during sample enrichment. As a consequence, reduced recoveries for (2) are attributed to signal suppression in the interface.

2.3 Results and discussion

2.3.1 Optimization and validation of LC-MS/MS conditions

The Zorbax Eclipse XDB-C8 (150 x 4.6 mm; 5 µm) column provided excellent retention and separation of all analytes under investigation. For faster sample analyses, the optimized method was transferred to a rapid resolution column with similar column characteristics. The Zorbax Eclipse XDB-C18 RRHT (50 x 4.6 mm; 1.8 µm) is packed with a microparticulate C18 material for high-speed RP-HPLC. The column enabled to reduce the time of analysis more than by half to 9 min with a slightly different gradient (see Table 2-5, and Table 2-6, Supplementary Material, for retention times and gradient programs and Figure 2-4 for the corresponding chromatogram).

Even though in MRM a definite precursor/product ion relationship exists and complete separation is not absolutely necessary in LC-MS/MS, retention time is still an important confirmation tool. An adequate separation is still desired as analytes like acesulfame and cyclamate produced rather unspecific product ions during fragmentation. For difficult environmental matrices like wastewater, we used the Hypercarb column, which provides a completely different retention mechanism (see Figure 2-5, Supplementary Material), to confirm positive results obtained with the Zorbax Eclipse XDB-C18 RRHT.

For all analytes negative electrospray ionization was used (Table 2-2), but even after optimization of the MS parameters, fragmentation and sensitivity remained poor for sucralose, aspartame and NHDC. As the chromatographic conditions were already in an optimum, we added TRIS post-column in order to increase the ionization yield. TRIS, as a strong base, facilitates deprotonation of the weakly acidic analytes. Higher intensity by the addition of TRIS buffer is explained by the high gas-phase proton affinity and the high proton consumption by TRIS in the buffer system (Shen et al., 2005). TRIS buffer was introduced in the flux with a conventional syringe pump connected with a T-piece directly to the interface of the mass spectrometer. With a concentration of 20 mM TRIS and a flow rate of 5 µL/min, signal enhancement ranging from 30 % for NHDC to 290 % for saccharin was achieved. Higher buffer concentrations increased the signal even three to four times for some compounds (see Table 2-7, Supplementary Material, for signal enhancement by addition of TRIS buffer). Gomides Freitas et al. (2004) observed an improvement in sensitivity for herbicides and their metabolites even by a factor of 13-22 when applying TRIS in ESI negative mode. In our case, 20 mM TRIS resulted in sufficient signal enhancement for the poorly ionizable compounds. For further experiments, we did not exceed this concentration to prevent the interface of the mass spectrometer from unnecessary contamination.

2.3.2 Method performance: linearity, recoveries, limits of detection (LOD) and limits of quantification (LOQ)

The calibration with directly injected standards was linear up to 2.25 ng on column for all analytes. All correlation coefficients of both calibration with directly injected standards for the evaluation of detector linearity and calibration including the entire analytical protocol, were higher than 0.995.

For five of seven analytes under investigation method recoveries >75 % were obtained for tap water (50 mL) with Bakerbond SDB 1 cartridges, which were eluted with methanol (see Table 2-8, Supplementary Material, for recoveries of artificial sweeteners in different matrices). For aspartame and NHDC, recoveries were 41 % and 59 %, respectively, which was sufficient for screening purposes. As these two compounds were not detected in any of the analyzed environmental samples, no further optimization of their method recoveries was necessary. In surface water, recoveries decreased slightly for acesulfame and saccharin. For all other compounds, the method proved to be robust for surface water. In waste water, recoveries for acesulfame could not be determined as the native concentration in undiluted STP effluent was still too high to obtain meaningful values for a spike amount of only 1 µg/L. For cyclamate, aspartame, and sucralose, recoveries were higher than 50 %, whereas recoveries for saccharin decreased to 30 %. Neotame was very robust against any matrix effect.

Except for aspartame, recoveries determined by spiking the sample prior to SPE (method recoveries) or during the reconstitution step (ionization recovery, Table 2-8) were similar for all analytes. Thus, reduced recoveries for six of the seven tested artificial sweeteners are rather due to ion suppression in the ESI process than due to losses during sample extraction. In contrast to the other analytes, the method recoveries of aspartame between 41 % and 55 % in all three matrices tested are mainly a consequence of losses during the extraction step.

Contrary to Loos et al. (2009), who reported signal suppression for sucralose of 80 % for waste water and 65% for river water extracted at pH 3, no severe ion suppression for this compound was observed with our method. Higher method recoveries for sucralose in our study (88% compared to 62% (Loos et al. 2009)) at a spiking level of 1 µg/L in tap water can be attributed to more suitable SPE material and/or to a reduced sample volume. We observed similar method recoveries for sucralose as the above mentioned study of about 60 % for Oasis

HLB cartridges at pH 7 but no severe decrease at reduced pH. Furthermore, we studied the effect of a higher sample volume on method recovery. When increasing the sample volume by a factor of 4 to 200 mL, acesulfame and cyclamate partly did break through the sorbent material. Method recoveries for saccharin and sucralose decreased with an increase of interfering substances, if larger sample volumes were extracted.

Based on a sample volume of 50 mL and applying 20 mM TRIS post column with a flow rate of 5 µL/min, limits of quantification were 1 ng/L for neotame, 2 ng/L for acesulfame and saccharin, 5 ng/L for cyclamate, and aspartame and 10 ng/L for sucralose and NHDC.

2.3.3 Occurrence and behavior in environmental samples

Behavior of sweeteners in waste water treatment

In the two German STPs investigated in this study, four of seven artificial sweeteners were detected (Figure 2-1). Influent concentrations were comparable in both STPs for each of the compounds. Concentrations ranged from 34 µg/L and 50 µg/L for acesulfame and saccharin, up to 190 µg/L for cyclamate and below 1 µg/L for sucralose. Elimination of acesulfame and sucralose is low. Acesulfame was removed up to 41 % in STP 1 and was discharged at concentrations higher than 20 µg/L into the receiving waters. Sucralose was eliminated only by about 20 % in both STPs. Our findings support the results obtained by Brorström-Lundén et al. (2007), who reported removal efficiency <10 % for sucralose in corresponding waste water samples. Saccharin and cyclamate were eliminated >90 % in both STPs, but due to high influent concentrations, STP effluent concentrations were still up to 2.8 µg/L. The trickling filter in STP 2 had no additional benefit for the poorly eliminated acesulfame and sucralose but contributed to the overall removal of saccharin and cyclamate. The results found in two STPs clearly show that, due to incomplete elimination during waste water treatment, acesulfame, saccharin, cyclamate, and sucralose are introduced via STPs into rivers and streams used as receiving waters. Other artificial sweeteners were not found in concentrations above the LOQ as they are metabolized in the body.

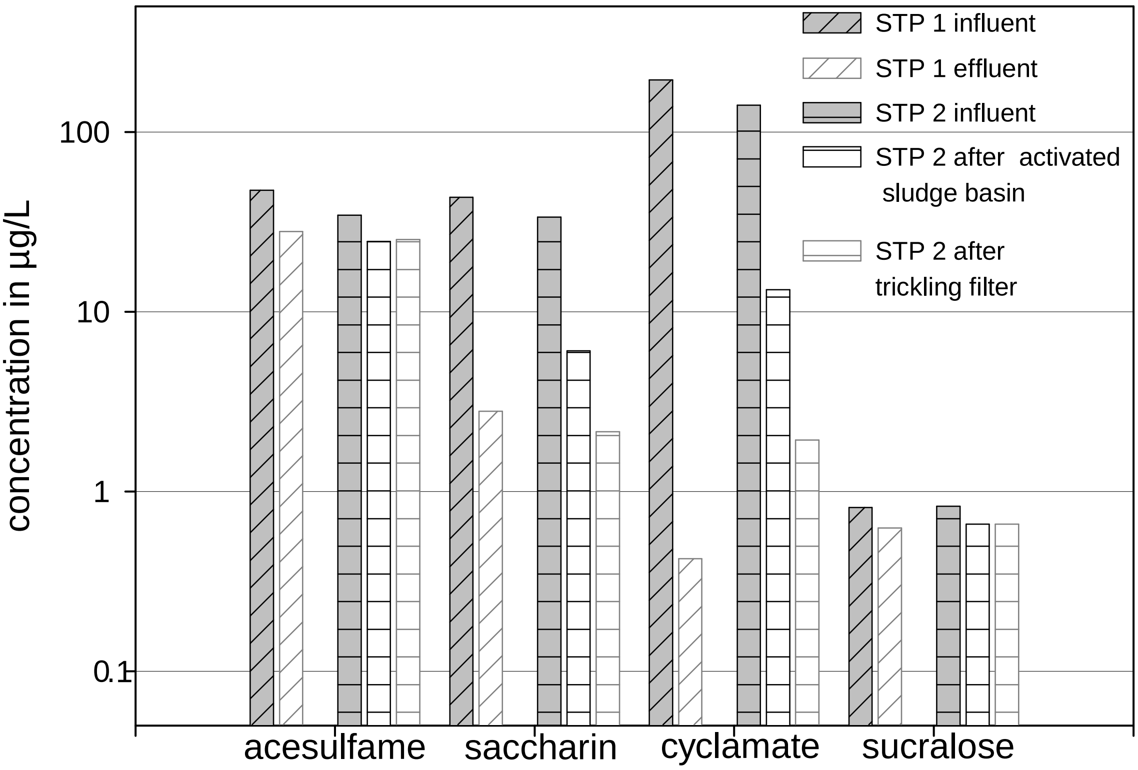


Figure 2-1 Influent and effluent concentrations of artificial sweeteners in two German municipal sewage treatment plants; samplings were in February 2009 for STP 1 and in March 2009 for STP 2

Based on the average flow of the two STPs, daily influent and effluent loads for artificial sweeteners were calculated (Table 2-3). Taking into account the influent concentrations and the number of inhabitants living in the catchment area of the two STPs, overall annual inputs into German STPs comprising 82 millions inhabitants, were extrapolated. Assuming no degradation in the sewer system, the figures give a rough estimation of the consumption of acesulfame, saccharin, cyclamate, and sucralose in Germany.

Table 2-3 Influent and effluent data of artificial sweeteners in two German municipal sewage treatment plants

	Influent load (g/day)	Effluent load (g/day)	Extrapolated total input into German STPs (t/year)	Extrapolated total input into German receiving waters (t/year)
STP Eggenstein-Leopoldshafen (waste water flow, 2,500 m^3/day; population served, 15,000)				
Acesulfame	120	70	240	140
Saccharin	110	7.0	220	14
Cyclamate	490	1.1	970	2.1
Sucralose	2.0	1.6	4.1	3.1
STP Karlsruhe (waste water flow, 96,000 m^3/day; population served, 350,000)				
Acesulfame	3,310	2,420	280	210
Saccharin	3,230	206	280	18
Cyclamate	13,540	185	1,160	16
Sucralose	80	63	6.8	5.4

Behavior of sweeteners during soil aquifer treatment (SAT)

In order to compare conventional waste water treatment as applied in the two German STPs and advanced waste water treatment by soil aquifer treatment (SAT), the behavior of artificial sweeteners was also studied at a SAT site in a Mediterranean country. Like in the German STP effluents, also in the effluent used for SAT, the highest concentrations of artificial sweeteners were found for acesulfame (Figure 2-2 and Table 2-9, Supplementary Material). However, the acesulfame/sucralose ratio in STP effluents in Germany was about 40, whereas it was only 3 for the waste water used for SAT. Assuming similar removal efficiency in conventional treatment in both countries, this finding suggests a significantly different usage pattern of both sweeteners. Acesulfame appeared to be more persistent during SAT than in conventional waste water treatment. In all three sampling campaigns, it was still found downgradient of the percolation basin in well 3, after a residence time of about 1.5 years after discharge at a level of more than 30 µg/L (Figure 2-2). In the STP effluent used for SAT, sucralose concentrations were remarkably higher than in the two German STP effluents. A significant decrease of the sucralose concentration in the aquifer occurred, but it was still present at a level of 1.4 µg/L at well 3 after more than one and a half years in the subsurface. The results for acesulfame and sucralose are remarkable compared to the results of previous studies dealing with the overall removal efficiency of the SAT process for other organic trace pollutants (Drewes et al., 2002; Quanrud et al., 2003; Yoo et al., 2006). Besides acesulfame and sucralose, which were shown here to resist SAT to a certain extent, only few other compounds like carbamazepine and primidone are known to persist during long-term SAT (Drewes et al., 2002). At the sampled SAT site, total organic carbon and most organic trace pollutants were eliminated to about ≥90 % already within the variably saturated vadose zone and are found only in traces in well 1, right below the percolation basin.

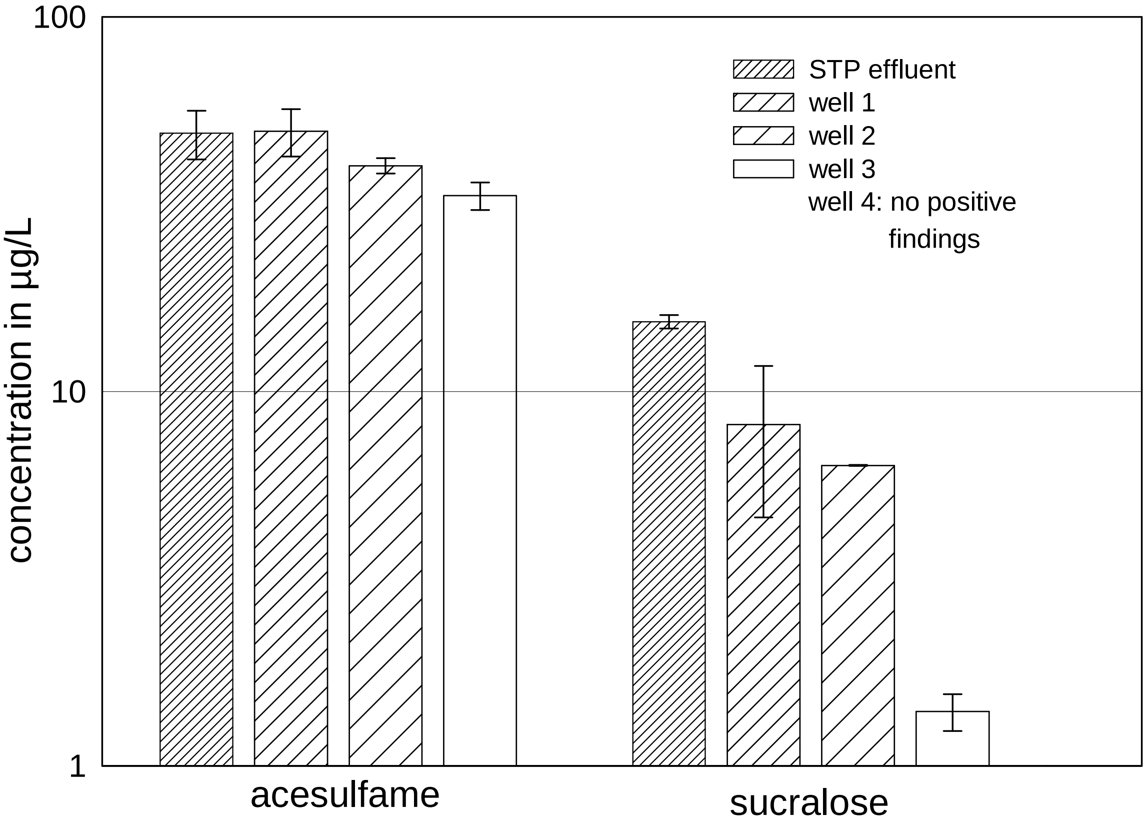


Figure 2-2 Occurrence of acesulfame and sucralose in samples from three sampling campaigns (June 2008, December 2008, February 2009) from a soil aquifer treatment site. Detention times to wells 1, 2, and 3 are approx. 1.5 months, 1 year and >1.5 years (*n* = 3 for recharge effluent, well 1 and well 3, *n* = 2 for well 2 and well 4)

The slow concentration decrease of sucralose is consistent with the slow and incomplete mineralization of sucralose in lake water and in sewage under aerobic conditions. Under anaerobic conditions, little or no mineralization was observed (Labare and Alexander, 1993; Labare and Alexander, 1994). The recalcitrant character of acesulfame and sucralose suggests their use as tracers for anthropogenic contamination of natural waters. Of the remaining studied sweeteners, only cyclamate and saccharin were found at levels up to 400 ng/L in the STP effluent used for SAT but were detected only in traces in the observation wells. As expected, in well 4, which is known to be separated by a hydraulic barrier from any waste water influence, none of the artificial sweeteners was detected.

Occurrence of sweeteners in German surface waters

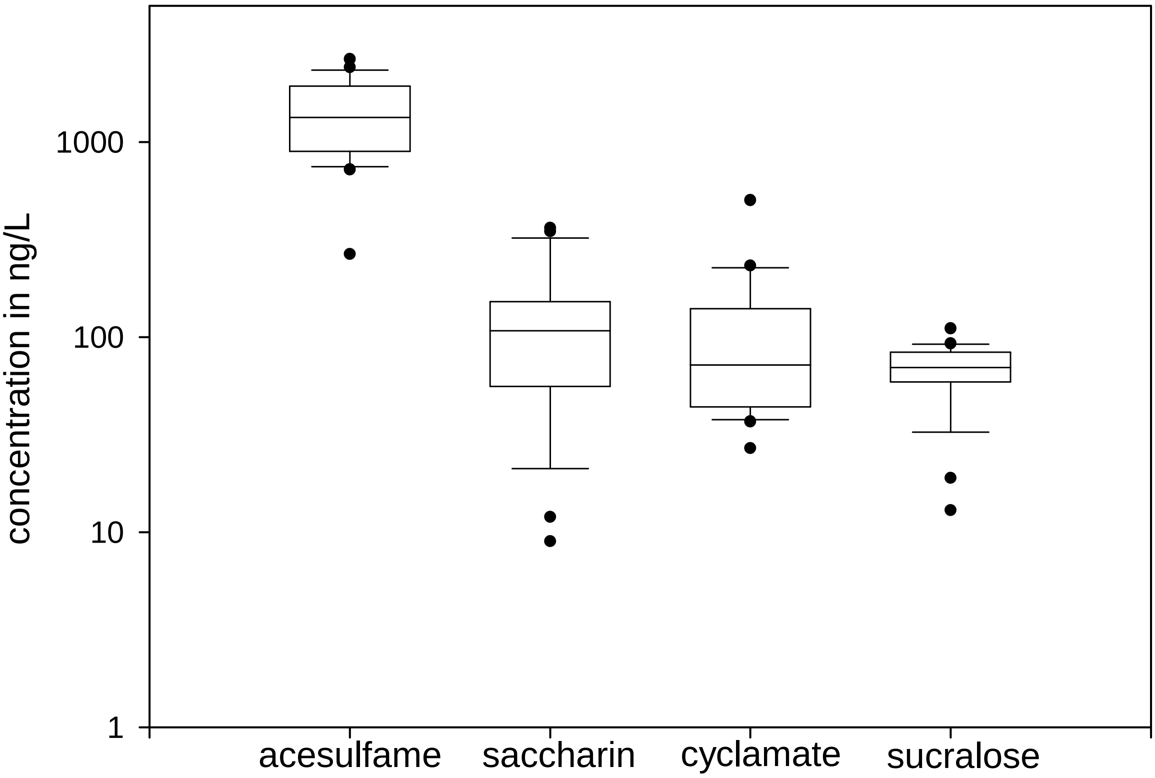


Figure 2-3 Concentrations of four artificial sweeteners in German surface waters (Rhine, Neckar, Danube, Main; *n* = 23)

In all German surface waters analyzed, acesulfame, saccharin, cyclamate, and sucralose were detected, which proved the observed incomplete removal in STPs (Figure 2-3 and Table 2-9, Supplementary Material). Sweetener levels in the investigated German rivers correspond to STP effluent concentrations when taking into account a dilution approximately between a factor of 10 and a factor of 100. Acesulfame was found in several samples in concentrations higher than 2 µg/L and, in most cases, occurred in about tenfold higher concentrations than other sweeteners. Saccharin and cyclamate were detected at levels between 50 and 150 ng/L in the majority of the river water samples. Findings of sucralose in German rivers were in excellent correlation to the values obtained for Germany in the EU wide monitoring program (Loos et al., 2009). Most samples showed sucralose concentrations between 60 and 80 ng/L with only one value exceeding 100 ng/L. Aspartame, neotame, and NHDC were again not detected in any analyzed sample.

2.4 Conclusions

The method developed allows the simultaneous extraction and analysis of seven artificial sweeteners from difficult environmental matrices, such as wastewater and surface water. Accurate quantification could be achieved by the use of a deuterated standard and sample dilution. Application of this method to wastewater samples, samples obtained from a soil aquifer treatment site, and surface water samples demonstrated incomplete removal of some of these compounds during wastewater purification. Due to their use as food additives, the occurrence of artificial sweetener traces in the aquatic environment might become a primary issue of consumer acceptance, especially as the aspect of drinking water quality, which might be negatively influenced by potential metabolites of these trace pollutants, is completely unknown yet.

2.5 Acknowledgements

This study was financially supported by the German Ministry of Education and Research as part of the project 02WA0901. The assistance by our project partners in organizing and execution of the sampling campaigns in the SAT field is kindly acknowledged. We thank the municipalities of Eggenstein-Leopoldshafen and Karlsruhe for providing waste water samples for this study. Furthermore, we thank Doreen Richter for the careful corrections and fruitful discussions on the manuscript.

2.6 Supplementary material

Table 2-4 Total recoveries for artificial sweeteners and different cartridge materials and pH values (sample volume 50 mL, spiked amount 200 ng/L, n=3)

cartridge	**pH**	**acesulfame**		**cyclamate**		**saccharin**		**aspartame**		**neotame**		**sucralose**		**NHDC**	
		recovery in %	**SD**	**recovery in %**	**SD**	**recovery in %**	**SD**	**recovery in %**	**SD**	**recovery in %**	**SD**	**recovery in %**	**SD**	**recovery in %**	**SD**
IST Isolute C18 (1 g)	7	3	1	21	4	19	3	52	1	82	2	75	2	n.a.	
	5	3	1	6	2	6	2	60	2	86	3	65	16	n.a.	
	2	5	0	15	1	18	1	63	1	82	4	60	1	n.a.	
Waters Oasis HLB (60 mg)	7	22	1	26	1	30	2	38	8	93	7	55	6	n.a.	
	2	30	3	24	4	25	1	64	1	105	4	62	2	n.a.	
	3	13	1	13	3	32	1	63	1	101	4	71	2	53	0
Waters Oasis HLB (200mg)	3	27	0	30	0	28	1	59	1	94	2	62	1	47	2
IST Isolute SDB 1 (200 mg)	7	34	0	47	0	96	1	0	0	30	1	88	3	15	7
	5	8	0	12	0	49	0	15	2	94	1	81	0	41	1
	3	75	1	88	4	71	0	78	1	96	3	87	3	56	1
	2	77	0	92	1	67	1	93	1	98	2	91	1	49	3
Phenomenex Strata X-AW (150 mg)	7	48	2	60	2	29	1	55	2	96	1	60	1	n.a.	
Waters Oasis MAX (60mg)	7	0	0	12	11	1	1	56	2	92	4	71	8	22	2
Waters Oasis WAX (60 mg)	7	65	13	72	4	48	12	17	1	99	0	74	5	43	2
	5	39	2	77	6	28	5	22	0	95	3	89	1	34	1
Phenomenex Strata-X (200 mg)	7	20	0	23	1	27	4	36	7	91	7	52	2	n.a.	
Varian PPL (200 mg)	7	22	0	27	1	31	4	41	8	96	7	60	2	n.a.	
IST Isolute ENV+ (200 mg)	7	14	1	12	2	38	5	10	2	57	11	79	6	n.a.	
Waters Oasis MCX (60 mg)	7	1	0	1	0	4	0	8	1	59	6	52	6	0	0

Table 2-5 Retention times (RT) of analytes under investigation on the three different liquid chromatography columns

	Zorbax Eclipse		**Hypercarb**
compound	**C18 RRHT**	**C 8**	
	RT	**RT**	**RT**
acesulfame	1.9	4.5	13.7
cyclamate	3.8	7.7	6.2
saccharin	3.1	6.0	20.9
aspartame	5.5	10.6	21.4
neotame	8.0	15.7	24.8
sucralose	5.1	9.4	26.0
NHDC	6.7	12.7	no elution

Table 2-6 Gradient programs for the three different liquid chromatography columns used for method development and confirmation of results

Zorbax Eclipse XDB-C18 RRHT (50 mm x 4.6 mm; 1.8 µm)

step	**time in min**	**flow rate in µL/min**	**buffer A[a] in %**	**buffer B[b] in %**
0	2	800	98	2
1	0	800	98	2
2	6	800	25	75
3	8	800	25	75
4	9	800	98	2

Zorbax Eclipse XDB-C8 column (150 mm x 4.6 mm; 5 µm)

step	**time in min**	**flow rate in µL/min**	**buffer A[a] in %**	**buffer B[b] in %**
0	7	800	98	2
1	0	800	98	2
2	13	800	25	75
3	17	800	25	75
4	18	800	98	2

Hypercarb (150 mm x 2.1 mm; 5 µm)

step	**time in min**	**flow rate in µL/min**	**buffer A[a] in %**	**buffer B[b] in %**
0	6	350	98	2
1	0	350	98	2
2	20	350	10	90
3	26	350	10	90
4	29	350	98	2

[a] 20 mM ammonium acetate

[b] 20 mM ammonium acetate in methanol

Table 2-7 Signal enhancement by addition of TRIS buffer

	signal enhancement in %			
compound	5 mM TRIS	20 mM TRIS	50 mM TRIS	100 mM TRIS
acesulfame	75	107	111	192
cyclamate	94	214	368	436
saccharin	180	287	330	330
aspartame	158	205	320	340
neotame	35	43	101	142
sucralose	171	222	229	215
NHDC	20	31	53	66

Table 2-8 Recoveries and standard deviations (SD) of artificial sweeteners in different matrices spiked i) prior to SPE or ii) spiked during reconstitution of dry SPE extract with HPLC eluent (initial condition). In both cases the final solutions for HPLC-ESI-MS/MS measurement contained the same theoretical amount of analytes, n=3)

	acesulfame		cyclamate		saccharin		aspartame		neotame		sucralose		NHDC	
	recovery in %	SD	recovery in %	SD	recovery in %	SD	recovery in %	SD	recovery in %	SD	recovery in %	SD	recovery in %	SD
Drinking water (Karlsruhe tap water)														
200 ng/L														
spiked prior to SPE	80	1	92	1	77	7	41	1	86	2	93	2	59	4
reconstituted with spiked buffer solution	93	1	87	8	86	1	82	2	101	2	95	5	75	3
1 µg/L														
spiked prior to SPE	81	1	87	2	76	2	58	1	94	1	88	0	57	2
reconstituted with spiked buffer solution	90	2	92	0	83	1	81	0	101	2	92	1	77	2
Surface water (Rhine river at Karlsruhe)														
200 ng/L														
spiked prior to SPE	60	12	89	3	61	3	46	3	90	3	98	1	63	2
reconstituted with spiked buffer solution	67	5	96	3	66	2	81	2	102	3	89	2	70	1
1 µg/L														
spiked prior to SPE	54	4	81	1	66	2	55	2	93	2	82	2	49	5
reconstituted with spiked buffer solution	52	9	91	3	66	8	76	1	100	2	86	3	69	1
Waste water (Municipal STP effluent)														
1 µg/L														
spiked prior to SPE	-[a]		52	11	30	1	51	1	106	5	48	6	374	31
reconstituted with spiked buffer solution	-		52	3	23	0	54	7	111	3	50	5	156	19

[a] Acesulfame concentrations in undiluted STP effluent samples were too high to determine recoveries, multiplier recording of the mass spectrometer reached upper limit.

Table 2-9 Concentrations of artificial sweeteners in µg/L in recharge effluent used for soil aquifer treatment (SAT) and in observation wells

sampling date sampling point	**acesulfame**	**saccharin**	**cyclamate**	**sucralose**
June 2008				
recharge effluent	53	0.32	0.22	15.4
well 1	47	0.006	<LOQ	4.1
well 2	41	<LOQ	0.018	6.3
well 3	35	0.006	0.031	1.2
well 4	<LOQ	<LOQ	<LOQ	<LOQ
December 2008				
recharge effluent	40	0.20	0.20	15
well 1	44	0.007	0.005	11
well 3	30	0.007	0.044	1.5
February 2009				
recharge effluent	53	0.34	0.40	16
well 1	58	0.021	0.16	9.5
well 2	39	0.015	0.050	6.4
well 3	35	0.022	0.036	1.5
well 4	<LOQ	<LOQ	<LOQ	<LOQ

Table 2-10 Mean concentrations of artificial sweeteners in µg/L in German rivers, n = 1 – 4

river /city	**acesulfame**	**saccharin**	**cyclamate**	**sucralose**
Neckar / Deizisau	1.9	0.12	0.07	0.06
Neckar / Kochendorf	2.2	0.11	0.04	0.09
Neckar / Mannheim	2.7	0.35	0.19	0.11
Main / Frankfurt	1.9	0.26	0.32	0.06
Main / Bischofsheim	2.3	0.20	0.17	0.08
Danube / Ulm	0.73	0.04	0.04	0.02
Rhine / Öhningen	0.27	0.01	0.03	0.01
Rhine / Basel	0.93	0.04	0.05	0.07
Rhine / Weil (Rhine)	0.91	0.08	0.05	0.08
Rhine / Karlsruhe	0.84	0.06	0.06	0.07
Rhine / Mainz	1.2	0.09	0.1	0.08
Rhine / Cologne	1.5	0.14	0.16	0.09
Rhine / Düsseldorf	1.5	0.14	0.14	0.08

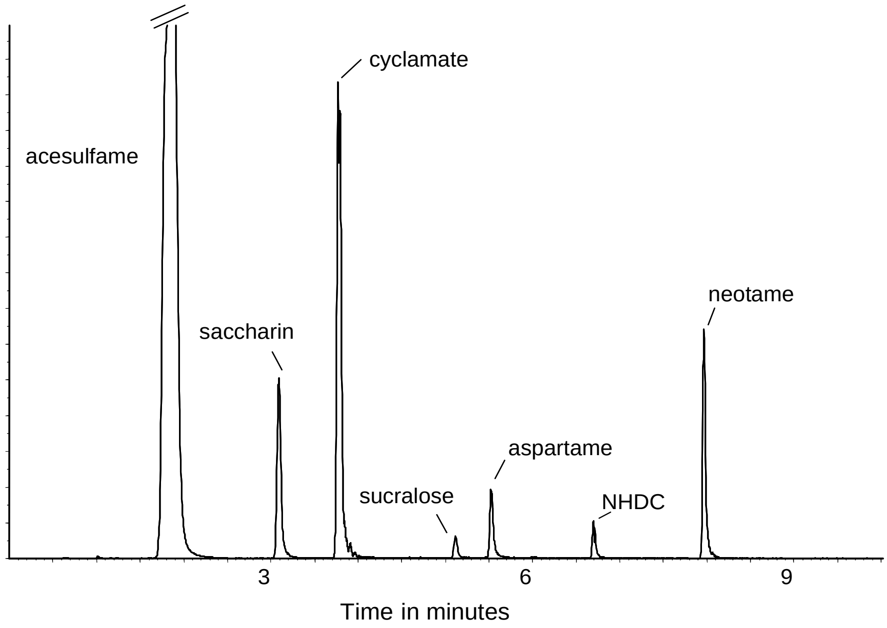


Figure 2-4 Chromatogram of artificial sweeteners on a Zorbax Eclipse XDB-C18 RRHT column (50 mm x 4.6 mm; 1.8 µm) obtained from a 100 ng/L calibration point, injection volume was 15 µL. For gradient program and eluent composition see Table 2-6

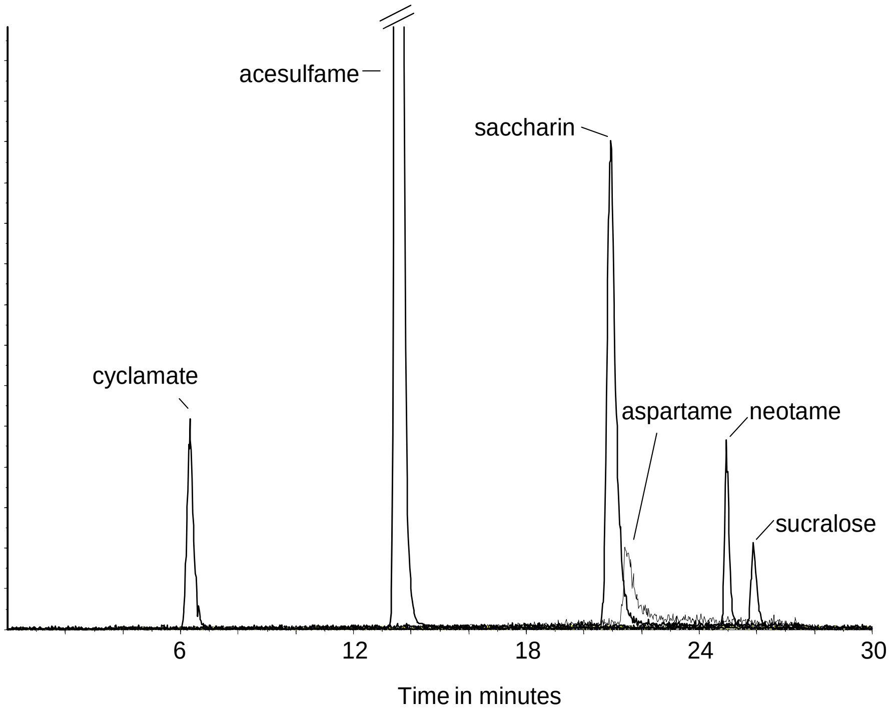


Figure 2-5 Chromatogram of artificial sweeteners on a Hypercarb column (150 mm x 2.1 mm; 5 µm) obtained from a 100 ng/L calibration point, injection volume was 15 µL. For gradient program and eluent composition see Table 2-6

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3

Correlation of six anthropogenic markers in waste water, surface water, bank filtrate, and soil aquifer treatment

Six trace contaminants (acesulfame (ACE), sucralose (SUC), carbamazepine (CBZ), diatrizoic acid (DTA), 1*H*-Benzotriazole (BTZ) and its 4-methyl analogue (4-TTri)) were traced from wastewater treatment plants (WWTPs) to receiving waters and further to river bank filtration (RBF) wells to evaluate their prediction power as potential wastewater markers. Furthermore, the persistence of some compounds was investigated in advanced wastewater treatment by soil aquifer treatment (SAT). During wastewater treatment in four conventional activated sludge WWTPs ACE, SUC, and CBZ showed a pronounced stability expressed by stable concentration ratios in influent (in) and effluent (out) (ACE/CBZ: in45, out40; SUC/CBZ: in1.8, out1.7; ACE/SUC: in24, out24). In a fifth WWTP, additional treatment with powdered activated carbon led to a strong elimination of CBZ, BTZ, and 4-TTri of about 80 % and consequently to a distinctive shift of their ratios with unaffected compounds. Data from a seven month monitoring program at seven sampling locations at the rivers Rhine and Main in Germany revealed the best concentration correlation for ACE and CBZ (r^2=0.94) and also a good correlation of ACE and CBZ concentrations to BTZ and 4-TTri levels (r^2 = 0.66 to 0.82). The comparison of ratios at different sampling sites allowed for the identification of a CBZ point source. Furthermore, in Switzerland a higher consumption of SUC compared to Germany can be assumed, as a steadily increasing ACE/SUC ratio along the river Rhine was observed. In RBF wells a good correlation (r^2=0.85) was again observed for ACE and CBZ. Both also showed the highest stability at a prolonged residence time in the subsurface of a SAT field. In the most peripheral wells ACE and CBZ were still detected with mean values higher than 36 µg/L and 1.3 µg/L, respectively. Although SUC concentrations in wastewater used for SAT decreased by more than 80 % from about 18 µg/L to 2.1 µg/L and 3.5 µg/L in these outlying wells, the compound was still adequate to indicate a wastewater impact in a qualitative way.

Scheurer, M., Storck, F.R., Graf, C., Brauch, H.-J., Ruck, W., Lev, O., Lange, F.T. (2011) Correlation of six anthropogenic markers in wastewater, surface water, bank filtrate, and soil aquifer. J Environ Monitor 13(4), 966-973.

3.1 Introduction

One of the main objectives of drinking water suppliers is to be aware of the influences on their raw water sources used for drinking water production to minimize water treatment effort and to ensure potable water of high quality. This is of particular importance, if an anthropogenic impact on raw water sources by diluted wastewater is given to a certain extent, *e.g.* when river bank filtrate is used as raw water. Therefore, it is necessary to identify markers that are able to provide an early indication of contamination and which can be used for the quantification of the wastewater burden. Furthermore, wastewater tracers can also be used to assess the hydrological fate of biodegradable compounds. The differentiation between transformation and dilution allows for the evaluation of purification processes during river bank filtration (RBF) or soil aquifer treatment (SAT).

A conservative tracer for wastewater impact should neither tend to degrade nor to adsorb. It must be source-specific for wastewater treatment plant (WWTP) effluents and has to be unaffected by the treatment steps applied in the WWTP. Furthermore, it should be persistent in the receiving waters and production wells, respectively. Both, a high effluent concentration as well as a sensitive analytical method, which allows low limits of quantification (LOQ), are also advantageous. For wells unaffected by wastewater the capability of an early detection of traces of the compound can be used as an early warning system of a wastewater breakthrough, *e.g.* when the pumping regime is altered or a leakage in the sewer system occurs.

Microbial indicators for water contamination lack selectivity and are often time-consuming in analysis (Glassmeyer et al., 2005). The approach of using trace elements (Babcock et al., 2006; Schreiber and Mitch, 2006) or their ratios (Vengosh and Pankratov, 1998; Rabiet et al., 2005; Alcala and Custodio, 2008) as conservative tracers raises the problem of distinguishing between their natural background concentrations and anthropogenic origin. A high reliability is only given when background levels are low and differences between their natural presence and wastewater borne concentrations are high.

Therefore, organic trace pollutants with anthropogenic origin have become more and more popular as markers for wastewater impact in the last decade. Caffeine was suggested by several authors (Seiler et al., 1999; Buerge et al., 2003b) due to high concentrations reported for WWTP influents, but its good biodegradability and sorption behaviour seem to limit its use as a potential marker for domestic wastewater. Schramm et al. (2006) reported about compost piles and the agricultural use of cesspool matter as another source of caffeine in addition to WWTP effluents.

Polycyclic musks and nicotine derivates were also presented as possible markers for domestic activities. The latter showed a high biodegradability in WWTPs but a good correlation was observed between the expected anthropogenic burden and the measured concentrations of the nicotine derivates in Swiss lakes (Buerge et al., 2008). Polycylic musks showed the correlation only in winter time whereas a significant loss in the summer occurred in the epilimnion due to their photochemical lability (Buerge et al., 2003a).

Pharmaceuticals and personal care products (PPCP) were also extensively surveyed (Nakada et al., 2008; Kahle et al., 2009; Kasprzyk-Hordern et al., 2009) as their occurrence in water bodies is mostly evoked by human usage. Diatrizoic acid (DTA), an X-ray contrast medium, was one of the most promising candidates among these compounds as it was found to be stable during wastewater treatment and simulated biological sewage treatment (Ternes and Hirsch, 2000; Haiss and Kümmerer, 2006). Another PPCP, which already proved its suitability as a possible wastewater marker due to a pronounced persistence in the aquatic environment, is the antiepileptic drug carbamazepine (CBZ) (Clara et al., 2004). Gasser et al. (Gasser et al., 2010) successfully used carbamazepine to calculate and estimate the impact on drinking water wells by infiltrated wastewater. An approach using benzotriazoles as tracers for domestic wastewater also seems to be promising. They are widely used as corrosion inhibitors, *e.g.* in anti-freezing and deicing fluids as well as in dishwashing liquids (Hart et al., 2004). One of the main benzotriazole chemicals is 1*H*-benzotriazole (BTZ). Its elimination during wastewater treatment is limited and high concentrations of several tens of µg/L in WWTP effluents were reported (Voutsa et al., 2006; Reemtsma et al., 2010). Consequently, BTZ was found in the range from several hundred ng/L in the Danube basin (Loos et al., 2010) up to several µg/L in a Swiss river (Giger et al., 2006). BTZ shows only little sorption onto soils (Jia et al., 2007) and biodegradation in the subsurface is slow as the compound was found in RBF production wells after a travel time of several months (Weiss et al., 2006). The 4-methyl analogue (4-methylbenzotriazole, tolyltriazole, 4-TTri) is even more persistent in the aquatic environment (Weiss et al., 2006).

In recent publications artificial sweeteners (ASs) have emerged as a new class of compounds applicable as anthropogenic markers (Buerge et al., 2009; Scheurer et al., 2009). Especially acesulfame (ACE) and sucralose (SUC), two sweeteners, which are persistent during wastewater treatment, were suggested as tracers. High concentrations of ACE of several µg/L in surface water, groundwater, and wells at river bank filtration sites support this approach. SUC seems to be persistent to a similar extent, but its usage within Europe is rather inhomogeneous based on concentrations measured in rivers and streams (Loos et al., 2009) and generally lower than in the USA.

The aim of this study was to prove the suitability of artificial sweeteners as wastewater markers and to assess their prediction power compared to other organic trace pollutants, in particular to CBZ and BTZ. For this reason we focused not only on one aquatic compartment but followed the ratio of several potential wastewater tracers from their source to receiving waters, RBF, and recovery wells of a SAT site, where treated wastewater is infiltrated.

3.2 Experimental

3.2.1 Materials

Chemical standards were purchased from the following suppliers: DTA (purity 100 %), CBZ, (analytical grade) and BTZ (99 %) from Sigma Aldrich (Steinheim, Germany), DTA-d_6 (98 %) from LGC (Luckenwalde, Germany); ACE potassium (99 %), SUC (>98 %) and CBZ-d_{10} (>99 %) from Dr. Ehrenstorfer GmbH (Augsburg, Germany), ACE-d_4 (98 %), SUC-d_6 (98 %), BTZ-d_4 (98 %) and 4-TTri-d_3 (98 %) from Campro Scientific GmbH (Berlin, Germany), 4-TTri (98 %) from Chemos (Regenstauf, Germany).

3.2.2 Analytical methods

All compounds were preconcentrated using solid phase extraction (SPE) and analyzed by high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS). Samples were pH adjusted in the lab at the day of extraction. Corresponding internal standards for all analytes were spiked into the samples before SPE and used for quantification. Limits of quantification (LOQ) were 10 ng/L in all cases, except for SUC (50 ng/L). The sample preparation and analysis of ASs were described previously in detail by Scheurer et al. (2009).

For DTA analysis 200 mL of a water sample (pH 3) were pre-concentrated using Bakerbond SDB 1 cartridges (200 mg / 6 mL from J.T. Baker, Deventer, The Netherlands). After extraction the cartridges were eluted with 5 mL methanol and 5 mL acetonitrile. The eluate was evaporated to dryness and reconstituted with 500 µL ultra pure water. The compound was separated by a Finnigan Surveyor HPLC on a Hypersil Gold column (150 mm x 2.1 mm; 3 µm) (both Thermo Scientific, Waltham, USA). The HPLC system was connected to a TSQ Quantum Ultra mass spectrometer (Thermo Scientific, Waltham, USA) operated in positive ionization mode.

For the pre-concentration of CBZ the same conditions as described above were applied, with the exception of a higher sample volume of 1 L. After extraction the cartridges were eluted with 10 mL acetone, the eluate was evaporated to dryness and reconstituted with 50 µL methanol followed by 50 µL ultra pure water. The compounds were separated by an Agilent 1100 HPLC (Agilent Technologies, Waldbronn, Germany) on a Luna C18 column (250 mm x 2 mm; 5 µm) (Phenomenex, Aschaffenburg, Gemany). An API 2000 mass spectrometer (AB Sciex, Foster City, USA) was used for detection and quantification of CBZ.

For BTZ and 4-TTri analyses a 10 mL water sample (pH 7) was extracted using Bond Elut PPL cartridges (200 mg / 3 mL from Varian, Palo Alto, USA). For elution of the analytes 6 mL methanol were used. After solvent exchange into 100 µL ultra pure water the compounds were separated by an Agilent 1200 HPLC (Agilent Technologies, Waldbronn, Germany) on a Kinetex PFP column (100 mm x 2.1 mm; 2.6 µm) (Phenomenex, Aschaffenburg, Germany). The HPLC system was connected to an API 4000 mass spectrometer (AB Sciex, Foster City, USA) operated in positive ionization mode.

For further details on chromatographic conditions see supplementary information in chapter 3.6.

3.2.3 Sampling sites and protocol

Wastewater Five WWTPs in Southwest Germany were sampled between May and July 2010. Samples for all WWTPs were corresponding 24 h composite samples. WWTP influents and effluents were stabilized by 100 mg/L NaN_3, stored at 4 °C and analyzed within two days after sampling.

WWTP 1 is applying conventional, *i.e.* mechanical and biological, treatment. It has a capacity of 20,000 population equivalents (PE) with about 15,000 inhabitants living in the catchment area and treats 2,500 to 3,500 m^3/d of domestic wastewater. Hydraulic retention time is about 5 h with an average sludge retention time of 20 d. WWTP 2 treats 40 million m^3 (capacity 875,000 PE) per year for about 350,000 inhabitants. It applies mechanical treatment with additional phosphate precipitation, followed by biological treatment with a denitrification/nitrification unit, equipped with a trickling filter. Hydraulic retention time is about one day for dry weather conditions. WWTP 3 has a rather high industrial impact and is designed for 75,000 PE with about 35,000 people living in the catchment area. The dry weather flow is approximately 12,000 m^3/d but was 28,500 m^3/d at the day of sampling due to heavy rain. Serving 12,000 people, WWTP 4 was the smallest of all WWTPs under investigation. The residence time in WWTP 4 is about 48 h. Therefore, influent and effluent 24 h composite samples are non-corresponding samples. As WWTP 4 was also sampled after a night of heavy rain this effect is even more pronounced. In WWTP 5 a new approach for the effective removal of organic trace pollutants during wastewater treatment is applied. The conventional mechanical and biological treatment is followed by the addition of 5-20 mg/L powdered activated carbon (PAC). After a contact time of >30 min a flocculant agent is dosed and the wastewater is directed to a sedimentation pond, from where some of the PAC is pumped back to the reactor. After sedimentation aluminum salt is added as a second flocculant and the remaining PAC is removed in a two-layer sand filter. WWTP 5 treats 380,000 PE with 220,000 residents living in the catchment area.

Surface water and river bank filtrate Samples from the rivers Rhine and Main were taken as grab samples between January and July 2010. The water was filled into 1 L brown glass bottles and cooled at 4 °C until analysis. Sampling locations were the cities of Basel (km 163.9), Karlsruhe (km 359.3), Mainz (km 500.6), Cologne (km 685.8), Düsseldorf (km 732.1), and Lobith (km 865) along the Rhine. The sampling point at the river Main was 30.1 km upstream from the confluence of Rhine and Main (Figure 3-6). The sampled RBF wells were all located at the river Rhine. They yield exclusively bank filtrate or a mixture of bank filtrate and landside groundwater. Measurements of redox sensitive parameters by the water suppliers confirmed aerobic conditions.

Other data used for evaluation were taken from Bayerisches Landesamt für Umwelt (LfU Bavaria, 2010) which sampled surface and well water in September 2009. Only those samples whose values for all compounds were >LOQ were taken into account. An evaluation of 4-TTri was not possible here, as a mixture of the methyl isomers was measured.

Soil aquifer treatment The SAT site is located in Israel and treats the secondary effluent from a WWTP that processes over 100 million m³/a of wastewater. Treatment includes mechanical treatment, followed by conventional activated sludge treatment including nitrification/denitrification and a limited biological phosphorous removal. The WWTP effluent is spread intermittently in percolation basins, where it infiltrates through an unsaturated zone, up to 40 m in depth. The effluent flows lateral in the saturated zone to observation and recovery wells located in a circle of up to 1,000 m in the periphery of the percolation basins. Samples from the SAT site were taken in the years 2009 to 2010 and comprised the WWTP effluent used for aquifer recharge, a sampling point located vertically below the percolation basin (V1) and four sampling points in the periphery, two in the west (W1 and W2) and two in the east (E1 and E2) of the percolation basin sampling site. Right after sampling the samples were cooled and shipped to Germany for analysis.

3.3 Results and discussion

3.3.1 Wastewater

Six organic trace contaminants CBZ, DTA, ACE, SUC, BTZ, and 4-TTri were analyzed in the sampled wastewater to investigate their suitability as wastewater tracers (Table 3-1). WWTP 1 and 2 were sampled during dry weather conditions in contrast to WWTP 3 and 4 where samples were collected after a night of heavy rain resulting in an almost doubled throughput. This is reflected by the influent concentrations of CBZ, ACE, and SUC in WWTP 3 and 4 which are only about 50% of the levels in the first two WWTPs.

Our results show that the concentration ratios of these three compounds are independent within the investigated German WWTPs. Due to constant concentrations during wastewater treatment, each individual ratio among two of those three compounds in WWTPs 1–4 was neither affected by the applied treatment technologies nor by any other boundary condition (Figure 3-1). A concentration change along the treatment train resulting in lower (or even higher) effluent concentrations is due to changing influent concentrations, and other uncertainties rather than to degradation.

Table 3-1 Influent and effluent data of six potential wastewater tracers in five German municipal WWTPs (concentrations rounded to two significant digits)

WWTP (population served)	**throughput** m^3/d	**influent concentration** µg/L	**calculated consumption** t/a in Germany	**effluent concentration** µg/L	**elimination** %	**input in receiving rivers** t/a in Germany
WWTP 1 (350,000)	70,000					
acesulfame		31	190	24	25	140
sucralose		1.3	7.8	1.1	15	6.6
carbamazepine		0.71	4.3	0.60	16	3.6
diatrizoic acid		4.4	26	2.3	48	14
1*H*-benzotriazole		10	60	5.1	49	30
4-methylbenzotriazole		4.2	25	2.8	33	17
WWTP 2 (15,000)	3,300					
acesulfame		37	240	39	-5	250
sucralose		1.5	9.9	1.53	-1	10
carbamazepine		1.2	7.9	1.0	17	6.6
diatrizoic acid		0.71	4.7	0.40	44	2.6
1*H*-benzotriazole		12	79	1.8	85	12
4-methylbenzotriazole		5.3	35	2.9	45	19
WWTP 3 (40,000)	28,500					
acesulfame		18	370	19	-9	400
sucralose		0.71	15	0.77	-9	16
carbamazepine		0.35	7.5	0.42	-20	9
diatrizoic acid		4.3	92	2.8	35	60
1*H*-benzotriazole		4.8	103	3.7	23	80
4-methylbenzotriazole		2.2	47	2.3	-5	49
WWTP 4 (12,000)	6,250					
acesulfame		17	270	19	-9	290
sucralose		0.7	11	0.77	-10	12
carbamazepine		0.32	5.0	0.50	-56	7.8
diatrizoic acid		<LOQ	-	0.06	-	0.9
1*H*-benzotriazole		4.5	70	2.0	56	31
4-methylbenzotriazole		1.8	28	2.2	-22	34
WWTP 5 (220,000)	151,000					
acesulfame		8.2	170	11	-34	230
sucralose		0.44	9	0.44	0	9.1
carbamazepine		0.33	6.8	0.053	84	1.1
diatrizoic acid		2.2	45	2.9	-32	60
1*H*-benzotriazole		3.9	80	0.87	78	18
4-methylbenzotriazole		2.4	49	0.33	86	7

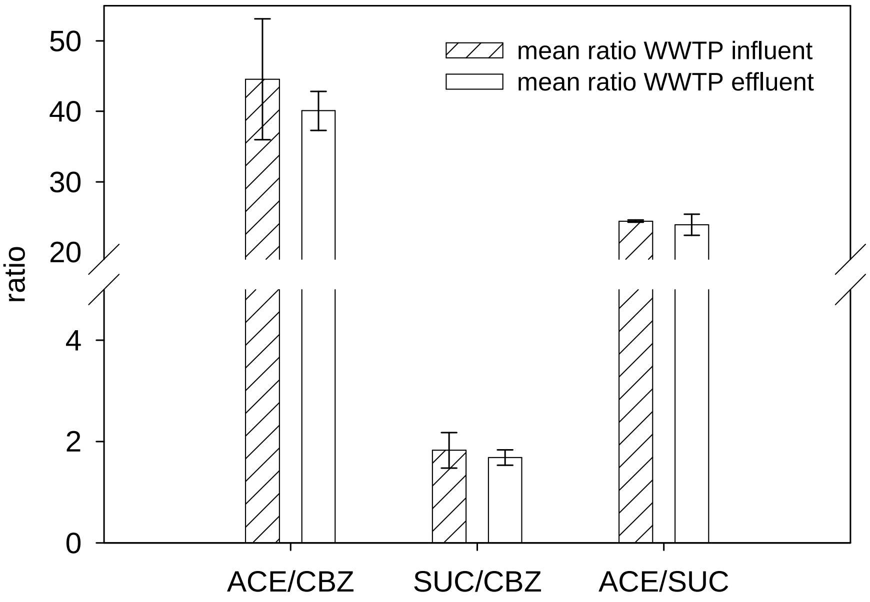

Figure 3-1 Mean ratios of ACE/CBZ, SUC/CBZ, and ACE/SUC based on the influent and effluent concentrations in WWTPs 1-4. Error bars indicate standard deviations

The highest DTA influent concentrations (4.4 µg/L and 4.3 µg/L) were measured in WWTP 1 and WWTP 3 which serve urbanized areas with several hospitals in the case of WWTP 1 and one in the case of WWTP 3 in the catchment area. X-ray contrast media are expected to be used to a much higher extent in hospitals than by physicians in private practice which can be an explanation for the higher values. Even if DTA is source specific to WWTPs and only partly degraded during wastewater treatment, its general use as a tracer seems to be limited and it cannot be used to predict the concentration of persistent trace pollutants. In fact, DTA is suitable to indicate a wastewater impact but the ratio to other organic trace pollutants will vary to a great extent as the usage of DTA depends on its application in the catchment area.

In WWTP 5 a significant shift of the ACE/CBZ and SUC/CBZ ratios from the influent to the effluent was observed. After the secondary sedimentation basin CBZ was still detected with a concentration of 0.38 µg/L (Table 3-5), indicating no biological degradation but a removal (84%) by activated carbon alone (Table 3-1). The input per year of CBZ in the receiving river, calculated by specific throughput conditions at the sampling day, is reduced from 21 kg to less than 3 kg in the case of WWTP 5. PAC seems to be a promising tool not only for minimizing the input of dissolved organic carbon into rivers and streams, but in the case of CBZ, also for organic trace contaminants which proved to be persistent in the aquatic environment. ASs were not affected by the PAC application and the ACE/SUC effluent ratio of 25 is in good correlation to the mean ratio for WWTPs1-4 effluents (23.9±1.5). A good SUC removal by activated carbon during drinking water production has been reported

(Scheurer et al., 2010). It appears that in wastewater treatment a pronounced competition for activated carbon sorption sites occurs and SUC tends to adsorb less strongly than many other wastewater constituents based on its physical-chemical properties.

It has to be noted that presently the application of activated carbon for wastewater treatment is not very common but steadily increasing (*e.g.* WWTP 2 decided to implement this approach in the near future). A widespread usage of activated carbon in WWTPs will consequently lead to a shift in the ratio of some organic trace pollutants which has to be considered in data interpretation in the future. Other treatments like ozonation discussed for the application in WWTPs can also lead to a distinctive transformation of certain compounds, *e.g.* ACE was transformed to a great extend during ozonation in drinking water treatment plants (Scheurer et al., 2010).

3.3.2 Surface water and river bank filtrate

The linear regression displayed in Figure 3-2 is based on all sampling sites along the river Rhine and Main (except for the one in Switzerland, see explanatory statement below), surface water data from LfU Bavaria and wastewater effluent samples. The data for CBZ and ACE correlate well ($r^2 = 0.977$) over the whole sampling period, although we measured values within a broad concentration range from 0.61 µg/L to 3.2 µg/L for ACE along the Rhine and Main rivers (image section of Figure 3-2). The slope of the linear regression indicates that about 28 times more ACE than CBZ was found on average in rivers. The concentrations of the data taken from LfU Bavaria are generally higher than the ones obtained for the river Rhine, where a reasonable dilution factor of the WWTP effluent concentrations of more than ten was observed. The small creeks in Bavaria have a higher wastewater burden due to a lower dilution in comparison to the Rhine, but the data fit to the regression is excellent. The slope of the linear regression when including data of the effluents of WWTPs 1-4 is about 38 which represents a higher ACE/CBZ ratio, although the same ratio in WWTP effluents and receiving rivers can be expected assuming a pronounced stability of ACE and CBZ, respectively. A possible explanation for this phenomenon is a higher consumption of ACE in the summer months as it is one of the main sweeteners in soft drinks. All WWTPs were sampled between May and July. However, data for river water comprise the first seven months in 2010 including rather cold periods when hot beverages are preferred, which are sweetened (when sweetened at all with ASs) more likely with cyclamate and saccharin, which are typical ASs in tabletop sweeteners. To confirm this assumption we used official stream flow values for the sampling point in Basel (values for Germany will not be published before

2011) to calculate the loads of ACE (see supplementary information Table 3-6). Comparing the daily load of the particular days of sampling an increase of more than 30 % to 70 kg/d in June and July compared to the winter months was observed for this sampling point. When using the ratios of two compounds as prediction tool for one of them, it seems that a seasonal variation has to be considered for certain compounds like ASs. The results should be confirmed by long term statistical series or by systematic seasonal sampling of wastewater to prove that the load of ACE is linked to the consumption of certain products based on the ambient temperature.

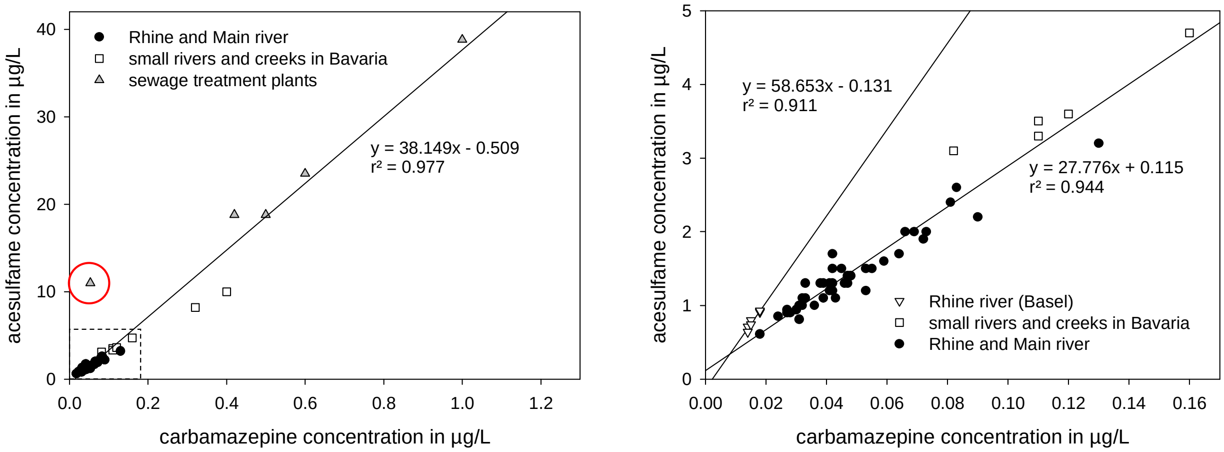

Figure 3-2 ACE over CBZ concentrations of six surface water sampling sites along the rivers Rhine and Main (each n=7), comparative surface water data obtained by LfU Bavaria and effluent concentrations of five WWTP. Circle indicates the application of powdered activated carbon in WWTP 5 (data point not included in linear regression (left)). Image section with corresponding linear regression including the additional sampling point in Basel, Switzerland (right)

The sampling point in Switzerland was not included in the linear regression displayed in Figure 3-2 because the ACE/CBZ ratio was about 59 and thus significantly different from the ratio of 28 along the river Rhine (Figure 3-3 A). Close upstream of the sampling point in Basel a leading producer and supplier of vitamins, personal care products, and pharmaceuticals is located, which we first suspected to be a point source of ACE. It was noticeable that the shifted ratio evened out completely already at the next sampling point downstream (Karlsruhe, distance about 200 km), although the increase in river flow is negligible. Therefore, we examined the shift of these potential tracers *versus* another rather persistent trace pollutant, BTZ (for a graphical presentation of the linear regression of ACE vs BTZ and CBZ vs BTZ see Figure 3-7 supporting information). The results showed almost no change in the ACE/BTZ ratio along the river Rhine sampling locations, including Basel (Figure 3-3 C). However, for the BTZ/CBZ ratio a sharp drop between the sampling points Basel and

Karlsruhe was evident (Figure 3-3 D), indicating a point source for CBZ in between as the only reason for the decrease of the ACE/CBZ ratio. The data set for the three ratios (ACE/CBZ, ACE/BTZ, and BTZ/CBZ) was statistically analyzed with the Tukey-Test ($p<0.05$) and revealed also a significant difference for the sampling point in Basel to all other sampling sites along the river Rhine for the ACE/CBZ and BTZ/CBZ ratios (Figure 3-8). In contrast, the Rhine sampling stations did not differ in the case of the ACE/BTZ ratio based on the same test.

The area of the CBZ input could be further localized by evaluating data from the Rhine monitoring station near the city of Basel, which is located downstream of our sampling point in Basel. At that station, CBZ was measured two to eight times per month between January and July 2010 with a mean value of 0.0281 ± 0.0082 µg/L (Basel-Stadt, 2010). This value is very similar to the mean concentration of our next sampling location (Karlsruhe; 0.0287 ± 0.0071 µg/L) and significantly higher than the one in Basel (0.0157 ± 0.0017 µg/L) narrowing the point source of CBZ down to the area of Basel.

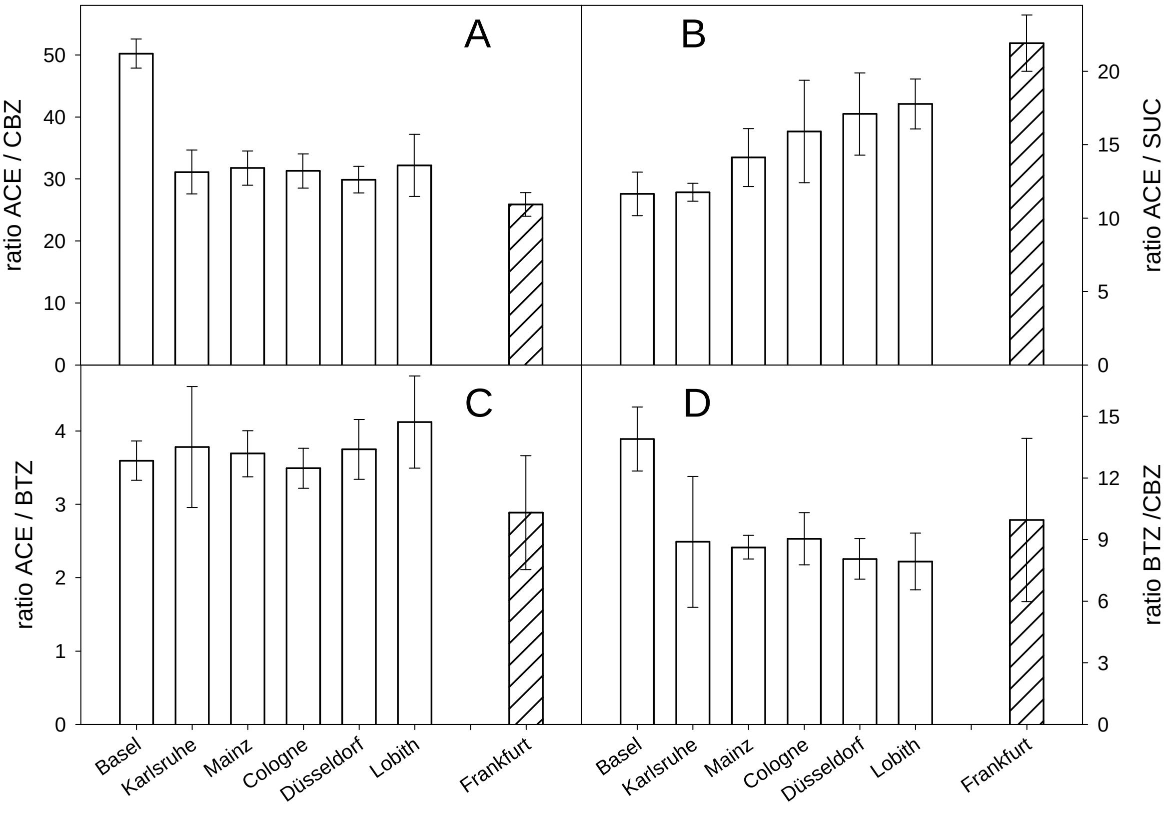

Figure 3-3 Ratios of ACE/CBZ (A), ACE/SUC (B), ACE/BTZ (C), and BTZ/CBZ (D) of six sampling points along the river Rhine (white bars) and at the river Main (striped bars), error bars indicating standard deviation. All sampling points *n*=7

Contemplating the ACE/SUC ratio (Figure 3-3 B), we observed that SUC seems to have a higher per capita consumption in Switzerland than in Germany based on the lower ratio. This effect was clearly leveling along the Rhine, most obviously at the sampling point Frankfurt/Main where no Swiss influence exists. Generally speaking, values from one sampling site to another directly downstream or upstream did not differ significantly, but from all others located further away (Figure 3-8). The observations are supported by Loos and co-authors (2009), who found SUC in every Swiss river water sample (n=7) with a maximum value of 0.45 µg/L, whereas only in seven out of 19 German river water samples the compound was detected above the LOQ with a maximum of 0.08 µg/L. As described above, the dilution factor of the WWTP effluent concentration in the receiving rivers was at least ten. For SUC this gives a concentration range between 0.05 µg/L and 0.17 µg/L, making ACE a much better tracer regarding detectable concentrations. A recent study about consumption related SUC emissions in Linköping/Sweden showed that daily household emissions decreased dramatically from 225 g/d in 2007 to 88 g/d in 2009 (Schmid-Neset et al., 2010). This was mainly attributed to the removal of the SUC containing Coca Cola light from the Swedish market in 2008. A further reduction, due to the announcement of local producers that the compound will also be removed from other consumer products, is expected. This example shows that a sudden pronounced ratio shift has to be investigated carefully as it can be not only a hint for a point source, but also an indication for modified usage patterns or the replacement and ban of certain compounds.

We followed several tracer concentration ratios further to river bank filtration wells to evaluate which compound ratio is most applicable to be used as a prediction tool. Both, ACE as well as CBZ concentrations, correlated well with BTZ in river water samples with r^2 of around 0.8 (Figure 3-7). In samples derived from the river bank filtration wells r^2 decreased dramatically (Figure 3-9), most likely due to a variable degradation of BTZ based on different residence time and environmental conditions in the subsurface. In contrary to BTZ, 4-TTri correlations to ACE and CBZ remained stable when comparing surface water and river bank filtration wells (Figure 3-10 and Figure 3-11) with a better fit for ACE (r^2 = 0.883). For both ratios including 4-TTri the slope of the linear regression shifted only slightly by about 15 %. The higher persistence of 4-TTri in comparison to BTZ in the environment, namely in a partially closed water cycle in Berlin, is supported by a previous study (Weiss et al., 2006). The authors observed a decreasing BTZ/4-TTri ratio from a WWTP effluent containing trench to a receiving lake and further to a transect used for bank filtration. A good correlation in both, river water samples (r^2 = 0.94) and bank filtrate (r^2 = 0.85), was achieved when comparing the two compounds most stable in wastewater treatment, ACE and CBZ (Figure 3-4). The slope of the best fit straight line of ACE and CBZ shifted slightly and simultaneously as observed for the ratios of these two compounds compared to BTZ and 4-TTri. Since ACE was measured in more than 22 times higher concentrations than CBZ, it seems to be a slightly better wastewater tracer, as the detection limits of both compounds are comparable. However, ACE findings should be evaluated carefully as it is widely used in sugar free soft drinks. A spill of 1 L soft drink, containing the maximum ACE level, valid for the European Union, of 350 mg/L (EU, 1994), would “contaminate” 35,000 m³ of water to the level of our LOQ (10 ng/L).

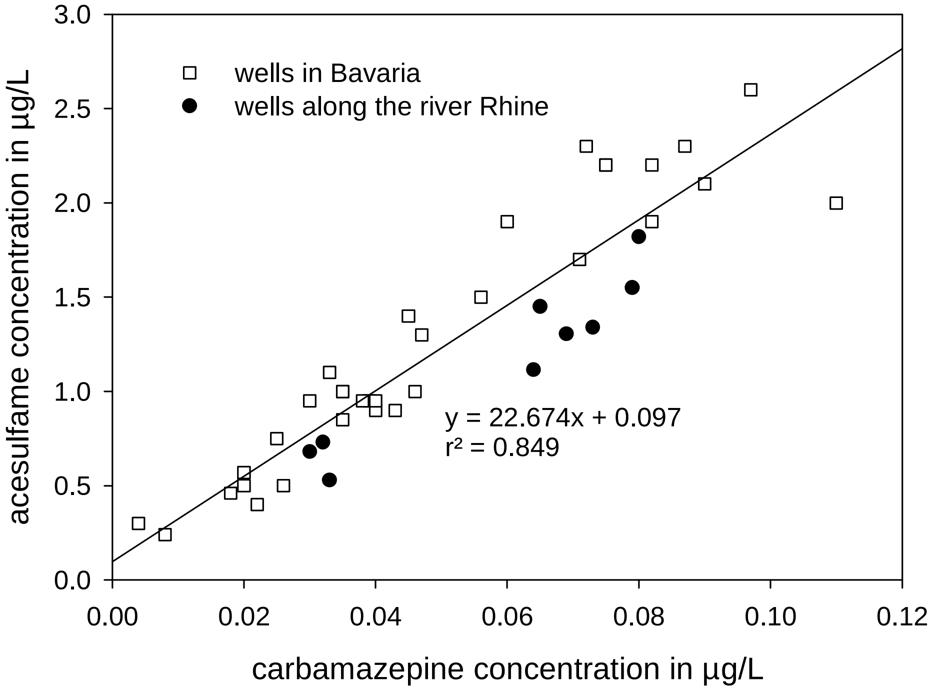

Figure 3-4 ACE over CBZ concentrations of RBF wells along the river Rhine (data from this study) and wells in Bavaria (data from LfU Bavaria, 2010)

3.3.3 Soil aquifer treatment

For the SAT site it became worthwhile to evaluate SUC as a possible tracer for wastewater, as in Israel a remarkably higher consumption than in Germany of this sweetener seems to exist. Whereas in Germany in four out of five WWTPs ACE was detected in about 25-fold higher concentrations (WWTP 5 19-fold) than SUC the ACE/SUC ratio was only about 3 in the WWTP effluent in Israel. Here, average concentrations of ACE and SUC were 55 ± 7.1 µg/L and 18 ± 2.4 µg/L, respectively, whereas CBZ concentrations were in both countries in the same range (1–2 µg/L) (Gasser et al., 2010; Gasser et al., 2011). For the ACE/CBZ ratio a pronounced stability in the variably saturated vadose zone (V1) and for the first recovery wells in the east and west (E1 and W1) was observed (Figure 3-5). The detention time to V1 is about 1.5 months and thus in the same range of riverbank filtrate retention times in Germany where the same persistence of both compounds was observed. Even in E1, which is about 250 m away from the closest recharge basin, no significant decrease of the ACE/CBZ ratio within more than one year of residence time in the subsurface occurred. In wells located further away from the recharge area, with residence times of about two years, a slight decrease of this ratio was monitored due to decreasing ACE concentrations in the periphery. However, in both peripheral wells ACE was still detected with several tens of µg/L. Thus this compound demonstrated its prediction power even at prolonged residence times as a breakthrough of wastewater in unaffected drinking water wells can be observed earlier with ACE rather than with CBZ based on comparable LOQs. For SUC a considerable concentration decrease of more than 50 % was observed in well V1, which is also displayed in a significant change of the ACE/SUC ratio. In laboratory batch experiments with three different soils with different percentages of organic carbon (0.74 %–2.99 %) sorption was neither observed for ACE nor for SUC when a water/soil ratio of 1 : 1 was applied (data not shown). These results support that in the case of SUC biodegradation or slow hydrolysis in the upper layer of the recharge basins is more likely than its removal by sorption processes. Further elimination on the way to the first observation wells (W1 and E1) was minor which revealed the mobility of the compound in the subsurface. Average SUC concentrations in the two outermost wells E2 and W2 were 2.1 µg/L and 3.5 µg/L, respectively, and therefore still higher than the findings for CBZ (1.3 µg/L and 1.6 µg/L).

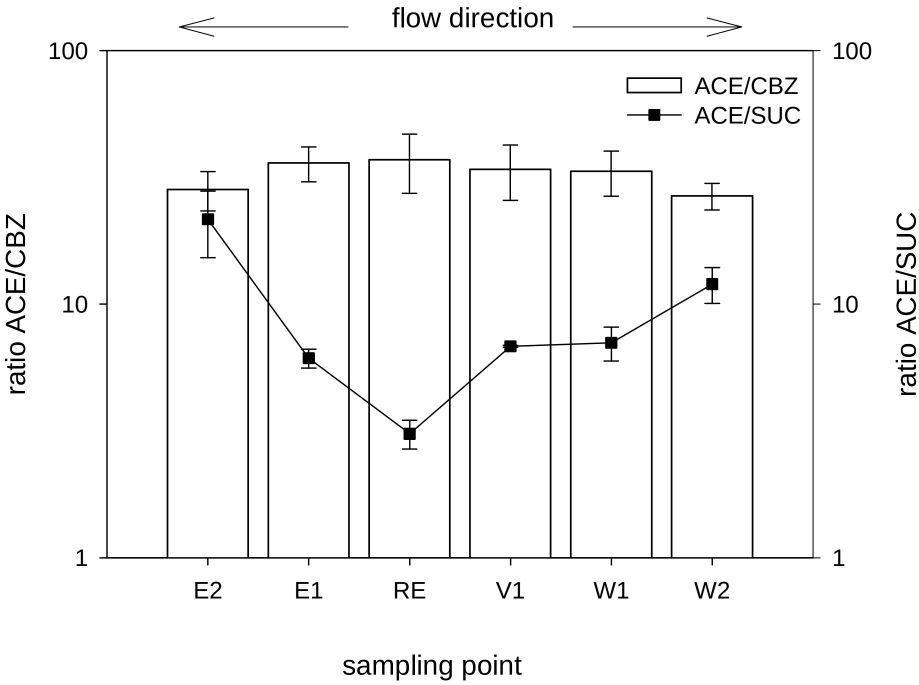

Figure 3-5 Ratios of ACE/CBZ and ACE/SUC in the recharged WWTP effluent (RE), an observation well below (V1), two wells east (E1 and E2) and two wells west (W1 and W2) of the percolation basin of a SAT site in Israel

3.4 Conclusions

Compounds with a pronounced stability during wastewater treatment were ACE, SUC, and CBZ which was consequently shown by their stable ratios within four WWTPs. However, already during wastewater treatment a shift of the ratios is possible when applying further treatment steps like activated carbon or ozonation. Ratios can also be affected by a country specific usage of certain compounds, like ASs. Other possible interferences are point sources deriving from production or application, *e.g.* deicing fluids at nearby airports in the case of BTZ. Even seasonal variations in consumption reflected in different ratios during summer and winter time seem possible in the case of ACE, which is one of the main sweeteners used in soft drinks. All the investigated compounds can give hints for a wastewater impact even for SUC, where a concentration decrease at prolonged residence time in the subsurface was found. The compound is still useful to indicate wastewater impact in a qualitative way for countries like Israel, where it is used in large amounts. The prediction power increases with increasing concentrations and decreasing LOQ. CBZ and ACE seem to have the strongest prediction power if the influence of point sources and regional differences are limited. In any case ratios of organic trace pollutants should not just be transferred from one sampling point to another for the calculation of predicted concentrations as they might vary. Once transferability is checked concentrations of one stable and mobile organic trace pollutant can be estimated with good accuracy based on measurements of another.

3.5 Acknowledgements

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3.6 Supplementary information

Table 3-2 Gradient program for the analysis of DTA

step	time in min	flow rate in µL/min	eluent A[a] in %	eluent B[b] in %	eluent C[c] in %
1	0.0	200	80	10	10
2	1.0	200	80	10	10
3	13.0	200	0	90	10
4	17.0	200	0	90	10
5	17.1	200	80	10	10
6	21.0	200	80	10	10

[a] water with 0.1% formic acid, [b] methanol with 0.1% formic acid, [c] 50 mM ammonium acetate

Table 3-3 Gradient program for the analysis of CBZ

step	time in min	flow rate in µL/min	eluent A[a] in %	eluent B[b] in %
1	0.0	200	80	20
2	20.0	200	0	100
3	28.0	200	0	100
4	29.0	200	80	20

[a] water with 20 mM ammonium formiate, [b] 2/3 acetonitrile 1/3 methanol with 20 mM ammonium acetate

Table 3-4 Gradient program for the analysis of BTZ and 4-Ttri

step	time in min	flow rate in µL/min	eluent A[a] in %	eluent B[b] in %
1	0.0	100	70	30
2	2.0	100	70	30
3	4.0	100	20	80
4	10.0	100	20	80
5	11.0	100	70	30

[a] water with 2 mM ammonium carbonate, [b] methanol with 2 mM ammonium carbonate

Table 3-5 Effluent concentrations of the secondary sedimentation basin in WWTP 5

WWTP (population served)	throughput	effluent concentration secondary sedimentation basin
	$m^3\ d^{-1}$	$\mu g\ L^{-1}$
WWTP 5 (220,000)		
acesulfame	151,200	9.0
sucralose		0.51
carbamazepine		0.38
diatrizoic acid		2.4
benzotriazole		3.2
4-methylbenzotriazole		2.4

Table 3-6 Daily loads of acesulfame in kg/d at the sampling point Basel/Switzerland based on the measured stream flow and detected concentrations at different sampling dates

sampling date	stream flow (Q) in m³/s	acesulfame in µg/L	load in kg/d
12.01.2010	781	0.80	54
11.02.2010	643	0.91	51
08.03.2010	701	0.93	56
07.05.2010	1074	0.71	66
07.04.2010	814	0.74	52
01.06.2010	1688	0.48	70
01.07.2010	1282	0.64	71

Figure 3-6 Sampling points at the rivers Rhine and Main

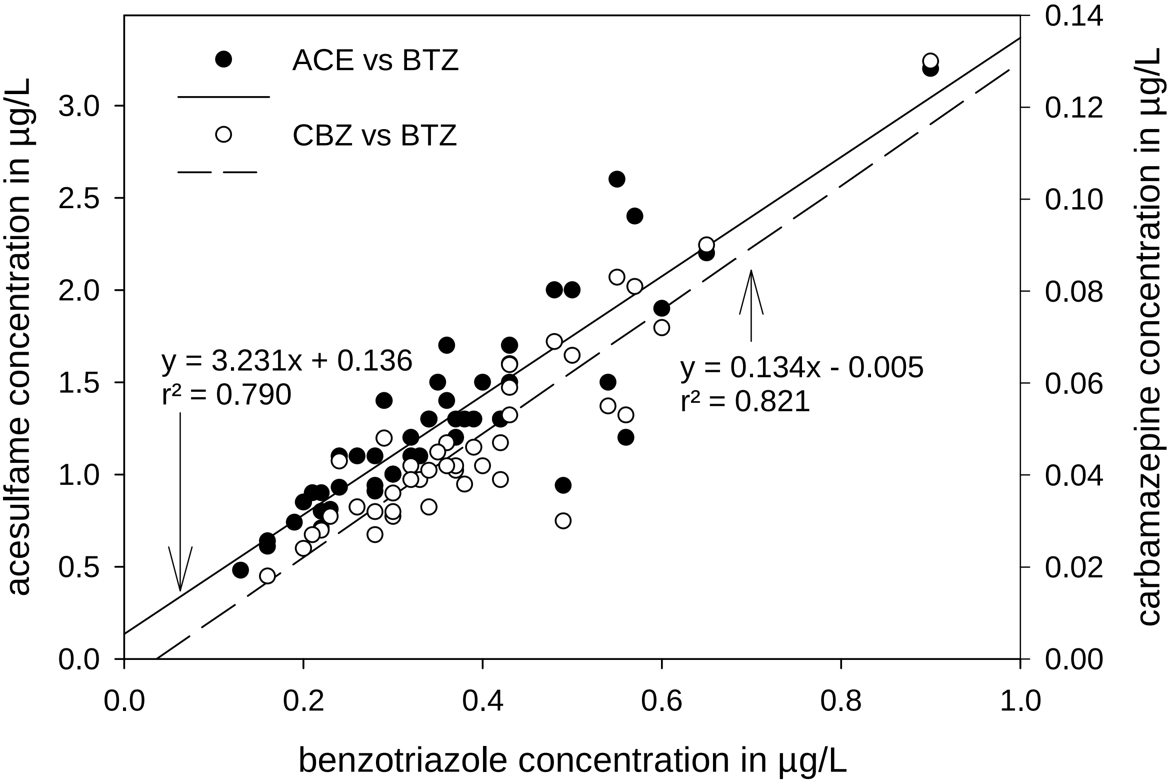

Figure 3-7 Linear regression of ACE vs BTZ and CBZ vs BTZ for all sampling points (except sampling point Basel for CBZ vs BTZ) at the rivers Rhine and Main

ratio ACE / CBZ	Basel	Karlsruhe	Mainz	Cologne	Düsseldorf	Lobith	Frankfurt
Basel		X	X	X	X	X	X
Karlsruhe	X		O	O	O	O	O
Mainz	X	O		O	O	O	X
Cologne	X	O	O		O	O	O
Düsseldorf	X	O	O	O		O	O
Lobith	X	O	O	O	O		X
Frankfurt	X	O	X	O	O	X	

ratio ACE / SUC	Basel	Karlsruhe	Mainz	Cologne	Düsseldorf	Lobith	Frankfurt
Basel		O	O	X	X	X	X
Karlsruhe	O		O	O	X	X	X
Mainz	O	O		O	O	O	X
Cologne	X	O	O		O	O	X
Düsseldorf	X	X	O	O		O	X
Lobith	X	X	O	O	O		O
Frankfurt	X	X	X	X	X	O	

ratio ACE / BTZ	Basel	Karlsruhe	Mainz	Cologne	Düsseldorf	Lobith	Frankfurt
Basel		O	O	O	O	O	O
Karlsruhe	O		O	O	O	O	O
Mainz	O	O		O	O	O	O
Cologne	O	O	O		O	O	O
Düsseldorf	O	O	O	O		O	O
Lobith	O	O	O	O	O		X
Frankfurt	O	O	O	O	O	X	

ratio BTZ / CBZ	Basel	Karlsruhe	Mainz	Cologne	Düsseldorf	Lobith	Frankfurt
Basel		X	X	X	X	X	O
Karlsruhe	X		O	O	O	O	O
Mainz	X	O		O	O	O	O
Cologne	X	O	O		O	O	O
Düsseldorf	X	O	O	O		O	O
Lobith	X	O	O	O	O		O
Frankfurt	O	O	O	O	O	O	

Figure 3-8 Statistical analysis of the calculated ratios by one-factor variance analyses and Tukey-Test with $p<0.05$. X = significantly different, O = not significantly different

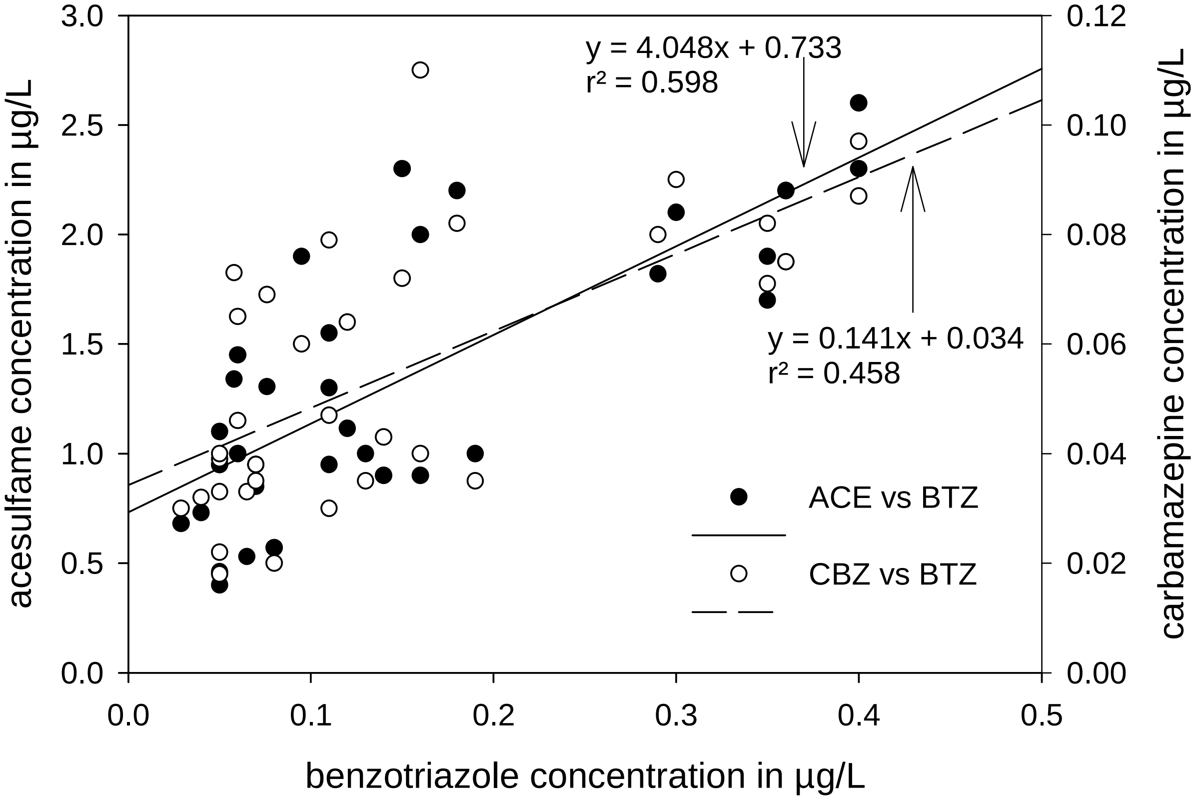

Figure 3-9 Linear regression of ACE vs BTZ and CBZ vs BTZ for RBF sampling points along the Rhine river and wells in Bavaria taken from Bayerisches Landesamt für Umwelt

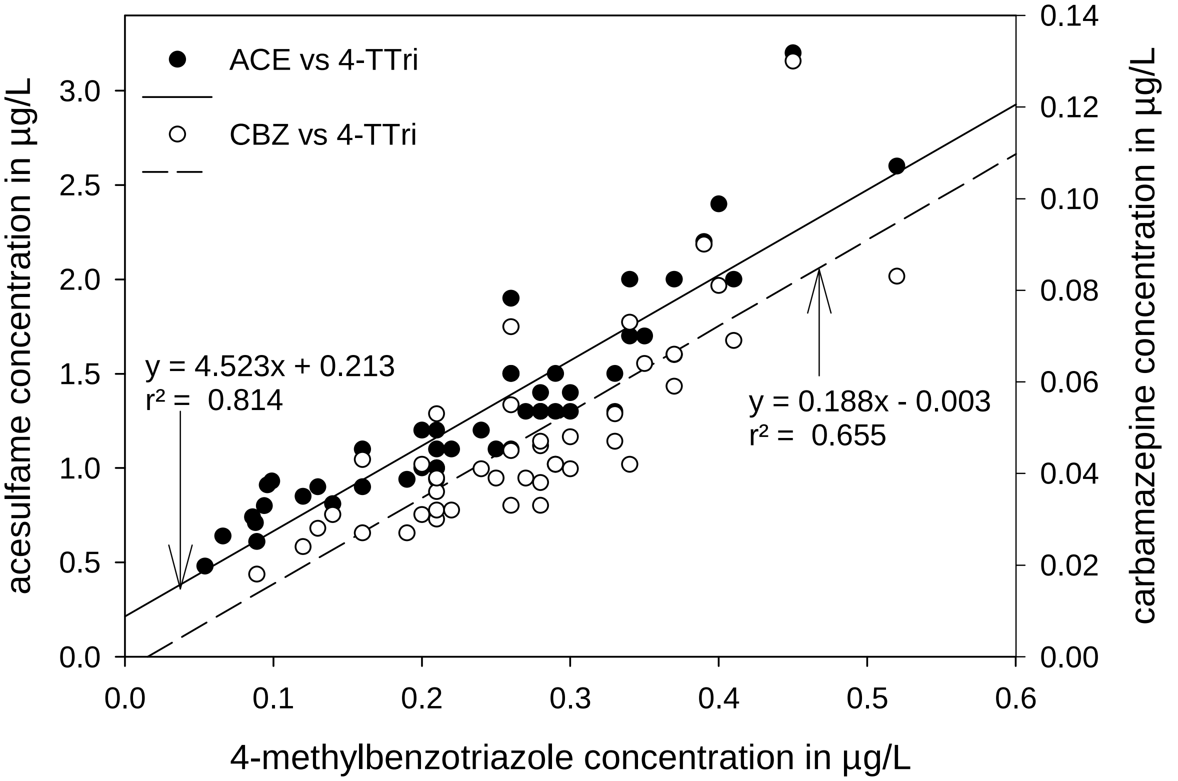

Figure 3-10 Linear regression of ACE vs 4-TTri and CBZ vs 4-TTri for all sampling points (except sampling point Basel for CBZ vs 4-TTri) at the rivers Rhine and Main

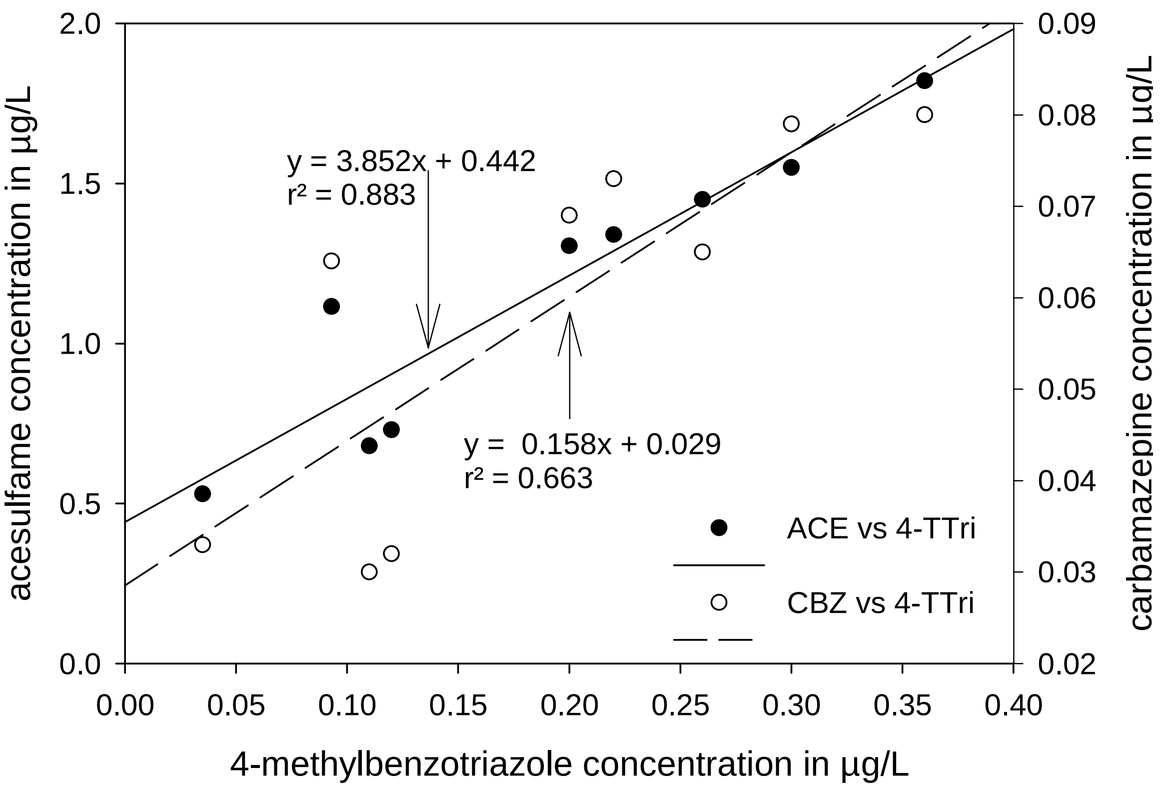

Figure 3-11 Linear regression of ACE vs 4-TTri and CBZ vs 4-TTri for all RBF sampling points at the Rhine river

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4

Performance of conventional multi-barrier drinking water treatment plants for the removal of four artificial sweeteners

Due to incomplete removal of artificial sweeteners in wastewater treatment plants some of these compounds end up in receiving surface waters, which are used for drinking water production. The sum of removal efficiency of single treatment steps in multi-barrier treatment systems affects the concentrations of these compounds in the provided drinking water. This is the first systematic study revealing the effectiveness of single treatment steps in laboratory experiments and in waterworks. Six full-scale waterworks using surface water influenced raw water were sampled up to ten times to study the fate of acesulfame, saccharin, cyclamate and sucralose. For the most important treatment technologies the results were confirmed by laboratory batch experiments. Saccharin and cyclamate proved to play a minor role for drinking water treatment plants as they were eliminated by nearly 100 % in all waterworks with biologically active treatment units like river bank filtration (RBF) or artificial groundwater recharge. Acesulfame and sucralose were not biodegraded during RBF and their suitability as wastewater tracers under aerobic conditions was confirmed. Sucralose proved to be persistent against ozone and its transformation was <20 % in lab and field investigations. Remaining traces were completely removed by subsequent granular activated carbon (GAC) filters. Acesulfame readily reacts with ozone (pseudo first-order rate constant $k = 1.3 \cdot 10^{-3}$ 1/s at 1 mg/L ozone concentration). However, the applied ozone concentrations and contact times under typical waterworks conditions only led to an incomplete removal (18-60 %) in the ozonation step. Acesulfame was efficiently removed by subsequent GAC filters with a low throughput of less than 30 m^3/kg, but removal strongly depended on the GAC preload. Thus, acesulfame was detected up to 0.76 µg/L in finished water.

Scheurer, M., Storck, F.R., Brauch, H.-J., Lange, F.T. (2010) Performance of conventional multi-barrier drinking water treatment plants for the removal of four artificial sweeteners. Water Res 44(12), 3573-3584.

4.1 Introduction

Due to the spreading of the liquid chromatography–tandem mass spectrometry (LC–MS/MS) technique in water analysis in the last decade, a strongly increasing number of polar trace pollutants and some of their transformation products were detected in the water cycle (Richardson and Ternes, 2005; Richardson, 2009). Examples comprise different compound classes, such as pharmaceuticals and personal care products (PPCPs) including X-ray contrast media, poly- and perfluorinated chemicals (PFC), and polar pesticide metabolites, such as N,N-dimethylsulfamide (DMS) (Schmidt and Brauch, 2008), desphenyl-chloridazone and methyldesphenyl-chloridazone (Weber et al., 2007), ethanesulfonic acid (ESA) and oxamylic acid (OSA) metabolites of chloroacetamide herbicides (Hladik et al., 2008). These findings of polar and persistent pollutants pose a new challenge to waterworks treating raw waters, which are directly (*e.g.* unknown sewer leakages) or indirectly (*e.g.* bank filtration or artificial groundwater recharge, AGR) affected by wastewater. Many of these chemicals, which can occur on the ng/L or low µg/L scale in the raw waters are harmless to humans at these trace levels (Snyder et al., 2008), but others are of special concern. The origin of concerns is either due to the toxic properties in animal experiments and accumulation potential in humans (*e.g.* some PFC, such as perfluorooctane sulfonate (PFOS) (Lau et al., 2007; Rumsby et al., 2009)) or because of other reasons, such as increased bacterial resistance through continuing exposure to antibiotics, or disruption of the endocrine system (Jørgensen and Halling-Sørensen, 2000). Another potential risk stems from the transformation potential of precursor compounds into toxic products, such as the carcinogenic N-nitrosodimethylamine (NDMA), generated upon the reaction of non-toxic DMS with ozone (Schmidt and Brauch, 2008).

Another class of compounds, recently detected in the water cycle, are artificial sweeteners (AS). These anthropogenic and xenobiotic compounds are high production volume chemicals, with supplies of saccharin, aspartame, acesulfame K, sucralose, and cyclamate accounting for 4.57 million tons of sucrose equivalents in 2007 in the U.S. (Haley et al., 2008). AS are used world-wide as table-top sweeteners as well as food additives to sweeten diet beverages, pharmaceuticals and some personal care products, such as tooth pastes and mouth washes (Weihrauch and Diehl, 2004; Zygler et al., 2009). Although minor direct inputs into the aquatic environment by improper disposal cannot completely be excluded, the typical entrance pathway of AS is via municipal wastewaters. Sucralose, the active ingredient of the well-known sweetener Splenda®, was the first AS analyzed as trace impurity in wastewater treatment plant (WWTP) effluents and surface waters in Europe (Brorström-Lundén et al., 2008; Loos et al., 2009). Recently, three additional AS, acesulfame, cyclamate, and saccharin, were detected in Swiss and German wastewaters (Buerge et al., 2009; Scheurer et al., 2009). In the latter investigations, acesulfame and sucralose turned out to be the most stable of the targeted AS. Traces of all four AS were also found in surface and groundwaters. In Germany and Switzerland the AS measured at the highest level was acesulfame. Its concentrations ranged up to 2.7 µg/L in river water (Neckar river) (Scheurer et al., 2009), 2.8 µg/L in lake water (lake Greifensee) (Buerge et al., 2009), and 4.7 µg/L in groundwaters (Buerge et al., 2009).

A first measurement of AS in a drinking water supply in Switzerland, where aeration of groundwater for iron and manganese removal was the only treatment step, revealed acesulfame to be present in tap water in concentrations up to 2.6 µg/L (Buerge et al., 2009). The toxicological properties of AS are well studied and show that µg/L quantities are harmless to humans (Weihrauch and Diehl, 2004). However, data on ecotoxicological properties of AS are scarce. For example, in the EU there is no obligatory environmental risk assessment for AS according to the European Parliament and Council directive on sweeteners for use in foodstuffs (EU, 1994), which is based on the framework directive for food additives (EU, 1988). Therefore, it is yet unknown, what the occurrence of these trace pollutants means to aquatic biocenoses. In sugarcane, sucralose can even inhibit the transport of the structurally strongly related sugar sucrose, an important transport and signal compound (Reinders et al., 2006). It was also speculated, that sucralose might interfere with plant photosynthesis (Lubick, 2008) or deteriorate functions such as orientation, food or partner localisation.

Both above-mentioned Swiss and German studies suggested acesulfame to be an ideal tracer to quantify the impact of wastewater derived proportions of raw waters. Besides this, there is first evidence that during the ozonation process in waterworks acesulfame readily reacts with ozone to not yet identified products (Buerge et al., 2009).

The aim of this study was to investigate in more detail the fate of the four mentioned AS in conventional multi-barrier treatment plants including bank filtration, artificial recharge, flocculation, ozonation, activated carbon filtration, and disinfection by chlorine and chlorine dioxide as treatment steps. For this purpose, the concentration decrease of AS was studied within six waterworks using river water, bank filtrate, or artificially recharged groundwater as raw water. The focus was directed to those four out of seven AS, which were found in surface waters in a preceding study (Scheurer et al., 2009). To confirm the results obtained from field studies, the main treatment steps were simulated in laboratory experiments.

4.2 Material and methods

4.2.1 Laboratory experiments

Analytical method

The AS were analyzed by high performance liquid chromatography–electrospray tandem mass spectrometry (HPLC–ESI–MS/MS) after solid-phase extraction according to a recently published method (Scheurer et al., 2009). In the present study acesulfame-d_4 was used as an additional internal standard for the quantification of acesulfame. The majority of samples were analyzed according to the described protocol. In addition, some samples of laboratory experiments with elevated concentrations >2 µg/L were analyzed by direct injection (15 µL) LC–ESI–MS/MS with external standard calibration. In the present study reporting limit of AS concentrations was 10 ng/L.

Fixed-bed bioreactor

A laboratory test filter unit, established to simulate aerobic degradation processes during river bank filtration was used to study the biodegradation of artificial sweeteners (Karrenbrock et al., 1999; Knepper et al., 1999a; Knepper et al., 1999b). A glass bottle used as a reservoir was filled with 10 L of unfiltered surface water (Rhine river at Karlsruhe) and spiked at a level of 1 µg/L of the test compounds in aqueous solution. The water was pumped bottom-up in recirculation with a flow rate of 17 mL/min over a filter column filled with sintered glass beads (SIRAN-Carrier no. 023/02/300, Schott Engineering GmbH, Mainz, Germany) used as carrier material. The porous material is non-adsorptive and provides optimum conditions for the formation of a biofilm with similar biological activity as observed in the colmation layer/infiltration zone during river bank filtration. The system was stored in the dark at room temperature (20 ± 2 °C) and aerated with compressed air to ensure aerobic conditions during the running period of over 90 days.

Flocculation

The removal of artificial sweeteners during flocculation was investigated in batch experiments with Karlsruhe tap water and water from the Rhine river. A Stuart flocculator SW6 (Bibby Sterlin Ltd., Stone Staffordshire, UK) was used for batch jar tests. The system consisted of six units with 1 L glass beakers and stirrers with a defined stirring depth. 800 mL of Karlsruhe tap water and river water were spiked at a level of 1 µg/L with the test compounds in aqueous solution. Polyaluminum chloride ($AlCl_3$) and iron chloride ($FeCl_3$) were used as flocculants and added in doses of 5, 10 and 15 mg/L. The batches were stirred at a velocity of 250 rpm for 30 s to achieve an evenly rapid distribution of the flocculation agents. No flocculation aid was added. The velocity was reduced to 50 rpm, held for 5 min and then further reduced to 25 rpm and held for 15 min for the formation and the growth of flocs. After sedimentation of the flocs for 60 min, 100 mL of supernatant was taken for the analysis of artificial sweeteners. The pH was controlled between 7 and 8 to ensure the proper precipitation of the Al or Fe hydroxides and low residual concentrations of dissolved Fe(III) or Al(III) (DVGW, 1998).

Ozonation

Ozonation experiments were performed as batch experiments in 5 L glass vessels filled with Karlsruhe tap water. This water was chosen as a matrix similar to that in waterworks. It had a pH of 7.3, a dissolved organic carbon (DOC) content of 0.9 mg/L, and a hydrogen carbonate concentration of 305 mg/L (5 mmol/L). The initial sweetener concentration was 1 µg/L and four different initial ozone concentrations (0.5, 1, 2, 5 mg/L) were applied. For this purpose 5 mL of a 1 µg/mL aqueous sweetener solution was dissolved in 2 L of tap water and filled up to a total volume of 5 L with tap water and, finally, with the necessary volume of a ozone stock solution (concentration between 17 and 28 mg/L). The test set up was slightly stirred on a magnetic stirrer to spread the ozone evenly. Samples (100 mL) were taken after contact times between 1 and 60 min and residual ozone was reduced by sodium sulfite. Complete ozone removal was confirmed by redox potential measurement.

Activated carbon small-scale filter test

The removability of artificial sweeteners by GAC filtration was investigated using ground (300-400 µm) and washed Filtrasorb 300 (F 300, Chemviron Carbon, Feluy, Belgium), a carbon type often applied in waterworks. To test the removability of artificial sweeteners from running water, a rapid small-scale filter column test, developed by Marcus (2005) and optimized by Happel et al. (2009), was used to assess the adsorbability of sweeteners. Substances were tested using i) a single compound solution with a sweetener concentration of 500 µg/L and ii) a mixture of the four sweeteners each with a concentration of 125 µg/L. To suppress biological activity, 100 mg/L sodium azide were added. The column feed concentrations reflect a compromise between realistic environmental concentrations and the applicability as a rapid laboratory test system. Substances are considered to be relevant for drinking water, if up to the point of 15,000 bed volumes treated (BVT), *i.e.* 30 m³/kg, a breakthrough of more than 10 % of the feed concentration occurs. With a flow rate of

8 mL/min the test can provide information about the general removability of a contaminant by GAC within one week.

Chlorination

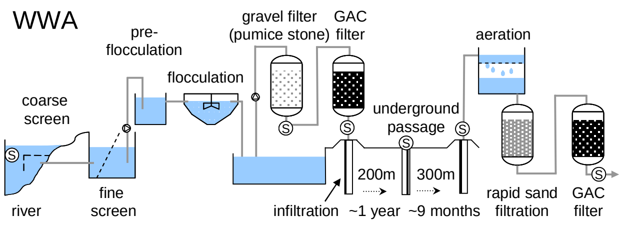
The behavior of artificial sweeteners during chlorination, a method which is frequently used for drinking water disinfection, was determined in a laboratory batch test using drinking water (pH 7) spiked at a level of 1 µg/L of artificial sweeteners. Free chlorine was added at two different levels of 0.2 and 1 mg/L by addition of a commercially available aqueous hypochlorite solution. Prior to use, the concentrations of HOCl stock solutions were determined photometrically after reaction with (N,N-diethyl-p–phenylendiamine, DPD). Samples (100 mL) were taken after contact times of 0.17, 2, 4, 8, 24, and 48 h and residual chlorine was reduced by 50 mg sodium thiosulfate.

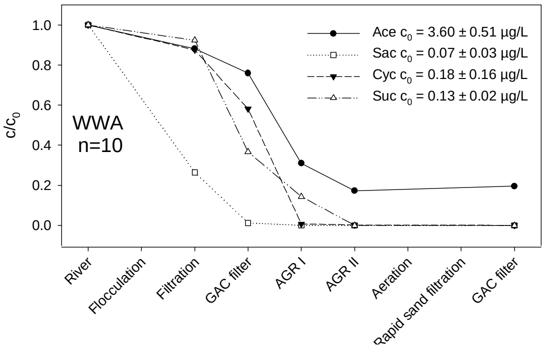
4.2.2 Sampling sites and protocols

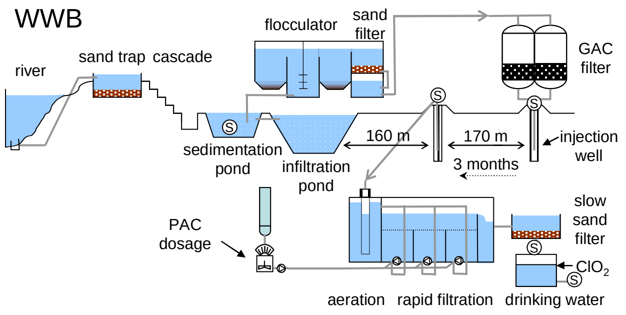
In order to clarify the behavior of artificial sweeteners under waterworks (WW) conditions, six facilities were sampled up to ten times between August and December 2009. These facilities are briefly described below (Figure 4-1).

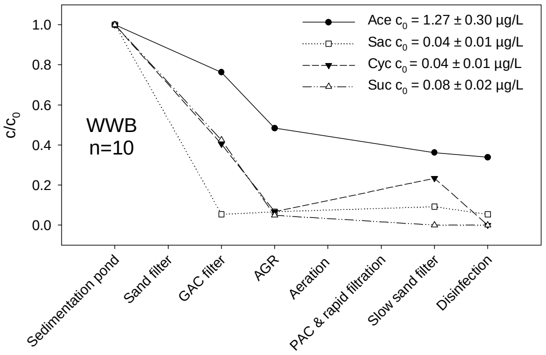
Waterworks A (WWA)

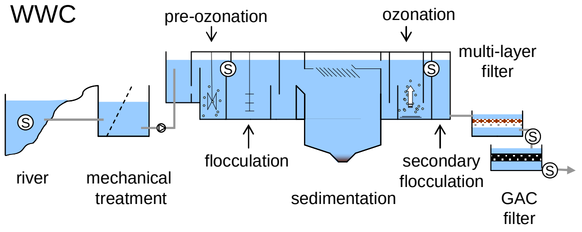
The facilities of WWA were mainly set up for groundwater recharge with treated water of the Main river and subsequent treatment of the recovered groundwater for drinking water production. The river water is purified by coarse and fine screens, flocculation, and filtration over a gravel filter and GAC. All eight GAC filters have a diameter of 3 m and were filled with activated carbon to a height of 3 m. The filter velocity was about 10 m/h. Every six months the GAC of four out of eight filters is regenerated when a mean load of approximately 60 m³/kg is reached. The infiltrated water passes a sandy soil layer zone of 500 m horizontal distance and 20 m of vertical distance and is recovered 16 to 24 months (usually about 21 months) after infiltration as a mixture of up to 70 % of infiltrate and 30 % of local groundwater. The recovered raw water is again treated by aeration, rapid sand filtration and an activated carbon filter unit. Sampling points at WWA within the surface water treatment facility were the raw water, the effluents of the gravel filter and the activated carbon filter. Within the groundwater treatment facility, an observation well half way to the recovery well and the recovered raw water before and after its repeated treatment with activated carbon were sampled. Samples were taken weekly over a ten week period (n=10).

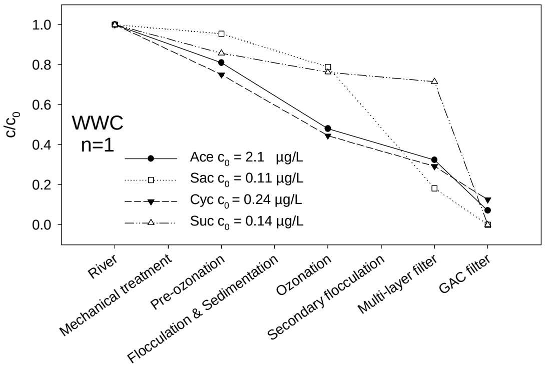

WWA
coarse screen
river
fine screen
pre-flocculation
flocculation
gravel filter (pumice stone)
GAC filter
underground passage
aeration
infiltration
200m
~1 year
300m
~9 months
rapid sand filtration
GAC filter
S

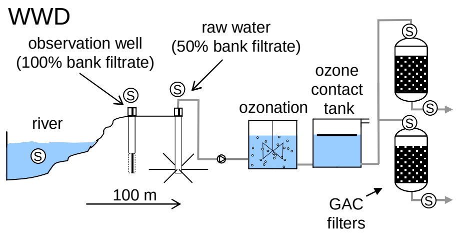

c/c0
WWA
n=10
Ace c0 = 3.60 ± 0.51 µg/L
Sac c0 = 0.07 ± 0.03 µg/L
Cyc c0 = 0.18 ± 0.16 µg/L
Suc c0 = 0.13 ± 0.02 µg/L
0.0
0.2
0.4
0.6
0.8
1.0
River
Flocculation
Filtration
GAC filter
AGR I
AGR II
Aeration
Rapid sand filtration
GAC filter

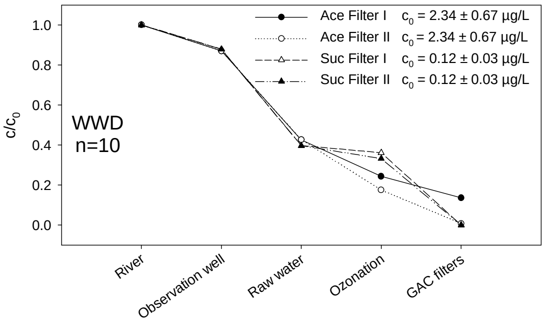

WWB
river
sand trap
cascade
flocculator
sand filter
GAC filter
sedimentation pond
infiltration pond
160 m
170 m
3 months
injection well
PAC dosage
slow sand filter
ClO2
aeration
rapid filtration
drinking water
S


c/c0
WWB
n=10
Ace c0 = 1.27 ± 0.30 µg/L
Sac c0 = 0.04 ± 0.01 µg/L
Cyc c0 = 0.04 ± 0.01 µg/L
Suc c0 = 0.08 ± 0.02 µg/L
0.0
0.2
0.4
0.6
0.8
1.0
Sedimentation pond
Sand filter
GAC filter
AGR
Aeration
PAC & rapid filtration
Slow sand filter
Disinfection


WWC
pre-ozonation
ozonation
multi-layer filter
river
mechanical treatment
flocculation
sedimentation
secondary flocculation
GAC filter
S


c/c0
WWC
n=1
Ace c0 = 2.1 µg/L
Sac c0 = 0.11 µg/L
Cyc c0 = 0.24 µg/L
Suc c0 = 0.14 µg/L
0.0
0.2
0.4
0.6
0.8
1.0
River
Mechanical treatment
Pre-ozonation
Flocculation & Sedimentation
Ozonation
Secondary flocculation
Multi-layer filter
GAC filter


WWD
observation well (100% bank filtrate)
raw water (50% bank filtrate)
river
ozonation
ozone contact tank
100 m
GAC filters
S


c/c0
WWD
n=10
Ace Filter I c0 = 2.34 ± 0.67 µg/L
Ace Filter II c0 = 2.34 ± 0.67 µg/L
Suc Filter I c0 = 0.12 ± 0.03 µg/L
Suc Filter II c0 = 0.12 ± 0.03 µg/L
0.0
0.2
0.4
0.6
0.8
1.0
River
Observation well
Raw water
Ozonation
GAC filters

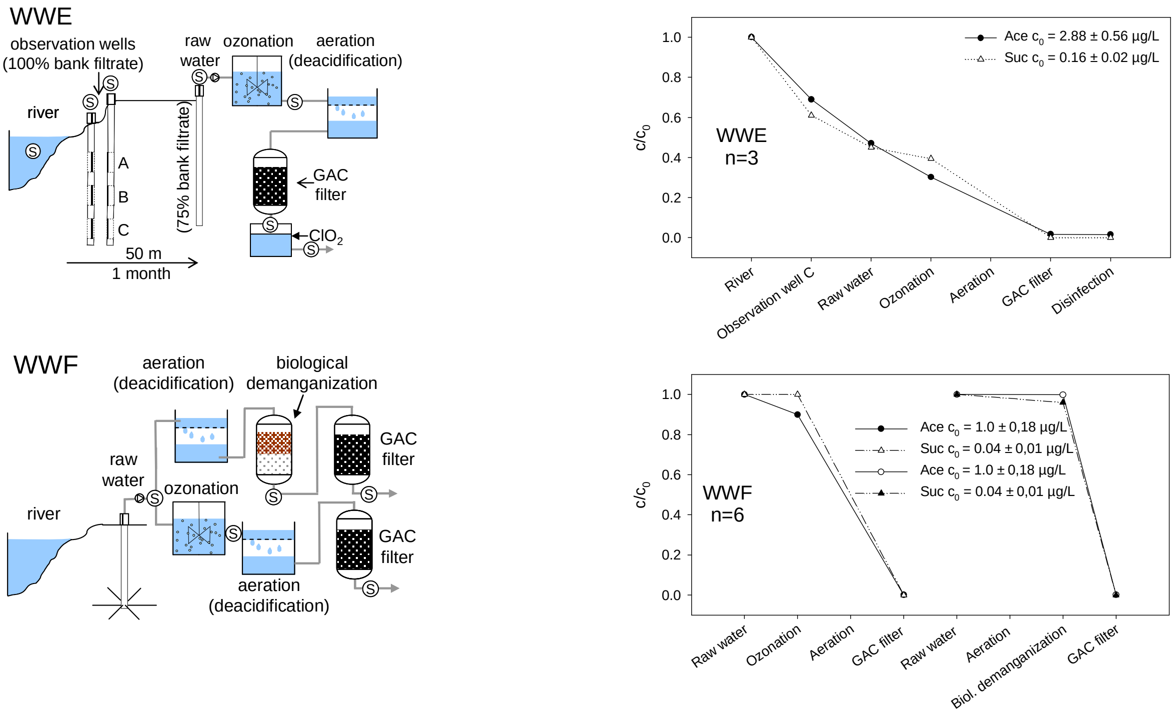


Figure 4-1 Schemes of the investigated water treatment plants and changes in artificial sweeteners concentrations (mean values) along the treatment train; "S" in circle denotes the location of the sampling sites. Ace denotes acesulfame, Sac saccharin, Cyc cyclamate, and Suc sucralose; Standard deviations (STD) of c/c_0 for Ace and Suc were: ≤0.12, and ≤0.11 in WWA, WWB, and WWD (n = 10), ≤0.20 and ≤0.19 in WWE (n = 3) and ≤0.22 and ≤0.27 in WWF (n = 6), respectively. Sac and Cyc concentrations were low and could only be followed along the treatment in WWA and WWB. Here, STD for Sac and Cyc were ≤0.23, except for three individual sampling points, where Cyc concentration in the raw water fluctuated by a factor of >10, resulting in a STD of c/c_0 ≤0.55 in WWA (sampling points: filtration, GAC) or was detected only in twice during ten samplings (STD ≤0.63 in WWB (sampling point: slow sand filter)

Waterworks B (WWB)

Similar to WWA, WWB consists of the pre-treatment of surface water from the Rhine river prior to infiltration and the final treatment of recovered groundwater. First, the river water passes a sand trap with a flow velocity of 0.3 m/s. Subsequently, the water flows over a cascade in order to increase the oxygen level and to remove volatile organic compounds. The aerated water is stored in sedimentation and infiltration ponds, where a part of it percolates through the ponds' bottom into the subsurface. Most of the water in the sedimentation ponds is pumped to the first treatment facility, which consists of flocculation, sand filtration, and GAC filters. The effluent from the GAC filters is used for the artificial recharge of groundwater. The infiltrated water has a residence time of three months in the aquifer, where it is mixed with local groundwater (20-30 %) and percolating water from the infiltration pond (10-20 %). In the second treatment facility the recovered groundwater is first aerated and then treated with powdered activated carbon (PAC, 12 g/m³), combined with a refiltration flocculation process. A subsequent sand filter is operated with a filtration velocity of 0.2-0.3 m/h. The last treatment step is disinfection with chlorine dioxide (0.15 mg/L). Sampling points were the river water, the purified surface water prior to infiltration, the recovered water, the effluent of PAC-treatment, and the finished water. Samples were taken weekly (n=10).

Waterworks C (WWC)

WWC represents a wide range of different treatment steps applied in drinking water production without any intermediary artificial groundwater recharge and recovery. First, water from the Rhine river is treated mechanically with different screens before pre-ozonation is applied to improve the subsequent primary flocculation. The next treatment steps are sedimentation, main ozonation (residual concentration 0.3 mg/L), and secondary flocculation. In a multi-layer filter flocks are retained and the remaining ozone is removed. GAC filters complete the treatment before the water is used for surface irrigation and artificial groundwater recharge. Sampling points at WWC were the raw water and the effluents of pre-ozonation, two lines of the main ozonation, the multi layer filter as well as the combined effluent of all activated carbon filters. WWC was sampled three times, but only at the last campaign all above mentioned sampling points were included. Additionally, effluents of four different activated carbon filters were sampled in order to compare the effectiveness at different filter loadings at the last sampling campaign.

Waterworks D (WWD)

In WWD bank filtrate of the Rhine river is used as raw water. The investigated well is located 100 m away of the Rhine’s right bank and horizontally screened at 18 m below ground. It yields a mixture of Rhine bank filtrate and landside groundwater (approximately 50 %). Measurements of redox sensitive parameters confirmed aerobic conditions. Sampling points at WWD were surface water, bank filtrate, and the influents (after ozonation) and corresponding effluents of two GAC filters, sampled weekly over a period of ten weeks (n=10). Both GAC filters differed in load and flux. After three of ten sampling campaigns an additional sampling point, unaffected by landside groundwater, was accomplished (n=7) to study the performance of river bank filtration.

Waterworks E (WWE)

WWE was sampled (n=3) in order to check the results from WWC and WWD with respect to the effectivity of ozonation. Additionally, it offered the opportunity to compare different residence times of bank filtrate at the same site. The facility consists of about 100 interconnected wells within a distance of 50 m to the bank of the Rhine. Water mainly infiltrates at the bottom of the river bed into a 10–12 m thick gravelly-sandy aquifer. At mean water level of the Rhine the retention time of the aerobic bank filtrate in the subsurface is about three to four weeks. Besides the Rhine water, two clusters of observation wells, situated between Rhine and the investigated production well, were sampled in three different depths. The approximate retention time to the particular sampling points is 60 d to the deepest observation well (C), 30 d to the second deepest (B), and 12 d to the observation well closest to the surface (A). There is no impact of landside groundwater on these sampling points. The next sampling point was the production well, which typically yields a mixture of 70–75 % of mixed bank filtrate of different retention times and 25–30 % of groundwater. Depending strongly on the surface water level, the proportion of groundwater is highly variable. Raw water is treated with 0.3-0.6 mg/L ozone in a contact tank for six minutes. On its way to the following aeration and subsequent GAC filters oxidation continues for more than 30 min. Additional sampling points were the effluents of two 50 m³ activated carbon filters and the water after disinfection with chlorine dioxide (50-60 µg/L).

Waterworks F (WWF)

WWF also uses bank filtrate from the Rhine river. Here, the investigation focused on the comparison of different treatment technologies. A part of the bank filtrate is treated by ozonation (0.15 mg/L ozone), followed by deacidification and finally by GAC filtration. Alternatively, and in order to abstain from the use of ozone, in WWF an additional synchronized pilot plant line exists, where biological manganese removal and GAC filtration are the only treatment steps after deacidification. Manganese removal is achieved in a two-layer filter filled with filter coal and filter quartz to a height of 1.5 m and operated at a velocity of 9 m/h. The GAC filters are 2.5 m in height and filled with reactivated GAC F 300. Samples for both treatment options were taken every two weeks over a period of ten weeks (n=5). Bank filtrate, effluents of the ozonation and demanganization step, and the GAC filters were sampled.

4.3 Results

4.3.1 Laboratory-scale experiments

Fixed-bed bioreactor

Saccharin and cyclamate were completely microbially degraded in the fixed-bed bioreactor after 20 and 15 days, respectively (Figure 4-2). After a lag-phase of about one week, the degradation rapidly proceeded, likely due to the adaption of microorganisms to the source of nutrition. In contrast, acesulfame and sucralose were not biodegraded even after a prolonged test time of 92 days. The results are in accordance with the reported behavior of artificial sweeteners in wastewater treatment plants (Buerge et al., 2009, Scheurer et al., 2009), even if the aerobic fixed-bed bioreactor experiment is not primarily a model for biodegradation under wastewater treatment conditions, but rather for biodegradation during river bank filtration.

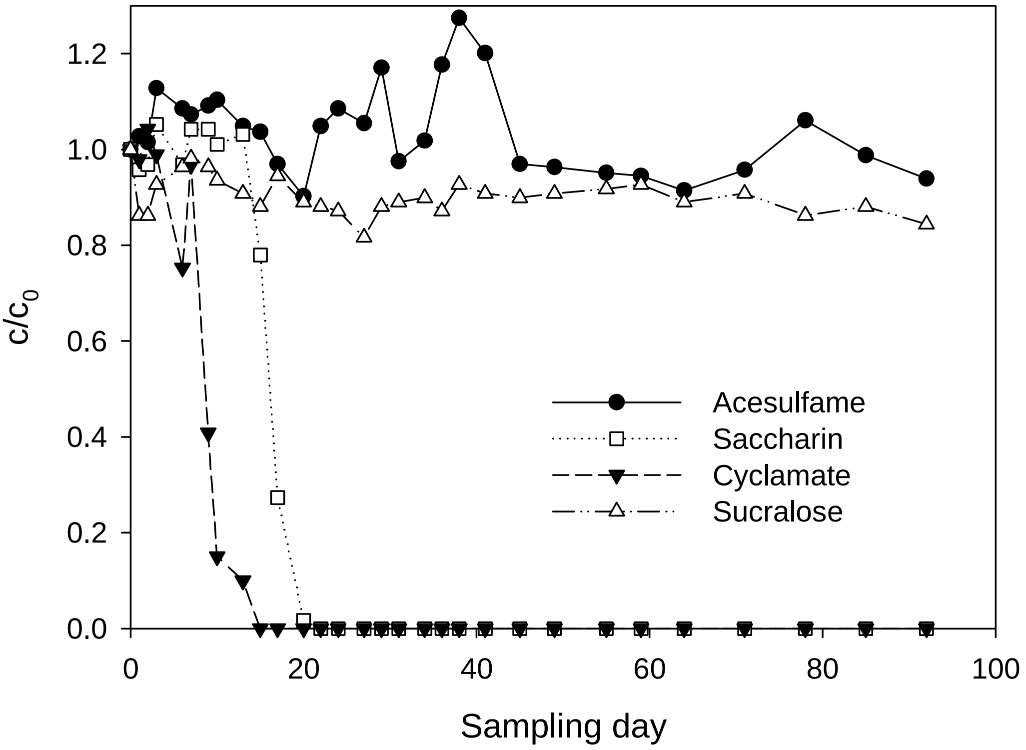


Figure 4-2 Behavior of artificial sweeteners in a fixed-bed bioreactor simulating aerobic river bank filtration. After day 40 the mass-labeled internal standard (IS) acesulfame-d_4 was used to quantify acesulfame. Variations of the acesulfame concentration after that date are mainly due to variations of the test and not due to analytical errors, which were compensated by the IS

Flocculation

Laboratory-scale jar test flocculation experiments with river and tap water and different concentrations of $AlCl_3$ and $FeCl_3$ resulted in insignificant elimination of AS from the aqueous phase. After flocculation, AS recoveries were between 79 and 117 % of the spiked concentration. AS background levels in the river water used were substracted. Although flocculation can remove other dissolved organic contaminants, the results are not surprising, as the removal mechanism is based on precipitation of the compounds or on sorption onto precipitates. Due to high water solubility and low sorption tendency of artificial sweeteners both removal mechanisms are not likely to occur. Log K_{OW} values for AS are scarce, and comprise a range of -1.8 to 0,3 (values from (Jenner and Smithson, 1989; Grice and Goldsmith, 2000), and calculated by ChemAxon, 2010). For comparison, the log K_{OW} of bentazone, a polar herbicidal active ingredient in plant protection products with some structural similarity to the sulfonamido type sweeteners, is -0.46 (EU, 2000b). Like the AS investigated bentazone also has a low sorption tendency (Delle Site, 2000 and references therein). For a graphical presentation of the obtained results see supporting material (Figure 4-5).

Ozonation

Acesulfame was the AS which was oxidized most readily. Its half life was approximately 15 min (Figure 4-3) at an ozone concentration of 0.5 mg/L.

After a contact time of 30–40 min, which is typical for waterworks practice, a residual concentration of ca. 30 % of the initial acesulfame still remained in the test solution. This is in good correlation to the results obtained for WWD (see 4.3.2). For short contact times cyclamate concentration decreased similar to the acesulfame concentration, but the residual concentration of cyclamate after 60 min was more than twice as high. Higher initial ozone concentrations resulted in a faster removal and lower residual concentration of both AS, but usually waterworks avoid ozone doses such high as 5 mg/L, the highest ozone level in these lab experiments. For saccharin and sucralose no significant concentration decrease was observed at an ozone concentration of 0.5 mg/L. This was also true when doubling the applied ozone concentration. Only with 5 mg/L ozone a depletion of about 20 % for saccharin and 30 % for sucralose was achieved.

The results indicate that sucralose and acesulfame are likely to be found in waterworks after ozone treatment, however, for different reasons: sucralose, because it is quite persistent against ozone attack and acesulfame, because it is present in relatively high concentration in the raw waters and, in addition, is incompletely degraded at typical ozone doses and contact times applied in waterworks.

The reaction of acesulfame with excess ozone followed pseudo first-order kinetics: $c(t) = c_0*e^{-kt}$; t: time; c(t):concentration at time t; k: rate constant . The coefficients of determination were $R^2>0.96$ (Figure 4-6, Table 4-2). At the highest ozone level of 5 mg/L, *i.e.* a molar ratio of ozone:acesulfame of approximately 17,000:1, the reaction was fastest with a rate constant of $9.7*10^{-3}$ 1/s, which decreased to $2.6*10^{-3}$ 1/s, $1.3*10^{-3}$ 1/s and $5.5*10^{-4}$ 1/s at 2, 1 and 0.5 mg/L, respectively.

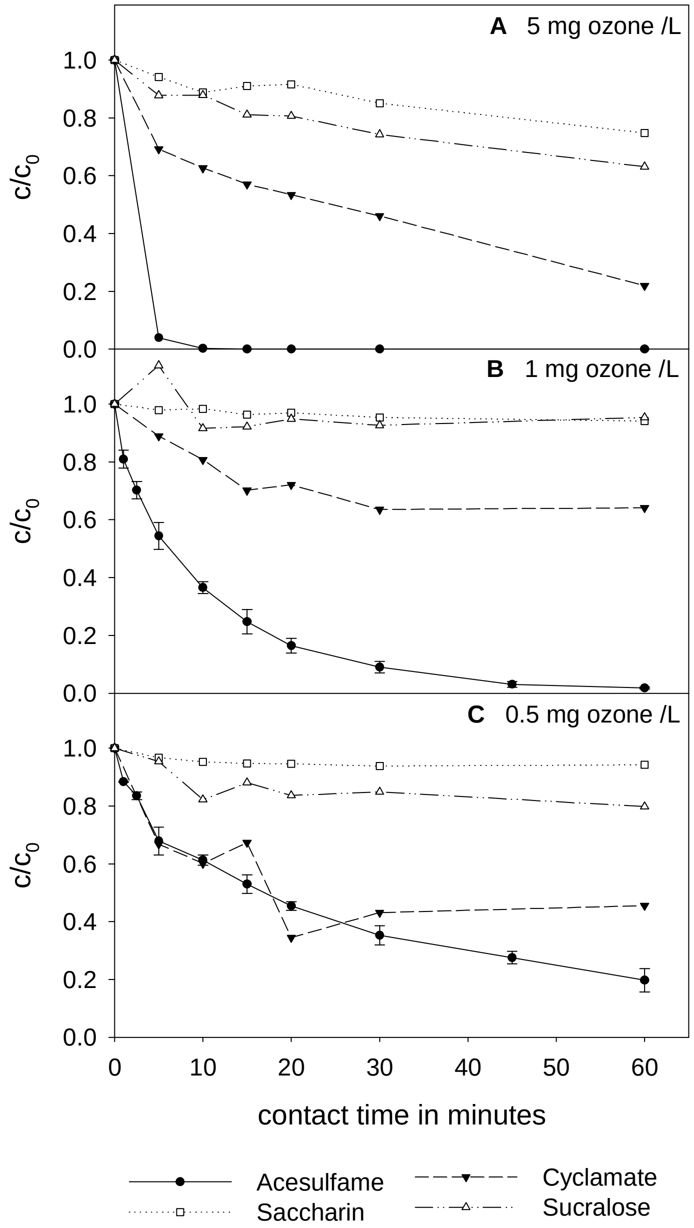


Figure 4-3 Degradation of artificial sweeteners in spiked drinking water treated with different ozone doses. Error bars for acesulfame indicate standard deviations (n=3; for 2.5, 5, and 45 min n=2)

Activated carbon small-scale filter test

A preliminary test with a mixture of all four AS gave a first impression of the suitability of GAC filtration in waterworks for the removal of AS (for graphical presentation see supplementary material, Figure 4-7). Cyclamate was only little retarded in the filter and a 10 % breakthrough occurred already after one day. Acesulfame and sucralose also crossed the 10 % line within 15,000 BVT, i.e. between day two and three. However, whereas for acesulfame and cyclamate a complete breakthrough was observed after about one week, sucralose was still retained by about 50 % after a prolonged runtime of more than 50,000 BVT. All these three sweeteners can be classified as relevant for drinking water in this preliminary test. Saccharin was retained slightly better than sucralose and, therefore, was the only sweetener with no drinking water relevance, based on the above definition. In batch tests with single AS solutions (Figure 4-4) the results of the preliminary test with the AS mixture were confirmed for every sweetener. The results indicate a breakthrough characteristic of cyclamate comparable to diatrizoic acid, an X-ray contrast medium, which is known to have almost no retention in the small-scale filter test and also in full-scale GAC filters in waterworks (Seitz et al., 2006). Saccharin could still be classified as "not relevant to drinking water", but after 30,000 BVT showed a higher breakthrough than in the multi-sweeteners batch experiments. A possible explanation is that sites on the activated carbon suited for saccharin adsorption are limited but still available at the low saccharin concentration of 125 µg/L in the multi-sweeteners batch test. For all AS, data from full-scale plants should be taken into consideration to clarify their behavior under practical conditions, as kinetics and loadings of full-scale filters can vary within a wide range. This is especially true for sucralose, which showed an early but low breakthrough combined with a good retention over weeks in the batch test.

Chlorination

AS were not transformed in chlorination experiments with spiked tap water. Recoveries of the compounds were in a narrow range from 91 to 107 % for all applied contact times and excess chlorine was still present at 1 mg/L Cl_2. The persistence of AS against chlorination was also proved in waterworks (see 4.3.2). For a graphical presentation of the obtained chlorination results see supporting material (Figure 4-8).

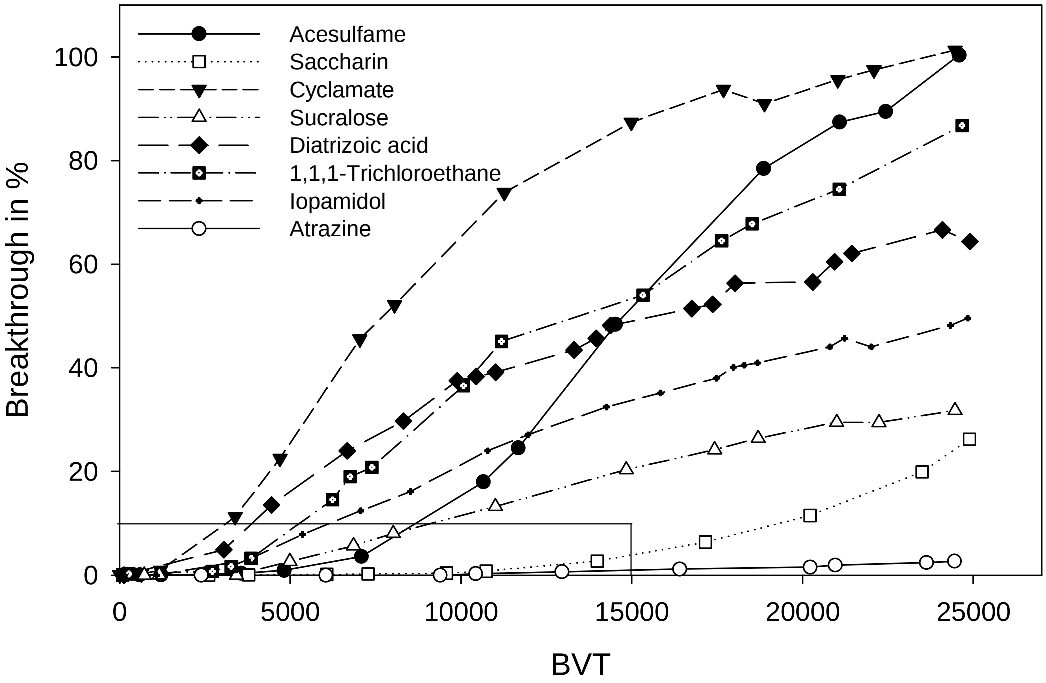


Figure 4-4 Breakthrough behavior of selected organic trace contaminants in an activated carbon small scale filter. Considering treatment with activated carbon filters, compounds are classified as relevant to drinking water, if a 10 % breakthrough occurs within 15,000 BVT (definition and data of diatrizoic acid, 1,1,1-trichloroethane, iopamidol, and atrazine from Marcus (2005))

4.3.2 Field studies

Waterworks A

The elimination of acesulfame and sucralose in the filtration unit of WWA was negligible and might be attributed to non-corresponding sampling and analytical uncertainties (Figure 4-1, WWA). For saccharin the first steps in WWA contributed to the overall elimination (74 % removal), most likely due to microbial activity in the gravel filter. Although the subsequent GAC filter was preloaded at 30–60 m³/kg, it was still effective for the removal of saccharin and sucralose. Saccharin was not detected in the filter effluent and sucralose was retained by more than 60 %. As observed in the batch experiments, acesulfame and cyclamate removal by GAC was limited. As the upper layers of the GAC filters were biological active, cyclamate removal can be understood as the combined effect of biological degradation and adsorption onto GAC. The decrease in the concentration of acesulfame (59 %) and sucralose (61 %) from the infiltration point to the observation well was almost identical for both compounds. Despite the fact that both AS proved to be persistent against microbial degradation in the fixed bed bioreactor experiments and adsorption onto soil should be limited based on their polar nature, this effect cannot be attributed solely to dilution with landside groundwater, because, according to information of the plant operator, there is almost 100 % infiltration water at this well. Final conclusions for this concentration decrease cannot be drawn, yet because the sampling period of about two months is quite short compared to the residence time of up to two years. Changes in raw water concentrations and/or treatment efficiency of the surface water could be possible explanations. Microbial degradation over such a long underground passage cannot be excluded, although no case of biodegradation of acesulfame has been reported yet. Additional dilution occurred on the way to the recovery well, but a comparison between the further behavior of these two AS was not possible, because sucralose concentrations dropped below the LOD. Acesulfame was the only sweetener detected in the recovered ground water. The GAC in the groundwater facility had a runtime of about four years and was not capable of retaining any acesulfame. In every sampling campaign even slightly higher concentrations in the GAC filter effluent were observed. Accumulation of acesulfame in the past and subsequent elution during the observation period (chromatographic effect), as known also from other trace pollutants, is the most likely explanation for the concentration increase in the filter effluent.

Waterworks B

Three artificial sweeteners were already eliminated by 60–100 % on their way from the river water to the effluent of the GAC filters (Figure 4-1, WWB). For saccharin and cyclamate the elimination was probably due to microbial degradation in the biological active parts of the facility, *i.e.* the sand trap and the sedimentation pond. As proven in the small-scale filter test, cyclamate should not have been adsorbed in the activated carbon filters to a great extent. No conclusion can be drawn, if traces of saccharin were eliminated in the GAC filters of this treatment train, as no sampling point at the influent of the filters was established. However, sampling in other waterworks (see results for WWC) and laboratory experiments proved that GAC had contributed to the elimination of saccharin. For the low biodegradable sucralose adsorption to GAC seemed to be the main elimination process here. Trace concentrations of cyclamate and sucralose were below LOD after recovering the infiltrated water. This can be attributed to dilution and in the case of cyclamate to further microbial degradation. Acesulfame was the most persistent sweetener and could be detected at a level of 34 % (highest value 0.46 µg/L) of the surface water concentration in the finished drinking water. After the GAC filters this compound was removed by about 20 %. Further concentration decrease was achieved by dilution on the way to the recovery well. Taking into account a known dilution with landside groundwater by 30 % and an impact of 10 % of the infiltration pond a theoretical concentration of 0.69 µg/L can be calculated for the recovered water. The measured mean value was 0.58±0.04 µg/L, which fits rather well to the expected value. The added PAC contributed by approximately 10 % to the overall removal of acesulfame, whereas the dosage of chlorine dioxide had almost no effect.

Waterworks C

In WWC due to a short contact time of 3 min, the pre-ozonation step had only a minor effect on the removal of AS (Figure 4-1, WWC). The AS most resistant to ozone was saccharin, which corresponds well with the ozonation batch experiments. The next sampling point in WWC summarizes the three treatment steps flocculation, sedimentation, and ozonation. Flocculation proved to have no effect on the removal of AS in batch tests, and thus, can be excluded to contribute to the overall elimination. Therefore, the subsequent main ozonation was the major barrier for acesulfame and cyclamate, whereas the oxidation of sucralose and saccharin was not very efficient again. Removal of saccharin can be attributed to microbial degradation in the multi-layer filter, while in this treatment step acesulfame and cyclamate were eliminated to a small proportion only. Sucralose was still detectable at a level of 71 % of the initial concentration after the multi-layer filter, but was completely eliminated in the subsequent GAC filter. GAC contributed by 17 to 25 % of the concentration initially present in the surface water to the removal of acesulfame, cyclamate and saccharin.

Acesulfame was the AS with the highest concentration of about 2 µg/L in the raw water. The entire treatment process removed this compound by about 90 %, but some acesulfame traces (0.15-0.20 µg/L) were still detectable in the finished water. When individual GAC filters were sampled separately, it became obvious, that some adsorbers could not retain this compound anymore due to a high filter load. In four different filters tested, the removal rates for acesulfame ranged from 100 to 43 %, corresponding with filter loads between 7 m^3/kg and 127 m^3/kg (Figure 4-9). Remaining traces of cyclamate in WWC, the only waterworks without any underground passage, emphasize the importance of a prolonged residence time in a biological active environment in order to remove this AS.

Waterworks D

WWD represents a waterworks, which uses bank filtrate derived from the Rhine river. As the raw water is a mixture of Rhine river bank filtrate and landside groundwater, an additional observation well was sampled, which delivers pure bank filtrate (Figure 4-1, WWD). Bank filtration proved to be very effective for the removal of saccharin and cyclamate, which were not detected above the LOD in any bank filtrate or raw water sample in WWD. Concentrations of these two sweeteners in the river water were 0.05 ± 0.03 µg/L and 0.10 ± 0.14 µg/L, respectively. High standard deviations are due to extremely high concentrations in the last sampling campaign. This phenomenon results from low water levels in the Rhine river and coincidentally heavy rainfalls, which were responsible for the discharge of untreated wastewater in the river. Acesulfame and sucralose concentrations continuously increased during the sampling campaign. Therefore, removal efficiencies of bank filtration were determined from median values (in contrast to Figure 4-1, where mean values are depicted). Recoveries of 102 % and 95 % were calculated for these two persistent AS in the observation well and of 40 % and 41 % in the raw water. These recoveries are largely consistent with the known proportion of bank filtrate in the observation well (100 %) and in the raw water (approximately 50 %), estimated based on historical water quality data.

On its way to the two activated carbon filters the water had different ozone contact times (ozone concentration 0.4 mg/L). The influent of filter I had a contact time of 18 min, filter II of 31 min, resulting in a significantly higher reduction of acesulfame concentration in filter II. This effect was observed in every series of samples and reduced the raw water concentration of acesulfame (0.95 ± 0.09 µg/L) by 44 % (0.53 ± 0.10 µg/L) in the influent of filter I and by 60 % (0.38 ± 0.08 µg/L) in influent of filter II, respectively. The results correlate well with the results obtained in the batch experiments, where a comparable ozone concentration (0.5 mg/L) led to an elimination of acesulfame of 47 % after 15 min and 65 % after 30 min contact time. Sucralose was more refractory against ozone attack, as already found in the batch experiments. Furthermore, the effect of different ozone contact times was less pronounced and not significant for this compound.

The subsequent granular activated carbon filters were differently loaded (56 m^3/kg for filter I and 10 m^3/kg for filter II, respectively after 9 of 10 sampling series), but both were still adequate to remove remaining traces of sucralose to concentrations <LOD. However, for acesulfame a decrease of the filter performance over the sampling period of ten weeks was observed for filter I, resulting in an elimination of only approximately 30 % at the last sampling. In the effluent of filter II only traces between 0.01 and 0.03 µg/L were observed. These findings suggest that fresh to moderately loaded GAC is an excellent option for the removal of remaining traces of acesulfame after ozonation. However, economic aspects have to be considered, too, before a decision on the frequency of GAC exchange can be made.

Waterworks E

Saccharin and cyclamate were present in every Rhine river water sample at concentrations between 0.03 and 0.18 µg/L. Nevertheless, these compounds were found neither in one of the bank filtrate samples from the observation wells, even after the shortest retention time of 12 days at observation well A, nor in the raw water from the production wells (Figure 4-1, WWE).

In contrast to all other investigated waterworks, in WWE apparently lower recoveries than 100 % were found for acesulfame (69 %) and sucralose (61 %) at the observation wells, which supply 100 % bank filtrate. However, this was due to non-corresponding samples and the high variability of the AS concentrations in the river (e.g. acesulfame concentration: 2.88 ± 0.56 µg/L) during the observation period. Nevertheless, the recalcitrant nature of these two AS is reflected by the nearly identical concentration decrease of about 32 % for acesulfame and 26 % for sucralose due to dilution from observation well C to the raw water wells, which parallels the observations in WWD.

As in WWC, WWD, and the batch test, acesulfame proved to react more readily with ozone than sucralose. The elimination of both compounds in two different GAC filters was excellent. No sucralose and only traces of acesulfame could be detected in the GAC filter effluents. A subsequent disinfection with chlorine dioxide was not capable of removing residual acesulfame: Therefore, at every sampling date acesulfame was detected in the finished water at concentrations up to 0.05 µg/L.

Waterworks F

Cyclamate and saccharin were not detected in the raw water as they were already removed during river bank filtration. The occurrence of acesulfame and sucralose in every raw water sample emphasizes their mobility and persistence. As observed in batch experiments, sucralose was more resistant to ozonation than acesulfame (Figure 4-1, WWF). Due to low ozone concentrations in WWF (0.15 mg/L) the elimination was incomplete (18 % elimination for acesulfame and 9 % for sucralose). The two-layer filter in the pilot plant did not contribute to the elimination of acesulfame and sucralose. However, both AS were removed by the GAC filters in both facilities. These filters were preloaded with about 13 m^3/kg and this load was similar to the load of filter II in WWD (10 m^3/kg after 9 samplings), which also effectively removed acesulfame and sucralose. WWF replaces its GAC at a load of about 30 m^3/kg, which represents a value between the two filter loads in WWD, where the filter loaded at 56 m^3/kg could not retain acesulfame completely anymore.

4.4 Discussion

Saccharin and cyclamate were removed by 100 % in waterworks using bank filtration or artificial groundwater recharge as one of their treatment steps in the multi-barrier system, even at rather short retention times of 12 days as in the case of observation well A at WWE. An aerobic laboratory degradation experiment yielded similar results, with complete removal of cyclamate and saccharin within 13 and 22 days, respectively. Biological active filters in the waterworks were capable of retaining 12–34 % of the cyclamate input and 74–77 % of saccharin. The good biodegradability of these two compounds was already reported for wastewater treatment plants (Buerge et al., 2009; Scheurer et al., 2009) and was thus confirmed by the present study for biological less active compartments like river bank filtration systems and biological active filters in waterworks. However, the biodegradation products of saccharin and cyclamate are still unknown. Therefore, no conclusions can be drawn, if those metabolites are also removed in drinking water treatment facilities.

Ozonation was applicable to a limited extend only to remove traces of both compounds from the raw water, but due to the effective removal at waterworks with bank filtration or artificial groundwater recharge, ozonation had to be studied in laboratory experiments. There, only <10 % of saccharin and 30 to 50 % of cyclamate was transformed within 30 min, requiring ozone concentrations of 5 mg/L.

GAC filters retained 100 % of saccharine and 23 % of cyclamate input at WWA. Consistently, the activated carbon small scale filter test revealed a behavior of cyclamate similar to diatrizoic acid and of saccharin similar to atrazine, but with an earlier breakthrough. However, in GAC filters with prolonged runtimes it is even likely that especially cyclamate is removed due to microbial degradation. As the initial concentrations in the raw waters are relatively low, only traces of both compounds are likely to be found in the finished water, even if treatment in a certain waterworks does not comprise an underground passage. For risk assessment purposes, the identification of oxidation products of cyclamate is of certain interest, as the compound occurs in higher concentrations than saccharin in the raw water and is less recalcitrant during ozone treatment.

Ozonation proved to be very effective for the removal of acesulfame. The compound was detected in the surface or raw waters in concentrations one order of magnitude higher than for all other investigated AS. Thus, the identification of ozonation products should be given high priority to clarify their removability during drinking water treatment and to assess potential health risks. The degradation of acesulfame and the other AS during ozonation is a function of the applied ozone concentration and contact time. This was proven in batch tests and also in waterworks, where the acesulfame concentrations were very susceptive to small changes in ozone contact times. In the waterworks investigated in this study ozonation incompletely removed acesulfame by 18–60 % due to insufficient ozone doses, but this treatment step contributed to a great extend to the overall elimination.

It is remarkable, that the structurally related sweeteners acesulfame and saccharin, both with a sulfonamide moiety in the ring structure, behaved completely different during the treatment with ozone. As ozonation products of AS are still unknown, it is also unclear what moiety of the molecule would represent the point of ozone attack. For comparison, bentazone a widely used active ingredient of herbicides, which has a structure similar to both sweeteners (phenyl ring plus sulfonamide unit in the ring structure) is very persistent against microbial degradation (Gonzalez et al., 2006) and is removed very well by ozone treatment (Fobbe et al. 2006; Bozkaya-Schrotter et al., 2008). The presence or absence of alkyl side chains play an important role in the reaction of these sulfonamides with ozone. For comparison, in photolysis experiments with bentazone (Peschka et al., 2007), the isopropyl moiety was hydroxylated and also oxidation and subsequent ring cleavage occurred. Similar reaction could be expected for acesulfame with a methyl side chain.

The removal of acesulfame by GAC filters was strongly dependent upon the filter load (compare Figure 4-4, Figure 4-7, and Figure 4-9). Activated carbon provided a very effective tool, as long as the loads were below about 30 m^3/kg, which is the limit of an economic use of activated carbon in order to remove single organic compounds in waterworks (Haist-Gulde et al. 1998). As a result, acesulfame was detected up to 0.76 µg/L in effluents of GAC filters, where short or no ozone treatment coincided with high filter loadings. As subsequent disinfection did not contribute to the acesulfame removal, similar concentrations were found in the finished water. Assuming a concentration of 1 µg/L acesulfame (potassium salt) in drinking water, a consumption of 2 L/d, and a body weight of 70 kg, a person would exceed the acceptable daily intake (ADI) of 630 mg/d (EU, 2000a) not until 863 years.

Conclusions of recent publications (Buerge et al., 2009; Scheurer et al., 2009), that acesulfame can be used as a tracer for anthropogenic contaminations, are confirmed by the present study, as its concentrations were only reduced by dilution during aerobic river bank filtration. Additionally, first measurements from anaerobic bank filtration sites at the Sawa river indicate that acesulfame is not degraded at anaerobic sites as well (data not shown). Therefore, this compound represents a new tool in drinking water production to predict the proportion of bank filtrate and landside groundwater in raw water of waterworks and to identify unknown sewer leakages.

The same seems to be true for sucralose, which was also very persistent during bank filtration and an aerobic degradation experiment. It also showed concentration decreases by dilution always in the same range as acesulfame. Brorström-Lundén et al., (2008) reported no significant accumulation for sucralose in sewage sludge, but detailed sorption studies of all AS are still missing. Even though sorption ought to play a minor role for the removal of AS in wastewater treatment or environmental systems, this gap of knowledge should be filled. Two consecutive studies by Labare and Alexander (1993 and 1994) proved that sucralose is not biologically inert, but is degraded in highly variable rates in soils and lake water. These results also have to be confirmed in future research to exclude that its tracer function can only be applied under certain environmental conditions. In waterworks sucralose was more persistent than acesulfame during ozonation, but was well removed in subsequent GAC filters even on activated carbon with higher loads. First comparative analyses of wastewaters from different countries (*e.g.* United States, Israel, Australia; data not presented here) revealed sucralose to still play a minor role among the sweeteners used in foodstuffs, pharmaceuticals and personal care products in Germany, yet. Higher consumption volumes, like in the above-mentioned countries, would cause higher raw water concentrations of this AS in waterworks using bank filtrate. As sucralose proved to be persistent during ozonation (removal of 8–15 % in waterworks and <20 % after 30 min in the batch test), higher influent concentrations in subsequent GAC filters would lead to an earlier detection in the finished drinking water.

In summary, the removal efficiency of single treatment steps applied in multi-barrier drinking water treatment plants can be classified as displayed in Table 4-1. The chance that sweeteners end up in finished drinking water is most likely for acesulfame but also possible for cyclamate and saccharin, when water treatment includes no underground passage.

Table 4-1 Removal efficiency of single treatment steps in multi-barrier drinking water treatment plants for the elimination of artificial sweeteners. + = good, 0 = moderate (also depending on boundary conditions, such as ozone concentration, load of GAC filters, etc.), − = poor

	Acesulfame	**Saccharin**	**Cyclamate**	**Sucralose**
Bank filtration	–	+	+	–
Flocculation	–	–	–	–
Ozonation	0/+	–	0	–
Activated carbon filtration	–/0	+	–	0/+
Chlorination	–	–	–	–

4.5 Conclusions

- The multi-barrier treatment system is effective for the removal of artificial sweeteners from raw water sources if certain boundary conditions are established.
- Saccharin and cyclamate are effectively removed in treatment steps with biological activity.
- Acesulfame and sucralose proved to be very recalcitrant against microbial degradation. Their role as possible tracers for wastewater contamination is confirmed by this study. However, detailed sorption studies are still lacking.
- Applied ozone doses and contact times in waterworks are not sufficient to entirely remove acesulfame, the sweetener with the highest concentrations, from the raw water. Subsequent activated carbon filters are an excellent option for its removal, provided the load is lower than approximately 30 m^3/kg.
- Acesulfame was the only artificial sweetener detected in finished drinking water up to several hundred ng/L. Cyclamate was only detected in finished water in a waterworks without any underground passage, which uses the water only for artificial groundwater recharge and irrigation.
- For other countries, where the concentration of sucralose in wastewater is up to two orders of magnitude higher than in Germany, the effectiveness of single treatment steps in waterworks should be further investigated as the only effective treatment for the compound seems to be GAC.
- Priority of research on the field of artificial sweeteners should be given to the identification of unknown transformation products, such as microbial degradation products of saccharin and cyclamate and, in particular, to ozonation products of acesulfame, as this compound occurred in the highest concentrations in the influents of ozonation units and reacted most readily with ozone.

4.6 Acknowledgments

We thank the colleagues of the involved waterworks for providing the samples, for the fruitful discussion of the results and for reviewing the manuscript.

4.7 Supplementary material

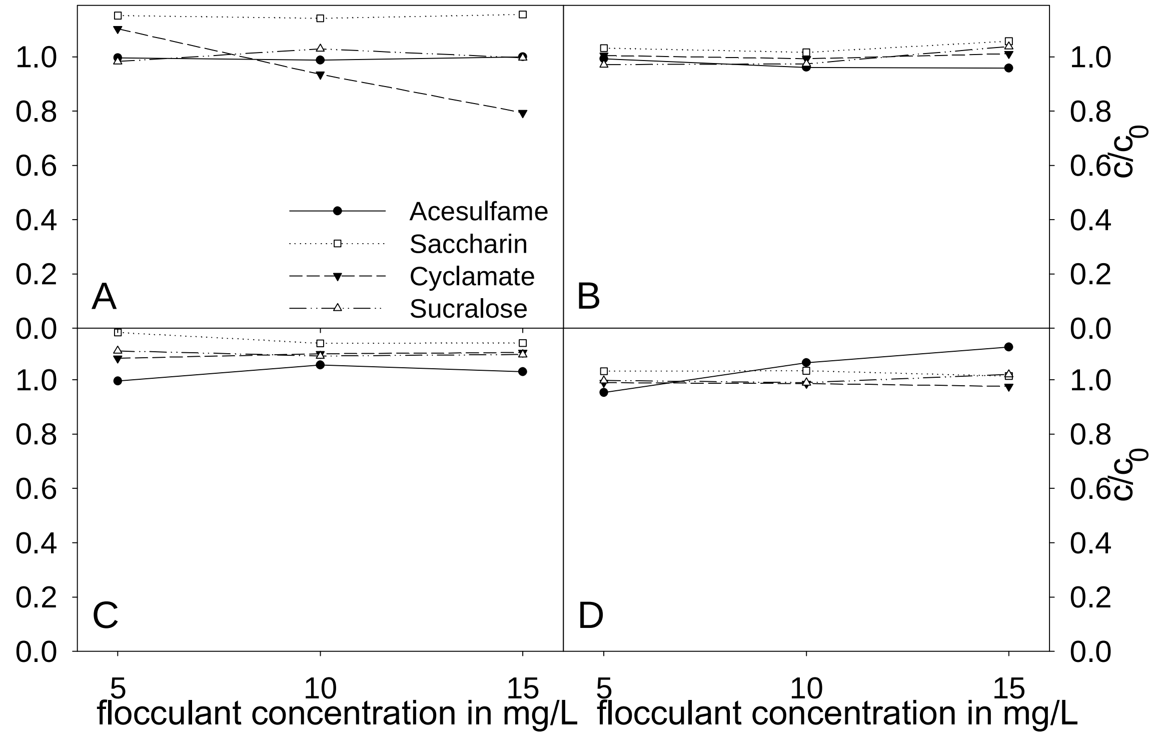


Figure 4-5 Behavior of artificial sweeteners in flocculation batch experiments with different doses of the respective flocculant. A: tap water with iron chloride ($FeCl_3$), B: river water with $FeCl_3$, C: tap water with polyaluminum chloride ($AlCl_3$) and D: surface water with $AlCl_3$

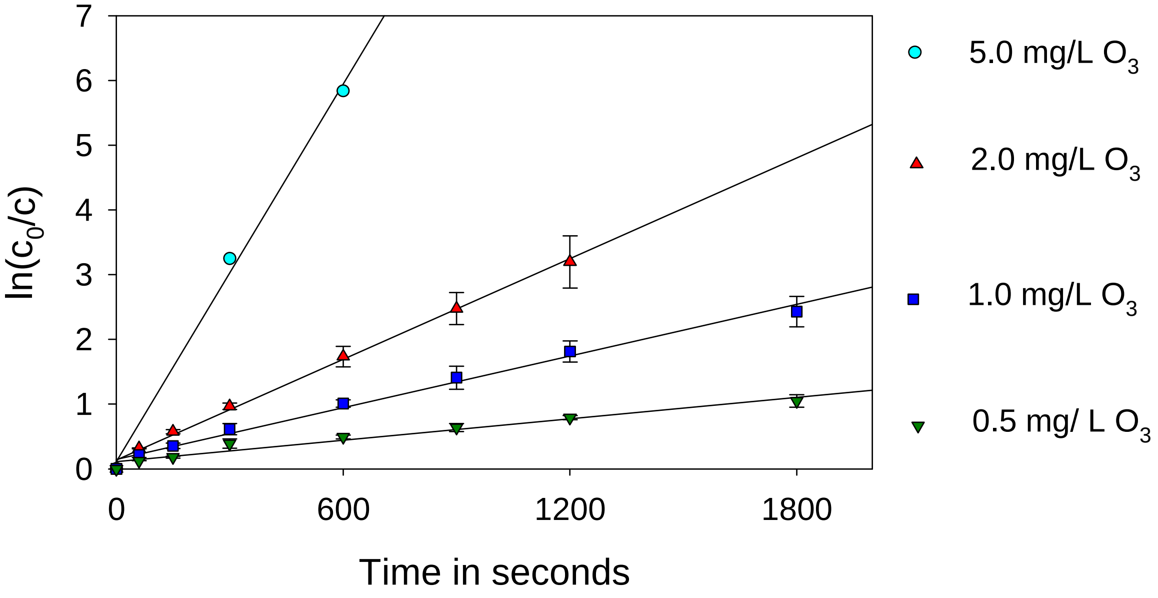


Figure 4-6 Pseudo-first order fit ($c(t) = c_0 * e^{-kt}$ or transformed $\ln(c_0/c(t)) = k*t$) for the reaction of acesulfame with excess ozone; t: time; c(t):concentration at time t; c_0: initial concentration (1 µg/L) at t = 0, k: rate constant. For number of experiments (n), rate constants (k), coefficient of determination (R^2) see Table 4-2

Table 4-2 Rate constants (k) of the oxidation of acesulfame for different ozone doses applied and coefficient of determination (R^2) of the linear model used to fit pseudo first-order kinetics; n represent number of batch experiments

Initial ozone concentration (mg/L)	n (-)	k Acesulfame (1/s)	R² (-)
5.0	1	$9.7*10^{-3}$	0.996
2.0	2	$2.6*10^{-3}$	0.997
1.0	3	$1.3*10^{-3}$	0.990
0.5	3	$5.5*10^{-4}$	0.964

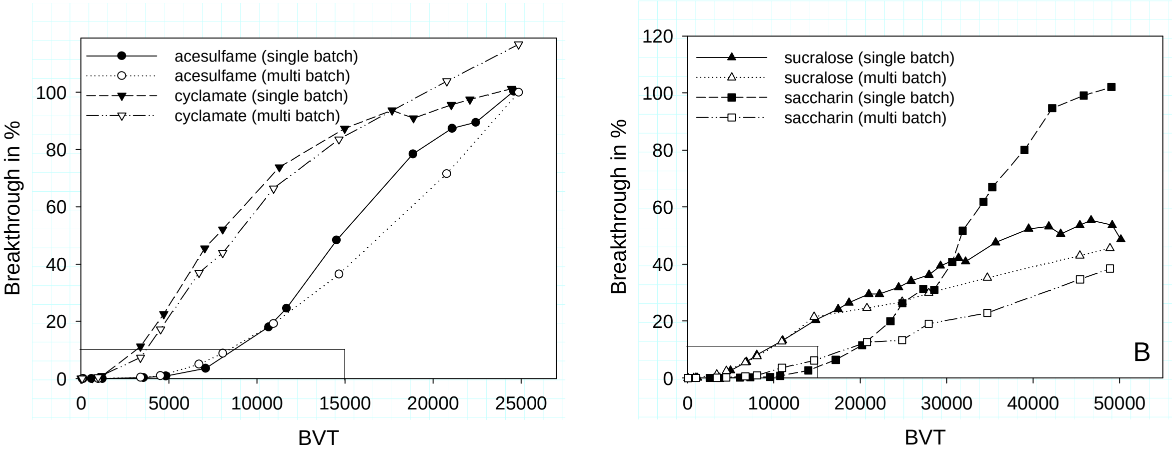


Figure 4-7 Breakthrough curves of acesulfame and cyclamate (A) as well as for sucralose and saccharin (B) in an activated carbon small-scale filter as a function of bed volumes treated (BVT). Spike amount in single batch experiments 500 µg/L, in multi batch experiments 125 µg/L (four sweeteners included). Matrix: drinking water

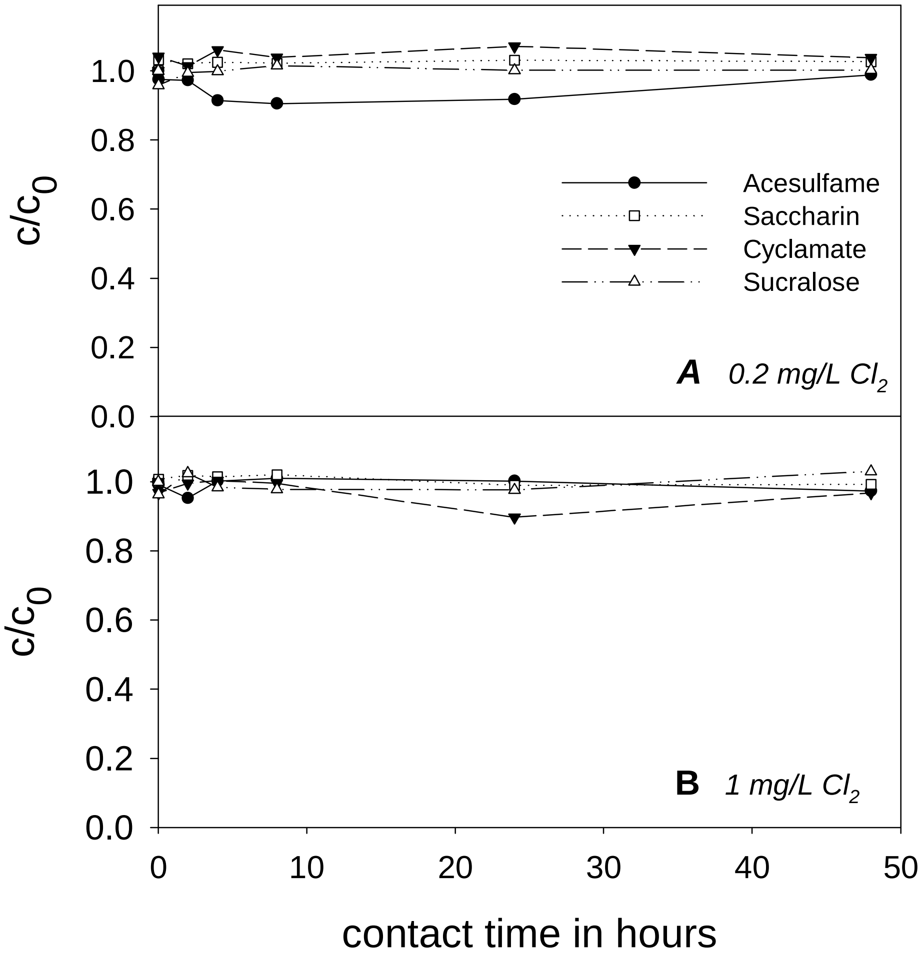


Figure 4-8 Behavior of artificial sweeteners during chlorination at two different chlorine doses in drinking water as function of time

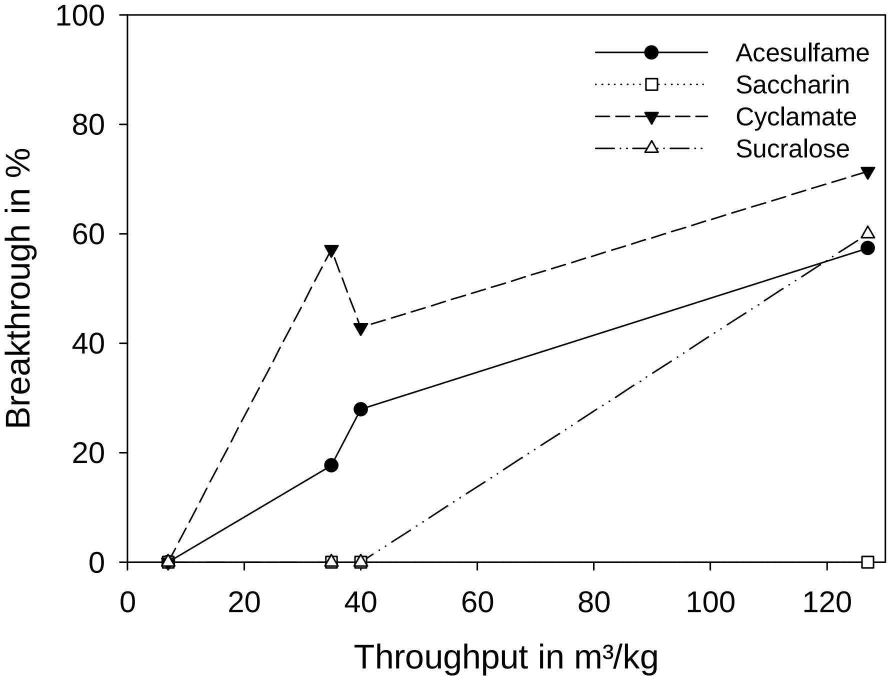


Figure 4-9 Breakthrough of artificial sweeteners through four GAC filters with different loadings in WWC

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5

Structural elucidation of main ozonation products of the artificial sweeteners cyclamate and acesulfame

Purpose The two artificial sweeteners cyclamate (CYC) and acesulfame (ACE) have been detected in wastewater and drinking water treatment plants. As in both facilities ozonation might be applied, it is important to find out, if undesired oxidation products (OPs) are formed.

Methods For the separation and detection of the OPs, several analytical techniques, including nuclear magnetic resonance experiments, were applied. In order to distinguish between direct ozone reaction and a radical mechanism, experiments were carried out at different pH values with and without scavenging OH radicals. Kinetic experiments were used for confirmation that the OPs are formed during short ozone contact time applied in waterworks. Samples from a waterworks using bank filtrate as raw water were analyzed in order to prove that the identified OPs are formed in real and full-scale ozone applications.

Results In the case of CYC, oxidation mainly occurs at the carbon atom, where the sulfonamide moiety is bound to the cyclohexyl ring. Consequently, amidosulfonic acid and cyclohexanone are formed as main OPs of CYC. When ozone reacts at another carbon atom of the ring, a keto moiety is introduced into the CYC molecule. Acetic acid and the product ACE OP170, an anionic compound with *m/z* 170 and an aldehyde hydrate moiety, were identified as the main OPs for ACE. The observed reaction products suggest an ozone reaction according to the Criegee mechanism due to the presence of a C=C double bond. ACE OP170 was also detected after the ozonation unit of a full-scale drinking water treatment plant which uses surface water-influenced bank filtrate as raw water.

Conclusions Acesulfame can be expected to be found in anthropogenic-influenced raw water used for drinking water production. However, when ACE OP170 is formed during ozonation, it is not expected to cause any problem for drinking water suppliers, because the primary findings suggest its removal in subsequent treatment steps, such as activated carbon filters.

Scheurer, M., Godejohann, M., Wick, A., Happel, O., Ternes, T.A., Brauch, H.-J., Ruck, W.K.L., Lange, F.T. (2011) Structural elucidation of main ozonation products of the artificial sweeteners cyclamate and acesulfame, Environ Sci Pollut Res, DOI 10.1007/s11356-011-0618-x.

5.1 Background, aim, and scope

In recent years, concerns arose about the environmental fate of so-called emerging contaminants released into the aquatic environment via wastewater treatment plants (WWTPs) due to incomplete removal during biological wastewater treatment. As a consequence, some of these organic trace pollutants were detected in the nanograms per litre to micrograms per liter range in WWTP effluents and were even found in raw waters used for drinking water production or in some cases also in finished potable water (Loraine and Pettigrove 2006; Benotti et al. 2009; Prasse et al. 2010). Acute toxic effects of low concentrations of these compounds are mostly unknown, but it should be aimed to minimize levels of such compounds in order to prevent from potential harmful and yet unknown risks.

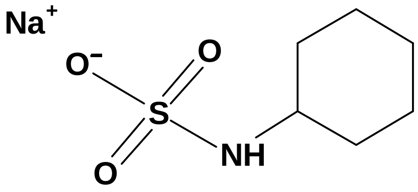
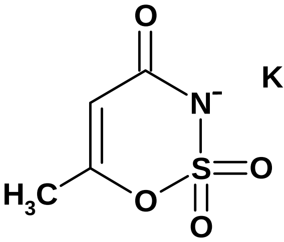
Different strategies to minimize the discharge of organic trace pollutants in the aquatic environment and for their removal during drinking water production are discussed in the literature. Upgrading WWTPs with powdered activated carbon or ozonation units as tertiary treatment seems to be a promising option. Several studies report not only about ozonation as an effective tool for the removal of pharmaceuticals, fragrances, X-ray contrast media, antibiotics, estrogens, plant-protecting agents, industrial chemicals, and food additives (Ternes et al. 2003; Huber et al. 2005; Dodd et al. 2006; Hollender et al. 2009; Rosal et al. 2010), but also about superior elimination of fecal and total coliforms (Ternes et al. 2003; Wert et al. 2007). Advanced wastewater treatment with ozone is considered to be cost effective and economically feasible (Joss et al. 2008; Schaefer et al. 2009). Ternes et al. (2003) assessed the costs for installation and ozone treatment with 10 g ozone/m³ wastewater to be ≤0.04 €/m³. An additional energy requirement of 12 % for upgrading a nutrient removal WWTP with a post-ozonation unit was reported by Hollender et al. (2009). The application of 0.6 g ozone per g dissolved organic carbon (DOC) was sufficient for the removal of the vast majority of 220 micropollutants in that case. Due to the benefits of micropollutant removal and disinfection, several full- or large-scale pilot plants, using ozonation as a tertiary wastewater treatment, are already established or planned for the near future.

The use of ozone in drinking water production has an even longer tradition and was already used for odor and color removal in the USA in the early 1900s (Rice 1999). Nowadays, ozonation is widely used for raw water treatment in drinking water treatment plants (DWTPs), in particular when indirectly surface water-influenced water (e.g. river bank filtrate) is used for the production of potable water.

The oxidation of organic trace pollutants during ozonation is often connected with a decrease of their original biological activity (Huber et al. 2004; Suarez et al. 2007; Dodd et al. 2009) and in most cases with the formation of more readily biodegradable compounds (Alvares et al. 2001). In aqueous solutions, there are two modes of attack to the trace pollutant: either by direct reaction of the O_3 molecule or after decomposition of O_3 by OH radicals. Direct reaction is favored by lower pH values and selectively occurs at sites with high electron density, such as double bonds or amines. The more nonselective reaction with OH radicals is the main oxidation pathway for some ozone-resistant micropollutants (Elovitz and von Gunten 1999). Although most of the hydroxyl radicals are likely to be scavenged in waters with a high matrix burden, they complicate the prediction of the nature of the oxidation products (OPs) even more. It was shown for wastewater and drinking water that ozonation can also lead to the formation of undesired by-products such as *N*-nitrosodimethylamine (NDMA) (Schmidt and Brauch 2008; Hollender et al. 2009). Thus, evidence is needed that harmless compounds are formed in ozonation units in WWTPs or DWTPs. The increasing use of ozone applications in water treatment resulted in an elevated number of publications dealing with the structural elucidation of potential OPs (e.g., Ramseier and von Gunten 2009; Benner and Ternes 2009a; Benner and Ternes 2009b; Dodd et al. 2010).

The aim of this work was to identify the main OPs of the artificial sweeteners cyclamate (CYC) and acesulfame (ACE) (Table 5-1), which were only recently detected in microgram-per-liter levels in WWTP effluents and which were also found in surface water as well as in raw water used for drinking water production (Buerge et al. 2009; Scheurer et al. 2010).

Table 5-1 Chemical structures of CYC and ACE with CAS registry number and monoisotopic masses

	
Sodium cyclamate	**Potassium acesulfame**
CAS: 139-05-9	CAS: 55589-62-3
Monoisotopic mass: 201.04 (sodium salt), 178.05 (anion)	Monoisotopic mass: 200.95 (potassium salt), 161.99 (anion)

5.2 Materials and methods

5.2.1 Chemicals

Acesulfame potassium (>99 %), tertiary butyl alcohol (*t*-BuOH; 99.7 %), and cyclohexanone (99.5 %) were purchased from Fluka (Steinheim, Germany), sodium cyclamate from Supelco (Bellefonte, PA, USA) and para-chlorobenzoic acid (pCBA; 99 %) from Sigma-Aldrich (Steinheim, Germany). Acetic acid, formic acid, and oxalic acid dihydrate (analytical grade) were purchased from Merck (Darmstadt, Germany). The solvents used for this study were all of analytical grade.

5.2.2 Experimental setup

Ozonation experiments were performed as batch experiments in 1 L glass vessels filled with ultrapure water (200 mL) containing 25 mg/L CYC (=140 µmol/L) and ACE (=153 µmol/L), respectively. The batch setups were prepared at pH 3 and 7.5 with phosphate buffer (25 mM) with or without *t*-BuOH as a radical scavenger to be able to distinguish between reactions mainly driven by ozone or OH radicals. To assess if scavenging of OH radicals was sufficient, samples were spiked with 300 µg/L pCBA. The ozone stock solution was prepared by sparging ozone gas through a cooled reactor column (5 °C) filled with ultrapure water. The ozone concentration of the stock solution (ca. 17 mg/L) was measured by the indigo method (Bader and Hoigne 1981) before adding it to the batch vessel. Different compound to ozone ratios were achieved by adding defined amounts of the aqueous ozone solution and taking into account the dilution factor for subsequent calculations. For an even spread of the ozone, the test setup was slightly stirred on a magnetic stirrer for about two minutes. The samples were placed in the fume hood over night and analyzed the next day.

Similar tests were performed with Karlsruhe tap water (DOC = 0.9 mg/L, pH = 7.3) and with treated wastewater from the WWTP in Eggenstein-Leopoldshafen (DOC = 8.6 mg/L, pH = 7.6). The batch tests in these real matrices were repeated in 100 times lower compound concentrations (250 µg/L) and with realistic ozone concentrations applied in water treatment plants (0.2 mg/L to 2 mg/L) and WWTPs (0.25 mg/L to 1 mg/L per mg DOC) to investigate the influence of the present DOC on a more realistic basis.

Kinetic experiments for ACE (200 µg/L) with a waterworks relevant ozone dose of 1 mg/L and a contact time of 25 min were performed in ultrapure and potable water. Samples were directly injected every 4 min for analysis by rapid-resolution liquid chromatography-mass spectrometry (LC-MS/MS) in order to track the degradation of the precursor and formation of identified OPs.

In order to perform nuclear magnetic resonance (NMR) experiments, higher concentrations (5 g/L) of both sweeteners were ozonated by passing the produced ozone gas directly through a gas-washing bottle head equipped with a filter disk into a separating funnel containing the test solution. Samples were taken after defined time intervals and stripped with nitrogen gas in order to remove remaining ozone.

5.2.3 Chromatographic separation and detection

Samples from the batch tests were analyzed with different LC and gas chromatography (GC) methods optimized for pCBA and the suspected OPs based on preliminary experiments. These separation techniques were coupled to different (also high resolution) mass spectrometers (MS) for detection and MS^2 experiments. MS^3 spectra were obtained by direct injection into the electrospray interface of the MS. Ion chromatography (IC) coupled with a conductivity detector was used to analyze carboxylic acids, which were suspected to be formed during ozonation. Furthermore, IC was also coupled with inductively coupled plasma mass spectrometry (ICP–MS) to rule out the formation of sulfate during ozonation of the sulfur containing sweeteners.

The organic carbon content of the batch samples was determined according to DIN EN 1484 (08/1997) with a total organic carbon (TOC) analyzer TOC-V_{CHP} equipped with an ASI-V auto sampler (both Shimadzu, Kyoto, Japan).

For structural elucidation of the proposed OPs, ^{1}H-NMR measurements were applied for the highly concentrated original aqueous solutions of OP mixtures. In a second approach, the results were confirmed by LC-HILIC-NMR/MS.

A detailed description of the applied methods is given in the supporting information (see chapter 5.5 and Table 5-2).

5.3 Results and discussion

The TOC balance for different CYC and ACE to ozone ratios for the batch test in ultrapure water without the addition of *t*-BuOH revealed that no significant loss of precursors or unknown organic oxidation products occurred till analysis. The results for the TOC measurements are displayed in Figure 5-7 in chapter 5.5.

For the batch tests where OH radicals were scavenged by the presence of *t*-BuOH, scavenging was controlled by pCBA measurements (method 3, Table 5-2). A constant pCBA concentration was observed for all tests where *t*-BuOH was added (data not shown).

5.3.1 Ozonation of cyclamate

For the CYC molecule, no preferred sites for a direct ozone attack could be expected. The cyclohexyl ring is saturated and the sulfonamide should be still protonated at pH 8 and therefore practically unreactive (Munoz and von Sonntag 2000). The ozonation of cyclohexane by OH radicals in gas-phase reactions or by ozonation has been reported before (Aschmann et al. 1997; Barletta et al. 1998). The second study describes the ozonation of cyclohexane as a pure compound and identified cyclohexanone and cyclohexanol as the main OPs.

In the case of CYC, we also identified cyclohexanone as one main ozonation product based on GC-MS/MS and ^{1}H-NMR measurements.

Figure 5-8 in chapter 5.5 shows the edited HSQC spectra of CYC and the main oxidation product after 80 min of ozonation. While CYC exhibits one single –CH proton resonating at 3.1 ppm, two pairs of equivalent –CH_2 groups and one –CH_2 according to the integral values between 1.1 and 2 ppm, the oxidation product shows only three NMR resonance signals corresponding to two pairs of equivalent –CH_2 groups and one additional –CH_2 between 1.7 ppm and 2.5 ppm. The loss of the downfield-shifted –CH, the significant downfield shift of the –CH_2 groups and the high degree of symmetry in the molecule is in agreement with the presence of cyclohexanone as being the major oxidation product of CYC. This has been corroborated by comparison with pure reference compound diluted in D_2O using NMR and GC-MS. Besides cyclohexanone, several minor NMR signals are present which are too low for further structural elucidation.

The formation of cyclohexanone was strongly dependent on the CYC to ozone ratio applied in the batch test (Figure 5-1). When ozone was present at the highest excess (1:10), 121 to 142 µmol/L cyclohexanone was formed in three out of four test series indicating an almost even mass balance on a molar scale. The non-scavenged batch test at pH 7.5 showed a lower conversion to cyclohexanone probably due to further oxidation by OH radicals. The formation of cyclohexanone represents a good example that several analytical techniques might be necessary for structural elucidation: from a highly polar precursor, predestinated for LC-MS, a typical GC compound is formed, which is not amenable for electrospray ionization anymore.

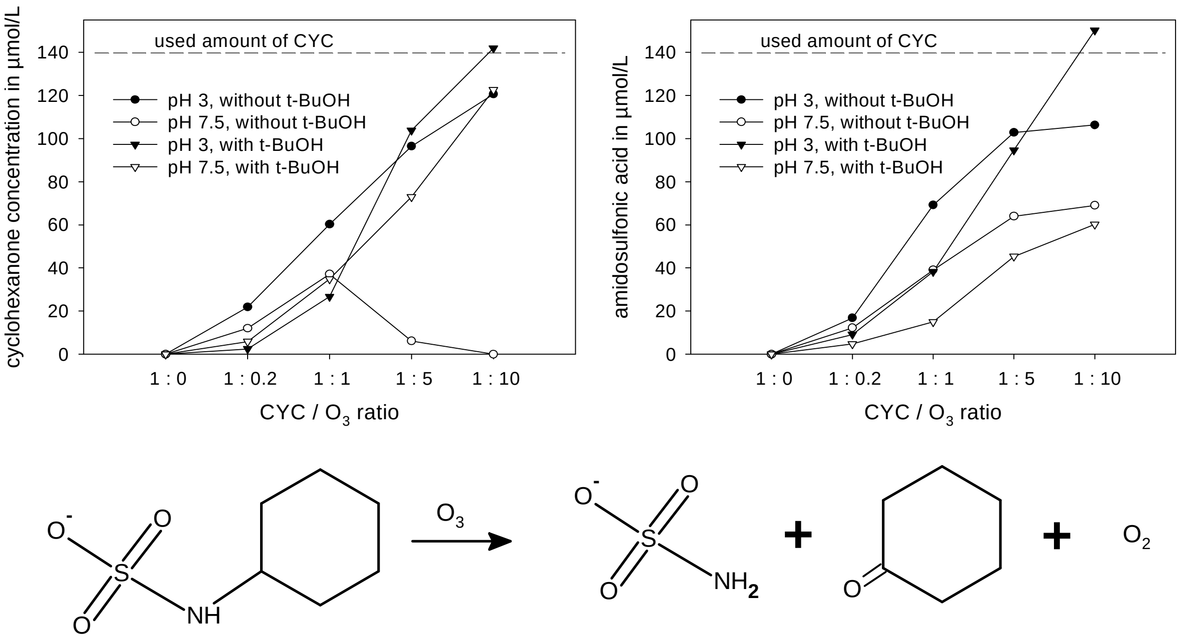


Figure 5-1 Formation of the OPs cyclohexanone (top left) and amidosulfonic acid (top right) in aqueous solution at different CYC to ozone ratios at pH 3 and 7.5 with and without the addition of *t*-BuOH as an OH radical scavenger (c_0 of CYC was 140 µmol/L and is indicated by the dashed line). Proposed main reaction pathway for the ozonation of CYC (bottom)

The fact that the ring of CYC yields cyclohexanone as one main OP, suggests that the cleaved side chain can lead to the formation of additional OPs. This led us to the identification of amidosulfonic acid as the second main OP of CYC (Figure 5-1). Quantification (method 2, Table 5-2) was possible with a reference standard. Amidosulfonic acid showed a higher yield at pH 3 compared with pH 7.5 for both the batch tests with and without the addition of *t*-BuOH. No further significant increase in concentration was observed between the CYC to ozone ratios of 1:5 and 1:10 in the non-scavenged batch, which corresponds to the complete reaction of CYC already at a ratio of 1:5. In batch tests with radical scavenging the yield of amidosulfonic acid increased with increasing CYC/ozone ratio due to residual precursor available for further conversion into amidosulfonic acid. The highest amidosulfonic acid concentration of about 140 µmol/L represents an almost even mass balance. Most likely an immediate oxidation of CYC to amidosulfonic acid occurs in the batch test without radical scavenging when adding ozone stock solution. Although the samples were measured the day after the ozone treatment, this time delay can be an explanation for lower concentrations due to a further hydrolysis of the OPs.

The OP amidosulfonic acid is also used as a precursor during production of both artificial sweeteners investigated in this study (Heravi et al. 2009; Yoshikubo and Suzuki 2000). In the case of CYC, cyclohexylamine and amidosulfonic acid are used for the synthesis (Alter and

Formann 1968). The US National Library of Medicine specifies the acute oral toxicity values (LD_{50}) to be 1,312 mg/kg for mouse and 3,160 mg/kg for rat (US NLM 2011).

IC-ICP/MS experiments with similar concentrations and a compound to ozone ratio of 1:2.5 proved that no or insignificant sulfate is formed during ozonation of cylamate neither with nor without scavenging of OH radicals. However, besides the main OPs of CYC, amidosulfonic acid and cyclohexanone, the formation of OPs with the same $m/z = 192$ (CYC OP192) was observed by high-resolution MS measurements (Figure 5-2 a, b and Figure 5-9). The molecular formula of $C_6H_{10}NO_4S$ indicated the introduction of a keto moiety most likely at the cyclohexyl ring of CYC forming three isomers of oxocylohexyl sulfamate. To confirm this assumption, we established a RP chromatographic separation (method 1,Table 5-2) for the three possible isomers. The elution order was assigned on the basis of the calculated octanol-water partition coefficients (logP) of the proposed structures (Chem Axon 2011). The three compounds revealed different fragmentation patterns also indicating the formation of three isomers. For CYC OP192, an α-cleavage next to the nitrogen heteroatom was observed for all three isomers. Amidosulfonate ($m/z = 96$) and the sulfite radical ($m/z = 80$) were generated as the main negatively charged fragments. The latter is known to be an important fragment in collision-induced dissociation of aliphatic sulfonates (Schultz et al. 2006; Frömel and Knepper 2008). Interestingly, the precursor CYC yields only the sulfite fragment and not amidosulfonate with the abovementioned instrumentation, which gives strong evidence that the introduced keto group influences the fragmentation pathway. In aliphatic compounds, an α-cleavage directly leads to the formation of fragment ions. However, in the case of alicyclic molecules a product ion with the same mass as the cyclic precursor ion can be formed by ring opening and formation of a C=C double bond, which might be the explanation for the high precursor signal in the spectrum of isomer 1.

In the case of isomer 2 with the keto group in β-position of the amidosulfonate group, an additional fragment ion with $m/z = 122$ was observed, indicating a ring cleavage at two positions. For isomer 3, the sulfite radical was the only MS fragment we observed under the applied fragmentation conditions. This might be explained by the position of the keto group, enabling the most stable possibility of a neutral aminocyclohexanone fragment of all isomers. Since the mass balance was almost even according to the quantitative results obtained from the two main OPs of CYC, cyclohexanone and amidosulfonic acid, the isolation of CYC OP192 was not feasible due to its low concentration. As a consequence, the quantification of the new OPs identified was not possible. However, we compared the peak area of a directly injected standard of the precursor CYC, which was commercially available as a reference standard, with the highest peak area of the three isomers (pH 7.5, CYC to ozone ratio of 1:5) which could be directly observed in the reaction mixtures. Although the three isomers of CYC OP192 and CYC elute at different retention times and hence at different ionization conditions, a semi-quantitative estimation gave a maximum yield in the range of less than 10 % for CYC OP192 in the batch tests.

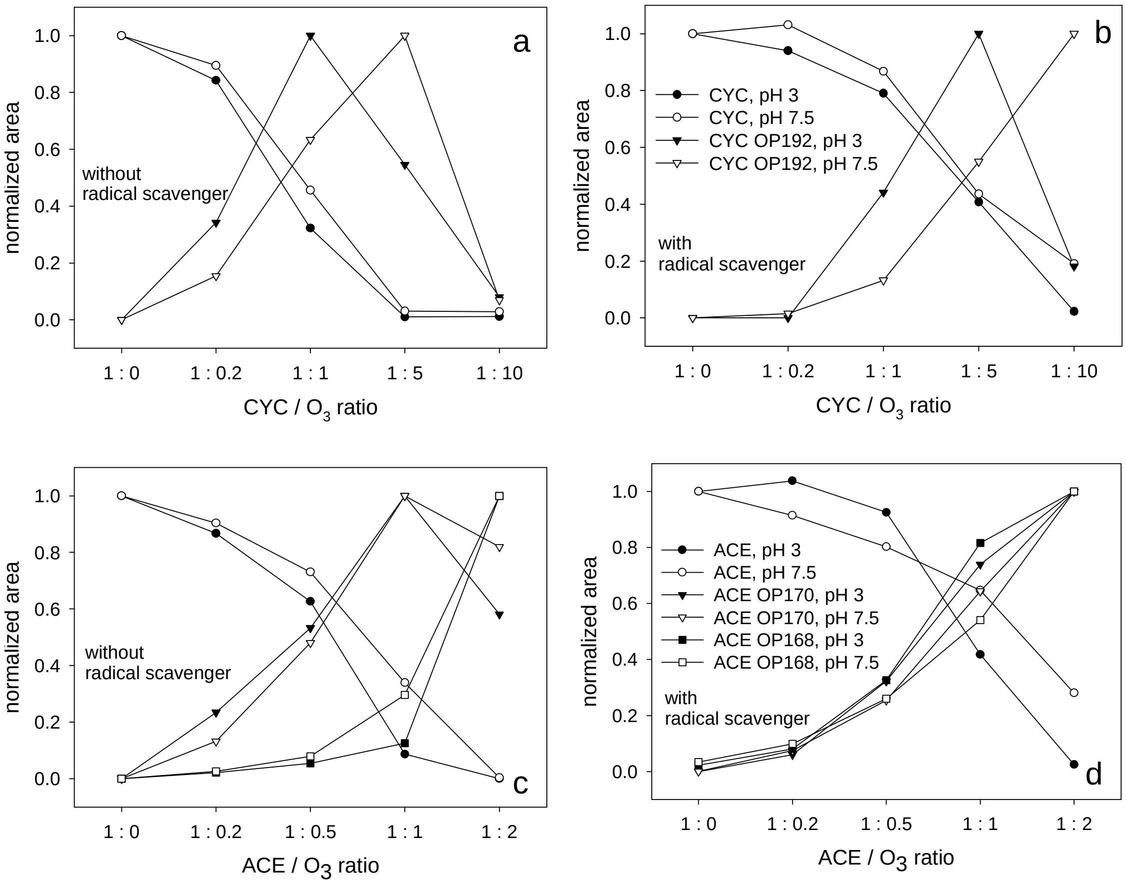


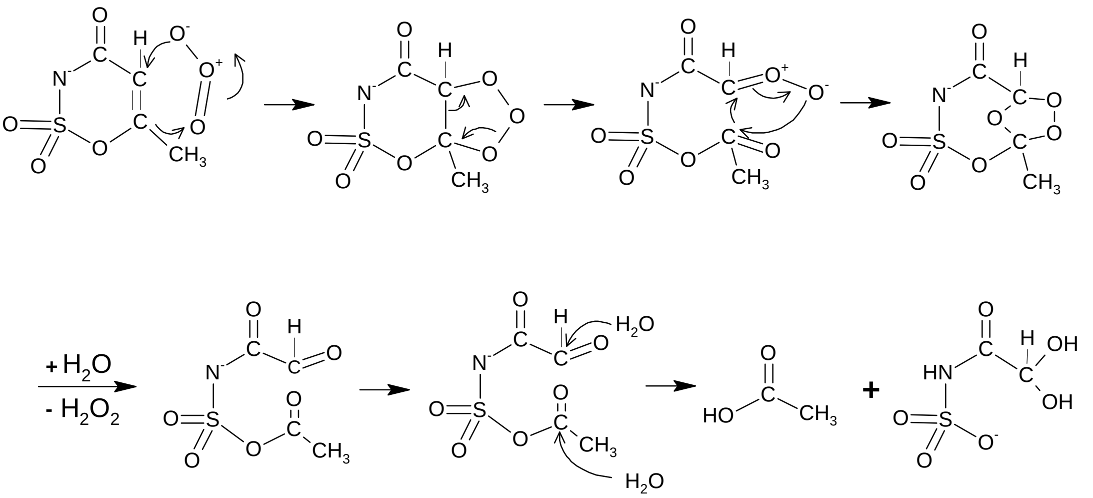
Figure 5-2 Normalized peak areas of CYC and CYC OP192 (a + b) and acesulfame and ACE OP170 and ACE OP168 (c + d) at two different pH values of the reaction mixture without (left) and with (right) scavenging of OH radicals. The normalized peak area of the suspected CYC OP192 refers to the most intensive isomer

5.3.2 Ozonation of acesulfame

According to our previous work on artificial sweeteners (Scheurer et al. 2010), ACE was oxidized more readily by ozone compared with CYC. This can be understood due to the presence of a C=C double bond, which is known to be easily attacked by ozone according to the Criegee mechanism (Criegee 1975). Therefore, compound to ozone ratios were applied in a lower range for the batch tests in the buffered ultrapure water. As displayed in Figure 5-2 c, d, a complete transformation of ACE was observed in three of four test setups already for a compound to ozone ration of 1:2 compared with a ratio of 1:10 necessary for comparable conversion of CYC. In the batch tests without *t*-BuOH, only slightly better oxidation yields were obtained, indicating that OH radicals play only a minor role in the ozonation of ACE. According to the LC-ESI-MS results, the ozonation of ACE yields anionic OPs with molecular masses of 170 and 168 Da. Based on MS^2 high-resolution measurements and MS^3 experiments, the structures, oxidation, and fragmentation pathways displayed in Scheme 1 and Figure 5-3 are proposed.

Following the Criegee mechanism, a ring cleavage of the primary ozonide at the C=C double bond and a decomposition into a carbonyl and carbonyl oxide moiety can be suspected. At the subsequently formed aldehyde, the addition of water is assumed leading to the proposed aldehyde hydrate. Compounds with two hydroxyl groups attached to one carbon in general are impossible to isolate due to the elimination of water. However, chemicals are known (e.g., chloral hydrate and ninhydrin), where strong electron withdrawing groups in α-position support the formation of an aldehyde hydrate in water. Thus, due to the α-carbonyl function in the proposed structure of ACE OP170, in aqueous solution, the formation of a fairly stable aldehyde hydrate is straightforward.

ACE OP168 seems to be the direct oxidation product of ACE OP170. As it can be seen in Figure 5-2 C, after complete oxidation of ACE the maximum of ACE OP170 is reached and it decreases slightly in favor of the formation of ACE OP168. It has to be noted that in Figure 5-2 peak areas were normalized to the highest intensity of the corresponding transition. Therefore, a quantitative comparison between OP concentrations is not possible, but a graphical presentation of relative intensities is given in the supporting information (Figure 5-10). In all batch tests, we observed that ACE OP168 is only a minor product in the ozonation of ACE. In all cases the main product was ACE OP170, which remained also rather stable when remaining ozone was present. This is supported by the fact that no sulfate formation was observed by IC-ICP/MS even after complete ozonation of ACE when applying an ozone to ACE ratio of 2.5:1.



Scheme 1 Proposed reaction pathway for the oxidation of ACE resulting in ACE OP170 and acetic acid

If really an aldehyde hydrate moiety is formed during ozonation, in the case of ACE OP170 could not be clarified by these measurements. However, a coelution of a reaction product with a precursor mass of (-)151.9 Da together with ACE OP170 was observed for all measurements with different MS conditions (ion source temperature, fragmentor voltage, etc.). The loss of 18 Da indicates a loss of water supporting the expected little stability of ACE OP170 and that the water loss occurs partly as an in-source fragmentation. Both products, ACE OP170 and ACE OP168, have one and two ring double-bond equivalents, respectively, which supports the proposed structures in the way that a ring structure after ozonation cannot be expected.

For further confirmation of these two structures, deuterium exchange experiments were run with MS^2 high-resolution measurements. For this purpose, eluent A of LC method 2 (Table 5-2) was replaced by 20 mmol/L ammonium formate dissolved in D_2O. The combination of evidence about the exchangeable hydrogen atoms and MS^2 experiments can provide valuable information if the proposed fragmentation pathways and structures are valid. For ACE OP170, the protons of the aldehyde hydrate moiety and the proton bound to the nitrogen should be most easily exchangeable and therefore result in m/z (-)172.9. For ACE OP168 both remaining protons can be expected to be exchanged.

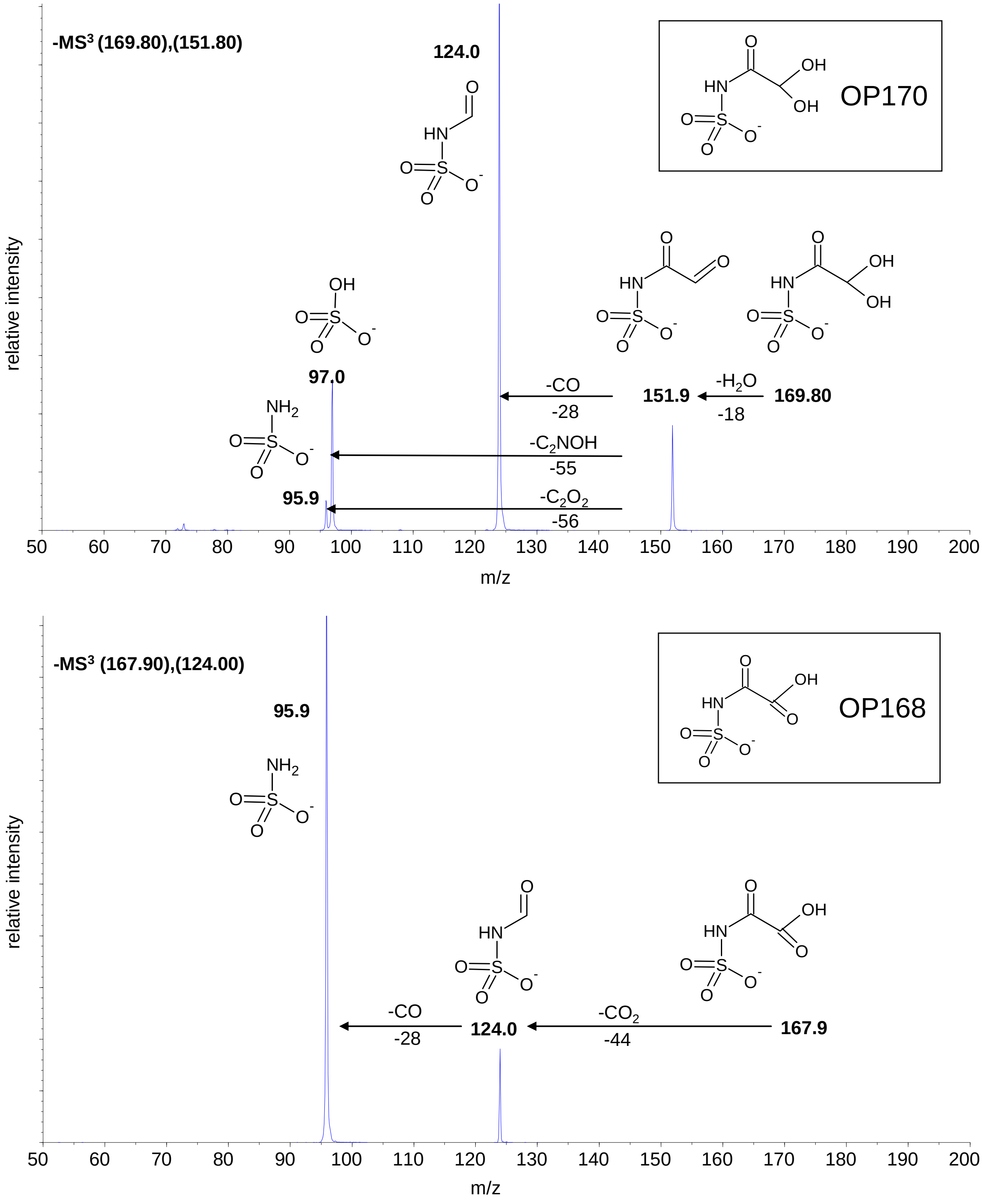


Figure 5-3 MS^3 fragmentation pathways for proposed ACE OP170 (top) and ACE OP168 (bottom) in negative ionization mode

As suspected for ACE OP170 an increase in m/z of three Da was observed with the modified eluent. The fragmentation led to a loss of 20 Da indicating a loss of D_2O, and consequently to the formation of a *m/z* fragment ion of (-)152.9 still carrying one nonexchangeable hydrogen from the precursor at the aldehyde moiety. The same was true for the formylsulfamate fragment (m/z = 124.9), which also showed some exchange at the aldehyde hydrogen. As expected also the masses of the hydrogen sulfate fragment and the amidosulfonic acid fragment increased in 1 and 2 Da, respectively. In the D_2O exchange experiments, the formation of an additional fragment ion with a mass of 73 Da was observed. High-resolution measurement revealed the formation of a molecular ion with the formula C_2HDNO_2 as a consequence of the loss of SO_3.

For ACE OP168, all hydrogens were exchanged. Interestingly, the formation of the deuterated formylsulfamte fragment was strongly hindered in this case. It has to be mentioned that MS^3 (Figure 5-3) and MS^2 fragmentation experiments were carried out on different MS systems and are therefore not directly comparable. The complete suppression of the neutral loss of CO_2 in favor of the formation of amidosulfonic acid and the sulfite radical in contrast to experiments with D_2O is surprising.

The loss of two carbon atoms in the above-discussed OPs, left only minor possibilities for further oxidation products. However, as it has been shown that carboxylic acids are important oxidation by-products when applying ozone to tertiary wastewater (Wert et al. 2007), screening on some of these compounds was also performed. In fact, we observed high concentration of acetic acid in the batch test (Figure 5-12), which corresponds well with the proposed structures above. Formic and oxalic acid formation was also observed but with concentrations an order of magnitude lower (data not shown). Both can be expected when ACE OPs 170 and 168 are further oxidized, most likely by OH radicals, but it was also shown that ACE OP170 is rather persistent against further ozone attack which explains the low yield of these two carboxylic acids. The acetic acid formation in potable water was similar to the batch test, giving first evidence on the transferability of the oxidation mechanism to real matrices.

In order to obtain more information about the structure of the proposed OPs, we performed first NMR screening experiments with a high concentration of ACE (5 g/L). The results displayed in Figure 5-4 show that two main OPs (regions 4 and 6) are formed. The chemical shifts of the NMR signals (Figure 5-11) can be attributed to the formation of formic acid (8.25 ppm, region 1) and acetic acid (2.09 ppm, region 6). As signals referring to regions 1 and 3 (5.49 ppm) correlate, they seem to originate from the same minor compound. The shift in intensity at 75 min ozonation time might be explained by the disturbance of tautomerism possibly due to missing buffer in the sample for NMR measurements. The sample with an ozonation time of 180 min was analyzed two more times by ^{1}H-NMR two and three weeks after the first measurement. The strong unknown signal at a shift of 5.25 ppm (region 4) was observed to be highly decomposed in favor of formic acid.

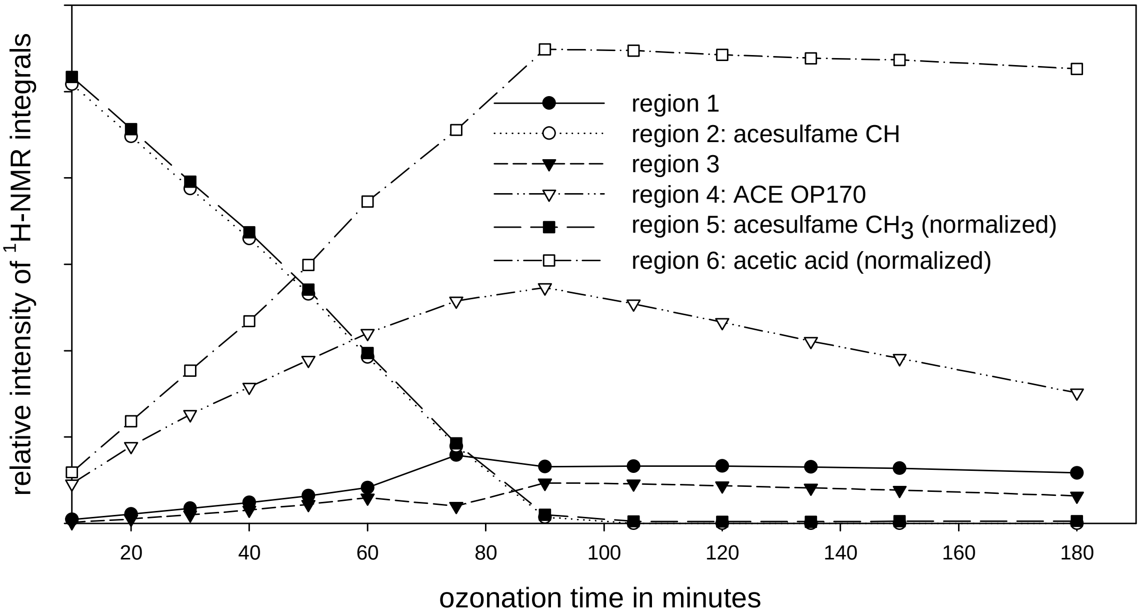


Figure 5-4 Integrated ^{1}H-NMR signals over ozonation time. Signals derived from more than one hydrogen are normalized. See also Figure 5-11 for corresponding ^{1}H-NMR spectra

In order to clarify if the remaining signal (region 4) can be assigned to ACE OP170, HPLC-NMR/MS-TOF were carried out using chromatographic conditions based on method 2 (replacement of water by D_2O for the aqueous buffer). Based on the optimized HILIC method and previous MS experiments, a certain retention time window could be used for storing the chromatographic fraction of interest into loops during the LC-NMR/MS run. The split of 2 % of the LC flow to the ion source of the mass spectrometer allowed for confirmation by the exact mass using the TOF-MS at the same time. After transfer of the loop corresponding to the peak with m/z = 170 into the NMR probe head and acquisition of an ^{1}H-NMR spectrum, a singlet with a chemical shift of 5.25 ppm could clearly be observed indicating a CH group with two hydroxyl moieties. This signal strongly corroborates the proposed dihydroxyacetyl sulfamate structure for ACE OP170 as shown in Figure 5-3. However, no resonance signals from ACE OP168 could be detected by ^{1}H-NMR. This is in agreement with the proposed structure of this compound (Figure 5-3) as the two remaining protons are fully exchangeable and hence cannot be detected in solutions with D_2O.

The formation of ACE OP170 and acetic acid is complete with the total oxidation of ACE (Figure 5-4). The further decrease of ACE OP170 can most likely be attributed to its further oxidation to oxalic acid and amidosulfonic acid (both detected by IC and LC screening).

Another possibility is the enhanced evaporation due to continuous sparging of ozone gas into the test solution which became strongly acidic (pH 2.2). Samples for NMR were also analyzed on carboxylic acids by IC and showed increasing oxalic acid and stable acetic and formic acid concentrations after complete ACE oxidation (Figure 5-13). The total sum of ^{1}H-NMR integrals decreased continuously over the test period to a final c/c_0 value of about 0.8 after 3 hours corroborating the assumption of evaporation.

For the reason of clarity, the identified OPs of both sweeteners and the tools used for identification are summarized in Table 5-3 in the supplementary material.

5.3.3 Ozonation of cyclamate and acesulfame in wastewater and tap water

In order to investigate ozonation of both artificial sweeteners in real matrices and at a more realistic environmental concentration, treated wastewater and tap water were spiked with 250 µg/L of the sweeteners and treated the same way as described above without the addition of *t*-BuOH. The wastewater samples were taken from a small WWTP described in detail in Scheurer et al. (2009) (STP 1). Influent and effluent samples were analyzed for both sweeteners. ACE concentrations in the STP influent and effluent were 39 and 34 µg/L, whereas for CYC, 145 and 0.58 µg/L were measured. The results confirm the persistence of ACE during conventional wastewater treatment and the good biodegradability for CYC reported before (Buerge et al. 2009; Scheurer et al. 2009). The applied ozone concentrations (0.2 mg/L to 5 mg/L ozone for drinking water and 0.25 mg/L to 2 mg/L ozone per mg DOC in wastewater) covered a range usually used in the corresponding treatment plants.

The results for ACE (Figure 5-14) in drinking water are in good agreement with our previous reported behavior of the compound, where 1 mg/L ozone was sufficient to remove 1 µg/L ACE from drinking water after a contact time of 40 min (Scheurer et al. 2010). As the DOC in the used drinking water is 0.9 mg/L, the results for drinking water and wastewater are comparable when contemplating the applied ozone dose per milligram DOC. A similar concentration was sufficient to remove ACE almost completely. However, 1 mg ozone per mg DOC in both drinking and wastewater treatment, represents a dosage rather in the upper range of the applied concentrations. Therefore, a considerable amount of ACE can be expected even after ozonation units.

In the case of CYC, the data for drinking water are in line with our previous findings, where 1 mg/L ozone led to a decrease of about 40 % CYC within one hour contact time (50 % after complete ozone depletion in this study), even though the compound concentration was lower. The formation of cyclohexanone was only observed for the drinking water samples and not for the treated wastewater, which is likely due to matrix effects or further reaction with the wastewater matrix rather than due to a completely different oxidation pathway. The highest yield of cyclohexanone formation was observed when applying 0.5 mg/L and 1 mg/L ozone indicating a further oxidation of cyclohexanone. This is supported by the finding of the non-scavanged batch test at pH 7.5, where cyclohexanone was already assumed to be further oxidized. In drinking water also, an increase of amidosulfonic acid was measured with decreasing CYC concentrations, supporting the finding of the batch test performed in ultra pure water. In the treated wastewater, the background concentration of amidosulfonic acid was too high to track the formation of this compound with increasing ozone dose.

A kinetic experiment was chosen as an additional approach for ACE, as the compound was found in concentrations of several micrograms per liter in raw waters used for drinking water production. For ACE, it was therefore more important to prove that no other intermediates occur during ozonation in waterworks. This was necessary, because the ozone contact time in DWTPs is usually not more than one hour, but it was one day in the batch test. After adding ozone stock solution in defined amounts, the samples were immediately measured by LC-MS/MS (method 4) representing an overall ozone contact time of about 25 min (see materials and method section). The oxidation of the precursor ACE was much faster in ultrapure water due to missing DOC (Figure 5-5). Both, ACE OPs 170 and 168, were formed under these conditions in ultra pure and tap water. OP yields in tap water were lower and signal intensity fluctuation of the OPs was more pronounced. This might be explained due to the fact that OPs and ACE were separated but the OPs eluted with the matrix peak which might be more important for drinking water. This corresponds with observations during method development for artificial sweeteners, where especially for ACE strongly suppressed signals were observed when injecting samples without SPE clean-up. A complex formation of the OPs with present cations could also be an explanation for that phenomenon, but no adducts were observed during this study.

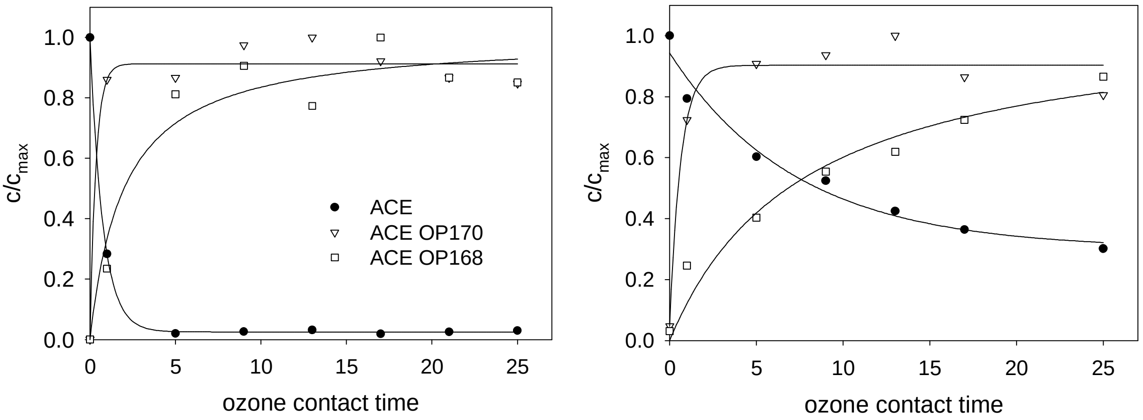


Figure 5-5 Degradation of ACE (200 µg/L) and formation of ACE OPs 170 and 168 in ultra_pure water (left) and tap water (right). Applied ozone concentration was 1 mg/L. Signals are normalized to the highest peak area observed for each compound

To confirm the relevance of ACE OP170 for real ozone applications, a DWTP located at the river Rhine was sampled. It uses a mixture of river bank filtrate and landside groundwater as raw water for drinking water production. Sampling points were the raw water, the water after the ozonation unit and after the following activated carbon filter. For detailed information about the treatment steps, see the description of waterworks D in Scheurer et al. 2010. The ozonated water was quenched with sodium sulfite in order to avoid further oxidation. In the raw water, no CYC and 0.51 µg/L ACE were measured. Due to insufficient ozone concentration and/or contact time, ACE was only partly removed and still present with 0.21 µg/L after the ozonation unit. Screening of ACE OP170 (after solid-phase extraction with a weak anion exchanger material and separation with LC method 2) confirmed the formation of the compound also in waterworks using ozonation, but also its removal by activated carbon (Figure 5-6). This is most likely due to biological activity in the upper part of the activated carbon filter and not due to the removal by adsorption. As the samples were not stabilized, a partial biological degradation of the OPs cannot be excluded, but the results are still adequate to prove the relevance for real water treatment processes.

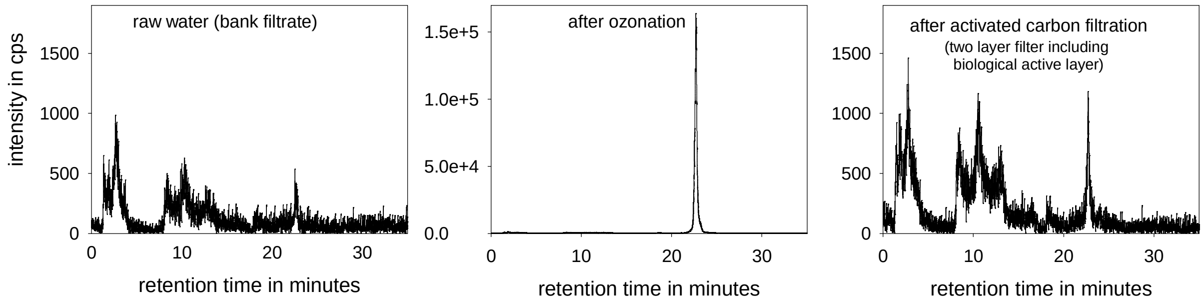


Figure 5-6 Chromatograms of MRM transition 169.8/151.8 (ACE OP170) of raw water (left), after ozonation (middle), and after subsequent activated carbon filtration (right) from a waterworks using bank filtrate of the Rhine river as raw water. Note the different ordinate scaling of the three graphs

5.4 Conclusions

For structural elucidation of unknown compounds, a wide range of analytical techniques for separation and detection is necessary. This work has shown that for polar analytes like artificial sweeteners, LC-MS is first choice, but it should be considered that also volatile compounds, more suitable for GC analysis, might be generated during ozonation. For the final structural elucidation, more advanced techniques like MS^n, high-resolution MS or even NMR measurements are necessary if no standards for the confirmation of the OPs are available.

In this study, the cleavage of the side chain of the CYC molecule during ozonation resulted in two main OPs, amidosulfonic acid and cyclohexanone. Based on the CYC concentrations found in WWTP effluents and the formation of these low to moderate toxic OPs during ozonation, the environmental relevance of ozone application in order to remove CYC seems little.

Based on previous studies, ACE has more environmental and drinking water relevance as CYC. As easily transformed during ozonation in DWTPs, it was important to elucidate that mainly acetic acid and ACE OP170, dihydroxyacetyl sulfamate, are formed in aqueous solution. This aldehyde hydrate might be a reactive compound, but primary findings showed its good removal in activated carbon filters downstream of an ozonation unit in a full-scale DWTP. Similar to the removal of other by-products formed during ozonation, such as NDMA, this combination of treatment steps can therefore be considered as an effective and safe tool to remove ACE from raw waters. For this purpose, ozone dose and contact time can be optimized, if necessary.

5.5 Supplementary material

Samples from the batch tests were analyzed with different liquid chromatography (LC), gas chromatography (GC), and ion chromatography (IC) methods coupled to different detection systems. The methods were optimized for pCBA and the suspected oxidation products (OPs) based on preliminary experiments.

Nuclear magnetic resonance (NMR), MS experiments and D_2O exchange experiments were used to confirm the structure of some OPs.

LC methods

For CYC and some OPs a Synergie Hydro RP column (250 x 3 mm, 4 µm) from Phenomenex (Aschaffenburg, Germany) was used for separation (method 1). More polar OPs were separated by a ZIC-HILIC column (150 x 2.1 mm, 3.5 µm) from dichrom (former SeQuant, Marl, Germany; (method 2)). Retention of pCBA (method 3) was achieved by using an Eclipse Plus C18 RRHD column (50 x 2.1 mm, 1.8 µm) from Agilent Technologies (Waldbronn, Germany). The same column was used for kinetic studies (method 4).

The optimized gradient programs and buffers used in the LC methods are summarized in Table 5-2. The analysis of ACE and CYC is described in detail in Scheurer et al. (2009).

The chromatographic methods were carried out on a series 1200 and an Infinity 1290 high performance (HP) LC system (both from Agilent Technologies, Waldbronn, Germany) equipped with a solvent cabinet, a micro vacuum degasser, a binary pump, a high-performance autosampler with two 54 vial plates, and a temperature controlled column compartment. The series 1200 HPLC system was connected to an API 4000 tandem mass spectrometer (Applied Biosystems / MDS Sciex Instruments, Concord, ON, Canada) with an electrospray interface and the series 1290 systems was coupled with a 6540 UHD Q-TOF mass spectrometer (Agilent Technologies, Waldbronn, Germany) both operated in negative ionization mode. However, screenings for unknown OPs were also performed in positive ionization mode. The Infinity 1290 system was additionally connected to a diode-array detector, which was operated at 241 nm for the detection of pCBA.

GC method

GC analysis for the suspected oxidation products was carried out with an Auto System XL gas chromatograph connected to a Turbo Mass Gold mass spectrometer (both Perkin Elmer, Waltham, MA, USA). A ZB Multi Residue 1 column (30 m x 0.25 mm from Phenomenex (Aschaffenburg, Germany) was used for the separation of the analytes (flow rate 1.0 mL/min). The temperature program started at 40 °C and was held for 4 min, ramped 5 °C/min to 120 °C (held for 0 min), and ramped 45 °C/min to 300 °C and held for another 6 min.

IC methods

Detection of carboxylic acids:

Separation of carboxylic acids was achieved by using an Ion Pac AS IC column and an ICS3000 high performance IC system coupled with a conductivity detector (both Dionex, Sunnyvale, CA, USA). Eluents used for the gradient were ultra pure water (A), 200 mmol/L NaOH (B), and 10 mmol/L NaOH (C). The gradient (flow rate 1 mL/min) started with 8 mmol/L NaOH and held isocratic for 10 min and was then increased over 13 min to 126 mmol/L NaOH and held isocratic for another 12 min (total run time 35 min). Equilibration time with the starting condition was 10 min. Injection volume was 500 µL.

Detection of sulfate and other sulfur containing anionic species:

The coupling of ion chromatography with inductively coupled plasma mass-spectrometry (IC-ICP–MS) was used to obtain information about the number of anionic sulphur containing OPs and the mass balance of sulfur during ozonation. For this purpose the IC system described above, equipped with an electrochemical suppressor (ASRS 300, 4 mm, Dionex) was coupled to an ICP-MS 7500 ce system (Agilent) for the detection of sulfur oxide ion SO^+ ($m/z = 48$). Separation was done under isocratic conditions (25 mmol/L NaOH, flow rate: 0.5 mL/min) on an Ion Pac AS 20, 4 x 250 mm (Dionex, Sunnyvale, CA, USA) analytical column

NMR

For NMR measurements, the original aqueous solutions were diluted with 10 % (v/v) 1.5 mol/L KH_2PO_4 buffer in D_2O adjusted to a pH of 3 in 5 mm NMR tubes. NMR spectra were acquired on a 600 MHz AVANCE II NMR spectrometer equipped with a 5 mm TCI cryo probe. For the 1D NMR experiments, the noesygppr1d pulse program was used. For all experiments continual water presaturation RF of 25 Hz was applied during relaxation delay D1. Moreover, the noesygppr1d sequence was acquired using a total of 8 scans for acesulfame and 32 scans for cyclamate reaction solutions. Data were acquired into 64 K complex data points, spectral width of 20 ppm, 10 ms of mixing time and relaxation delay of 10 s. With all types of experiments receiver gain was kept at constant value of 128. Free induction decays (FID)s were multiplied by an exponential function equivalent to that of a 0.3 Hz line-broadening factor and then Fourier transformed. Taking advantage from the baseopt digitalization mode, spectra were automatically phased, baseline corrected and referenced using Topspin. On representative samples we also acquired 2D-^{1}H-^{13}C-HSQC (Heteronuclear Single Quantum Coherence) and 2D-^{1}H-^{13}C-HMBC (Hetero Multiple Bond Correlation) experiments. 8 to 64 FIDs were acquired for each of the 400 increments. Sweep widths were adjusted according to the requirements obtained from the ^{1}H-NMR spectra.

For confirmation of the results HILIC-NMR/MS was used according to method 2 described above, but eluent A was replaced by D_2O (Deutero GmbH, Kastellaun) with 20 mmol/L NH_4COOH. The system consists of an Agilent 1200 HPLC system including a quaternary HPLC pump, auto sampler and diode array detector. The chromatography was done after injection of 30 µL of sample. 2 % of the post chromatographic flow was split to a MicroTOF time of flight mass spectrometer (Bruker, Rheinstetten, Germany) equipped with an electrospray ion source and operated in negative ionization mode. Calibration was done by infusion of 20 mmol/L lithium formate solution prior to the chromatographic separation. Peaks detected were stored in BPSU-loops (Bruker peak sampling unit, Bruker Biospin, Rheinstetten, Germany) prior to the transfer into a room temperature selective inverse NMR flow probe connected to an AVANCE III 500 MHz NMR spectrometer (Bruker Biospin, Rheinstetten Germany). NMR measurements were done using a WET solvent suppression pulse program (Smallcombe et al., 1995). Up to 1024 scans were collected into 32 K complex data points over a sweep width of 20 ppm and a relaxation delay of 3 s. Prior to Fourier transformation the free induction decay was multiplied to an exponential function leading to a peak broadening of 1 Hz.

Table 5-2 Compilation of liquid chromatographic methods used throughout this study

step	time				flow rate				eluent A			
	in minutes				in mL/min				in %			
Method No.	1[a]	2[b]	3[c]	4[a]	1[a]	2[b]	3[c]	4[a]	1[a]	2[b]	3[c]	4[a]
step 1	0	0	0	0	0.3	0.35	0.4	0.25	100	5	90	98
step 2	5	5	1	3	0.3	0.35	0.4	0.25	100	5	90	98
step 3	20	30	3		0.3	0.35	0.4		65	40	50	
step 4	23	32	6		0.3	0.35	0.4		100	40	50	
step 5		35	7			0.35	0.4			5	10	

[a] eluent A: water, eluent B: methanol; A and B with 20 mM ammonium acetate

[b] eluent A: water + 20 mM ammonium formate, eluent B: acetonitrile (for LC-NMR: water substituted by D_2O)

[c] eluent A: water, eluent B: methanol; A and B with 0.1% formic acid

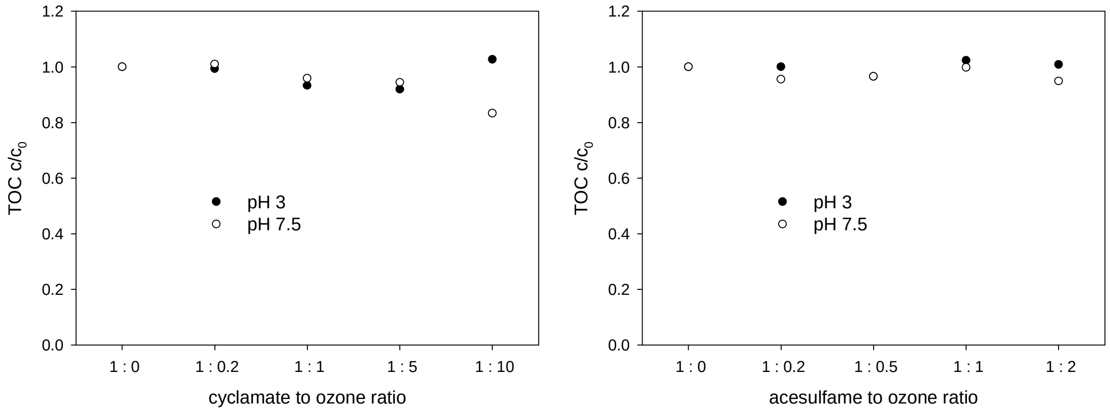


Figure 5-7 Normalized TOC concentrations after treatment with ozone for different cyclamate and acesulfame to ozone ratios and two different pH values in batch tests without t-BuOH radical scavenger

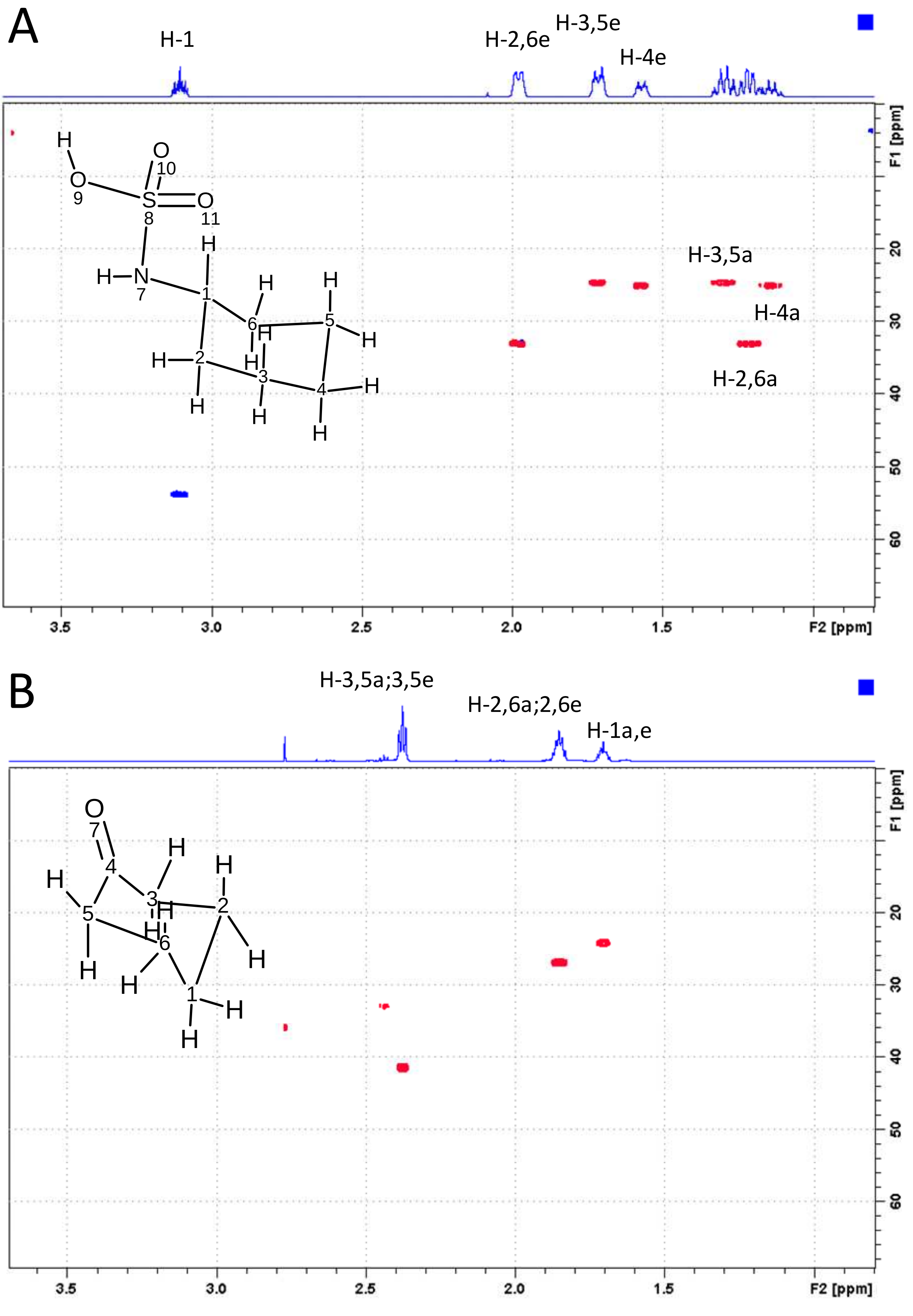


Figure 5-8 Edited HSQC spectrum of CYC before ozonation (A) and after 80 min ozonation (B). Blue signals indicate –CH groups, while red signals correspond to –CH_2 groups. The labeling for CYC distinguishes between axial (a) and equatorial (e) protons of the cyclohexane moiety

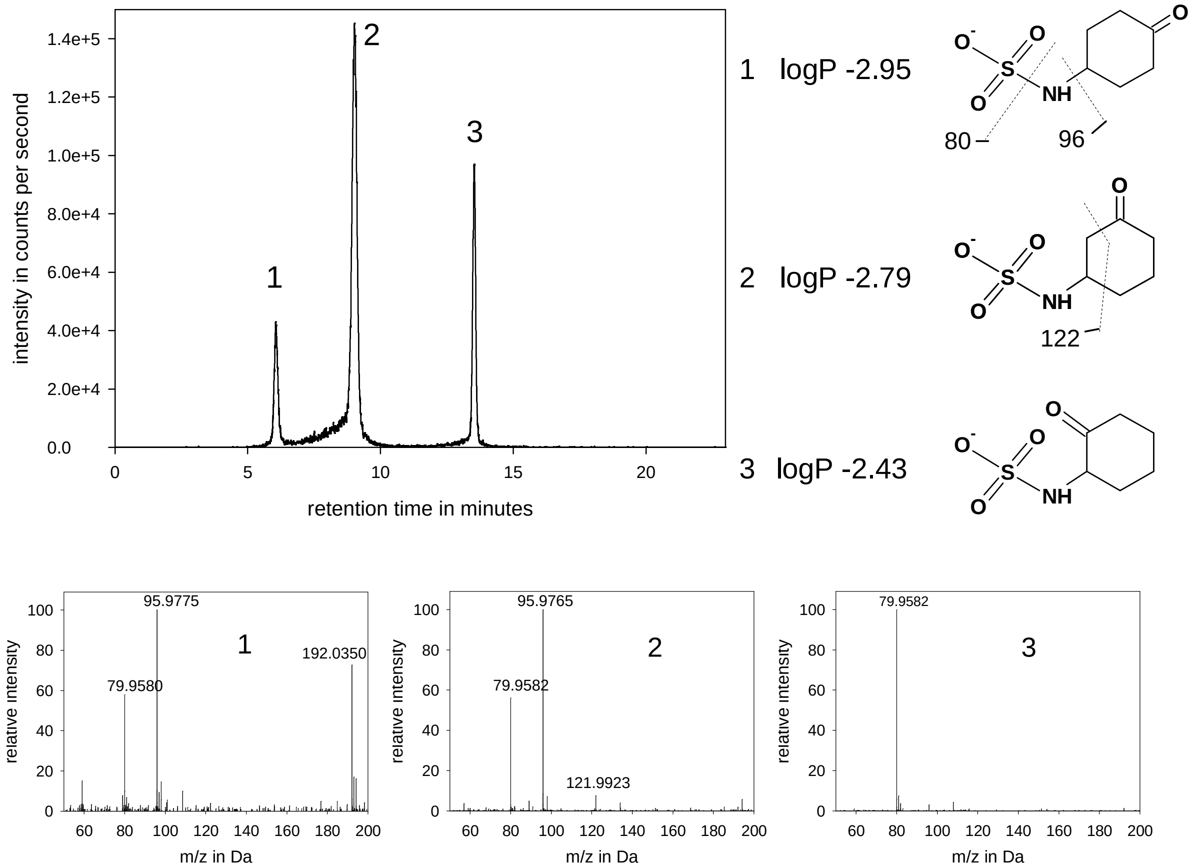


Figure 5-9 Extracted ion chromatogram of *m/z* = 192.03. Elution order of the expected isomers of the proposed structure of CYC OP192 was assigned on calculated logP values (ChemAxon, 2011) (top). MS/MS spectra from left to right correspond to the three peaks in the order of their elution (bottom)

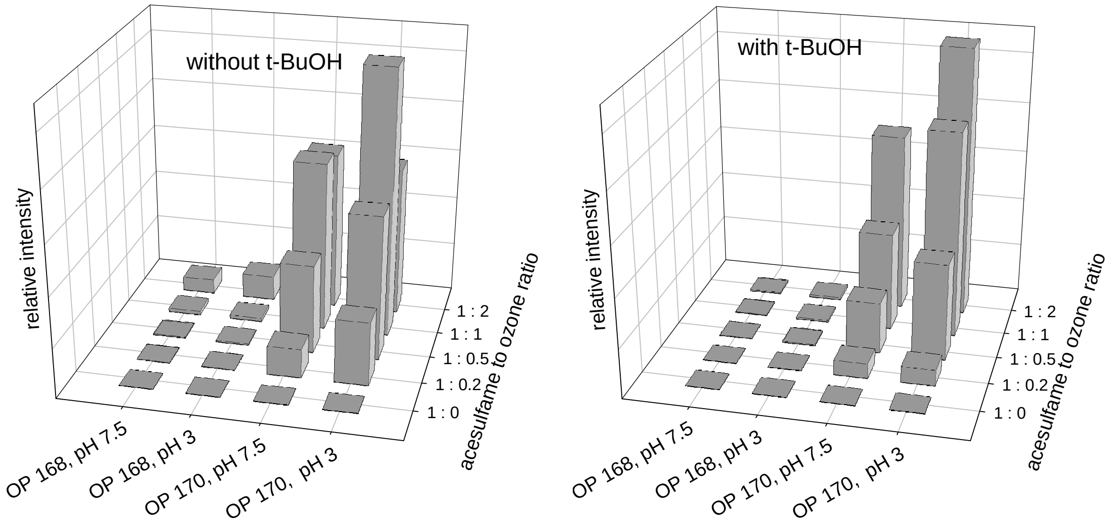


Figure 5-10 Relative intensities of ACE OPs 170 and 168 in batch tests with different pH values and with or without radical scavenging. Both figures are normalized to the same absolute peak area and are therefore directly comparable

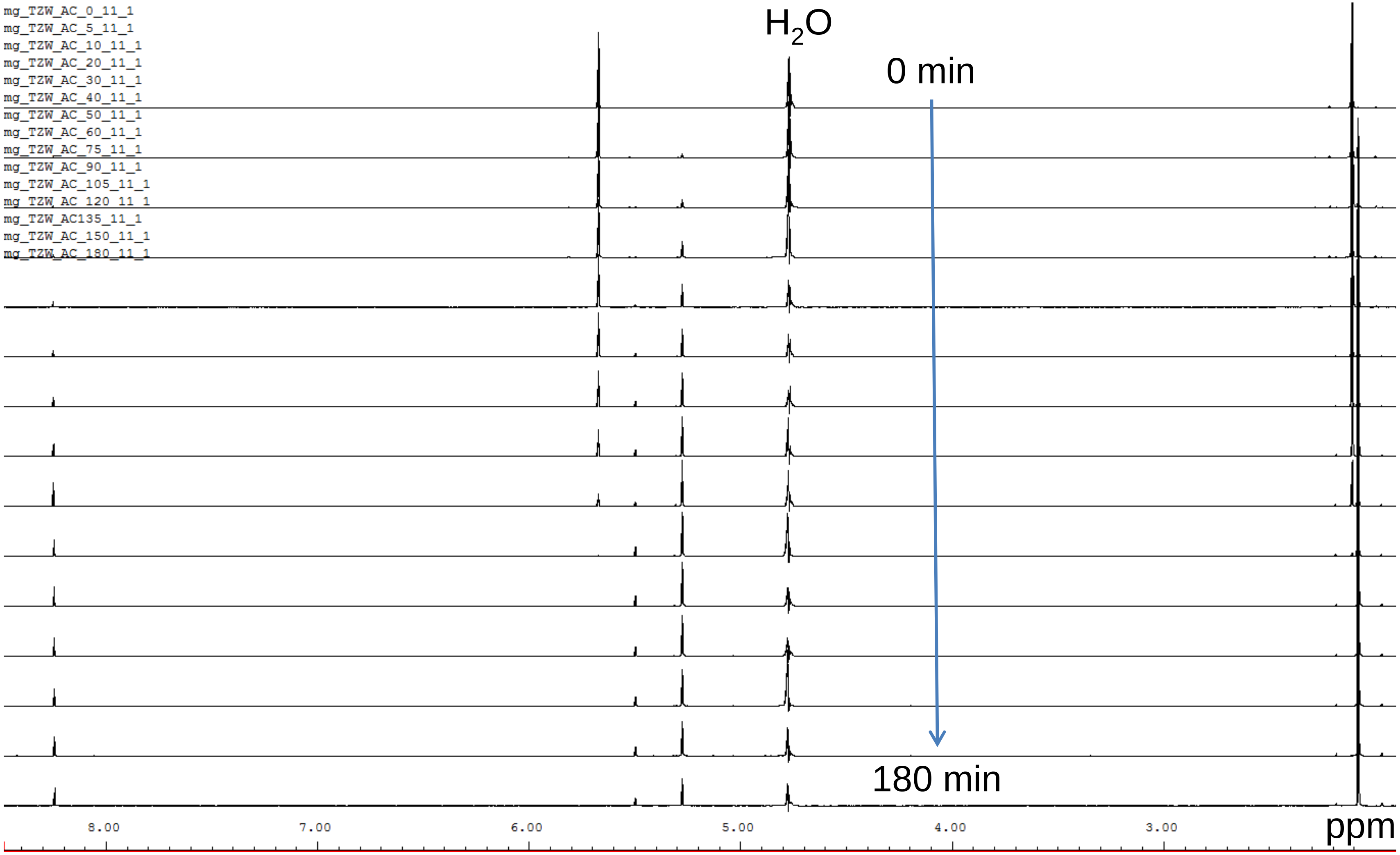


Figure 5-11 ^{1}H-NMR spectra for the ozonation of ACE after different ozonation times. Ozone gas was directly sparged into the aqueous test solution (C_0 ACE= 5g/L), maximum ozonation time was 180 min

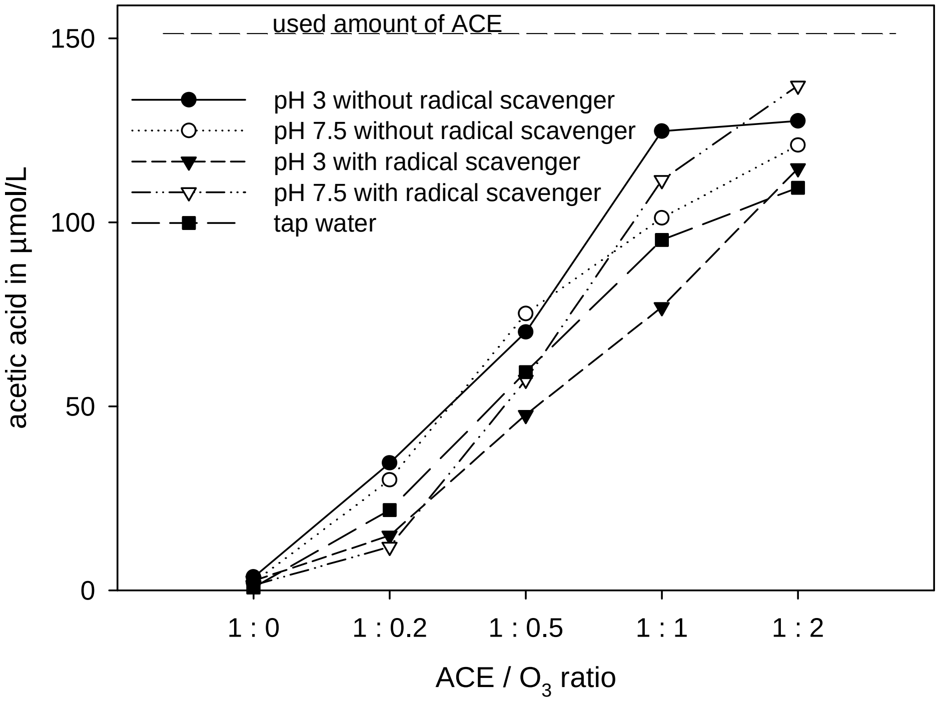


Figure 5-12 Formation of acetic acid in the batch test in ultra pure and tap water at different ACE to ozone ratios (c_0 of ACE was 153 µmol/L and is indicated by the dashed line)

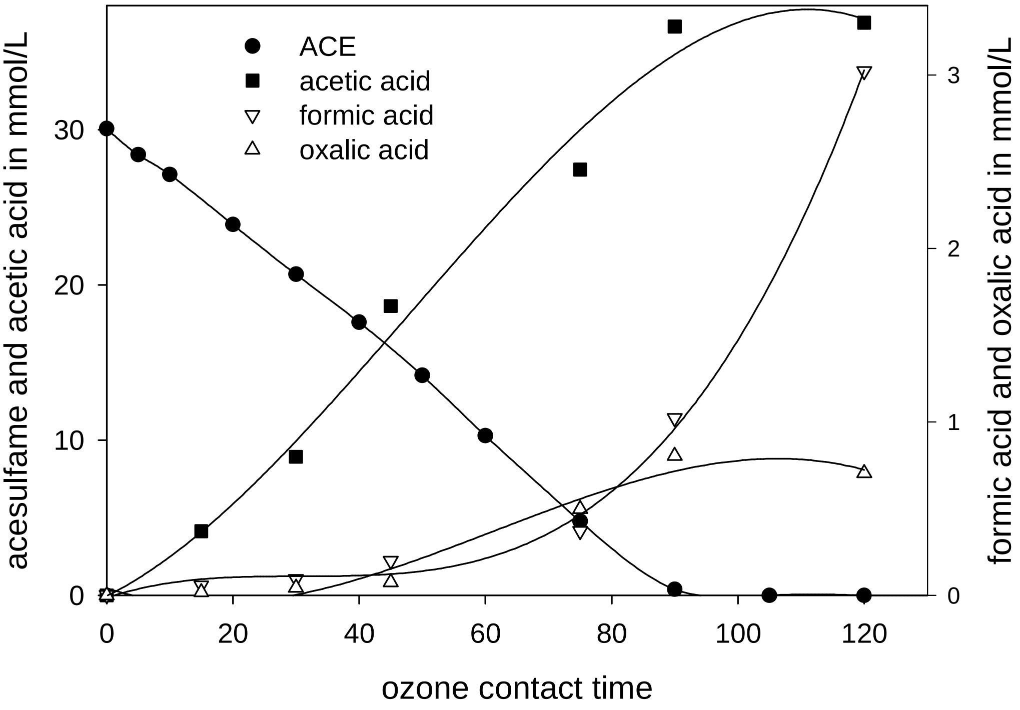
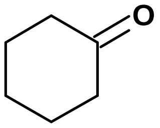
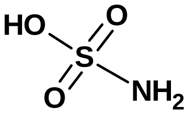
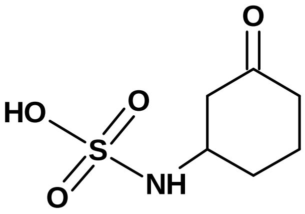
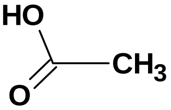
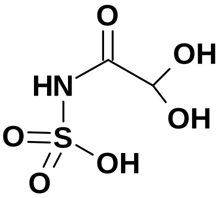
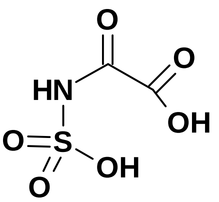
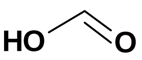
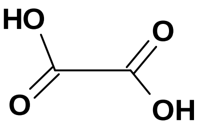


Figure 5-13 Formation of carboxylic acids during ozonation of ACE in ultra pure water, without radical scavenger. Ozone gas was directly sparged into the aqueous test solution

Table 5-3 Summary of the identified oxidation products of cyclamate and acesulfame and the used tools for their identification

name	**chemical structure**	**tools used for identification**
identified oxidation products of cyclamate		
cyclohexanone		- reference standard - ^{1}H-NMR - GC-MS and NIST database
amidosulfonic acid (sulfamic acid)		- reference standard - high resolution LC-Q-TOF
CYC OP192 (3-oxocyclohexyl)sulfamate*		- high resolution LC-Q-TOF - MS^{2} experiments (high resolution L-Q-TOF)
identified oxidation products of acesulfame		
acetic acid		- reference standard - ^{1}H-NMR
ACE OP170 (dihydroxyacetyl) sulfamate		- ^{1}H-NMR - HILIC-LC ^{1}H-NMR - MS^{2} experiments (high resolution LC-Q-TOF) - MS^{3} experiments by direct injection - D$_{2}$O MS^{2} experiments (high resolution LC-Q-TOF)
ACE OP168 (carboxycarbonyl) sulfamate		- MS^{2} experiments (high resolution LC-Q-TOF) - MS^{3} experiments by direct injection - D$_{2}$O MS^{2} experiments with high resolution Q-TOF
formic acid		- reference standard - ^{1}H-NMR
oxalic acid		- reference standard - ^{13}C-NMR

* displayed is only one of three possible isomers, (2-oxocyclohexyl)sulfamate and (4-oxocyclohexyl)sulfamate are also identified oxidation products

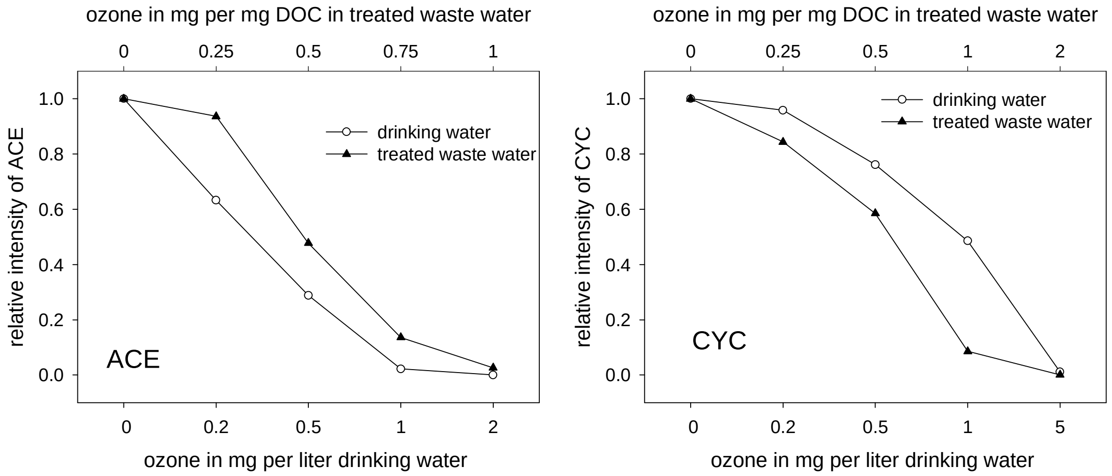


Figure 5-14 Decrease of ACE (left) and CYC (right) concentration in drinking water and treated waste water spiked with different ozone concentrations. Note the different ozone doses applied for the two sweeteners

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Dodd MC, Rentsch D, Singer HP, Kohler HP, von Gunten U (2010) Transformation of beta-lactam antibacterial agents during aqueous ozonation: reaction pathways and quantitative bioassay of biologically-active oxidation products. Environ Sci Technol 44:5940-5948.

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6

Final conclusions and outlook

The original search for a reliable tracer compound for the indication of a wastewater impact in a SAT-field in Israel identified artificial sweeteners as emerging contaminants. The developed analytical method, based on LC-MS/MS, allows for the rapid and sensitive analysis of seven artificial sweeteners in aqueous environmental matrices, such as wastewater, surface water, groundwater, and drinking water. Accurate quantification could be achieved by the use of deuterated standards and appropriate sample dilution. The first application of this method demonstrated the ubiquitary occurrence of acesulfame, sucralose, saccharin, and cyclamate in German wastewaters and surface waters. Although all four sweeteners are mainly excreted unchanged by humans, their behavior in WWTPs is different. A very good biodegradability was observed for saccharin and cyclamate, but due to high influent concentrations they are still released into recipient waters with concentrations up to the µg/L range. The limited use of sucralose in consumer products in Germany constrains its relevance, although the compound showed a pronounced stability during wastewater treatment. However, in other countries sucralose is already the dominant sweetener with concentrations more than one order of magnitude higher than in Germany. Acesulfame is by far the most important sweetener of this study. The compound has high concentrations in wastewater and is practically not removed by secondary or even tertiary treatment by powdered activated carbon.

As a direct consequence of their stability and mobility only sucralose and acesulfame are relevant for drinking water treatment plants, whereas remaining traces of saccharin and cyclamate are effectively removed during river bank filtration (RBF). The multi-barrier treatment system is essential and only effective for the removal of sucralose and acesulfame from raw water sources if certain boundary conditions are met. For sucralose activated carbon filtration is very effective and no detectable traces can be expected in drinking water at this stage of usage in Germany. However, for other countries, where the concentrations of sucralose are much higher, the effectiveness of single treatment steps in waterworks should be further investigated as other treatment steps seem to be ineffective. Chemical treatment with ozone is more effective for acesulfame, but the applied ozone doses and contact times in waterworks are not sufficient for a complete oxidation of the compound. It was shown for the first time within this thesis that acesulfame is only removed by activated carbon with a low pre-load and that it can break through into finished drinking water. Concerns about the formation of undesired compounds during ozonation of acesulfame were allayed by the structural elucidation of acetic acid and dihydroxyacetyl sulfamate, a compound with an aldehyde hydrate moiety, as major oxidation products. In fact, the latter was detected in a full scale waterworks after the ozonation of river bank filtrate, but also effectively eliminated by activated carbon filtration, most likely by biological degradation in the filter.

The most important outcome of this thesis is the identification of acesulfame as an almost ideal tracer for contaminations of water sources by domestic wastewater. The usage of acesulfame implicates a high specificity to wastewater and its persistence and mobility enables the evaluation of a contamination in a quantitative manner. Based on an average concentration of about 20 µg/L in treated wastewater and on a limit of quantification of 10 ng/L the detection of only 0.05 % of wastewater derived water is possible. Contemplating all four sweeteners found in wastewater it might be even possible to use this compound class for the differentiation of untreated and treated sewage. Saccharin and cyclamate are short-lived when passing WWTPs and can therefore indicate an impact of untreated wastewater when they are detected in high concentrations comparable to the levels found in WWTP influents.

Further work is worthwhile for sucralose. The compound proved to be rather persistent during aerobic RBF but the concentrations decreased during extended residence time in the subsurface of the SAT field in Israel. As sucralose is used in much higher quantities in Israel compared to Germany, it was still detected in the µg/L-range even after a reduction of about 90 % of the initial concentrations and after almost two years in the subsurface. This fact should be studied carefully in future research as sucralose could be one of very few compounds providing information about the age of SAT treated wastewater depending on the degradation extend. By using acesulfame as a wastewater tracer it was possible to assess the purification power of the Israeli SAT process regarding organic trace pollutants. A very effective removal of a broad range of compound classes like pharmaceuticals, X-ray contrast agents, aromatic sulfonates, etc. was observed (Lange et al., 2011), whereas the acesulfame concentrations remained constant even after a residence time of more than one year in the subsurface.

The publication in chapter 2 and the contemporaneously published article of Buerge et al. (2009) were the first putting forward the research on artificial sweeteners in common and on sucralose and acesulfame in particular. They were followed by numerous other studies on artificial sweeteners in the environment during the last two years. Mead and co-workers (2009) presented the first data for sucralose for North America. Sucralose was found in two orders of magnitude higher concentrations in wastewater compared to Europe and was even detected in oceanic waters. The presence of sucralose was attributed to its persistence against bacterial decomposition in coastal environments and it was suggested as a potential tracer for anthropogenic activities and for the oceanic circulation. The presence in U.S. inland surface waters, its suitability as an anthropogenic marker and the ineffectiveness of advanced wastewater treatment techniques for the removal of sucralose was demonstrated in subsequent publications (Oppenheimer et al., 2011; Torres et al., 2011; Soh et al., 2011). The sucralose emissions for the Swedish city of Linköping were evaluated by Schmidt Neset et al. (2010). The authors proved that the removal of only one sucralose-sweetened product from the market (Coca Cola light) reduced the daily household emissions by more than 60 %. This has to be considered when using artificial sweeteners as anthropogenic markers and one should keep in mind that the wastewater load might also be a matter of the market launch or removal of sweetened products. In a wastewater plume in a Canadian aquifer acesulfame correlated positively with chloride, revealing its abundance and prediction power also for North America (Van Stempvoort et al., 2011). In Switzerland acesulfame was used to confirm the correlation of perfluoroalkyl acids in river water with the population living in the catchment area (Müller et al., 2011).

However, further work about artificial sweeteners is still needed to fill gaps concerning their behavior under different redox conditions and to identify possible degradation products. If acesulfame proves to be stable in any environmentally relevant redox environment, it could even indicate changing redox conditions, e.g. when concentration ratios with other persistent trace pollutants, which are degraded only anaerobically, are shifted. This is becoming apparent by first own measurements at anaerobic river bank filtration sites.

In the Israeli study area a pronounced decrease of the sucralose concentration was observed, but no degradation products are identified yet. Both, aerobic as well as anaerobic degradation, e.g. by dehalogenation, have to be taken into account in future research.

Although acesulfame concentrations correlated well with the levels of other conservative markers like CBZ and chloride, uncertainty studies about its prediction power are still missing. The wastewater specificity of acesulfame could be slightly lower compared to other organic trace pollutants due to its widespread usage and possible spills. Even a seasonal fluctuation seems possible. This should be evaluated in terms of the variability of WWTP effluent and background concentrations. For this reason two projects financially supported by the German Technical and Scientific Association for Gas and Water (DVGW) and the Federal Ministry of Education and Research (BMBF) are already approved or proposed to fill these gaps of knowledge.

Apart from the fate and the tracer function of sucralose and acesulfame first research groups already addressed their work to ecotoxicological effects of artificial sweeteners. As used as food additives, sweeteners have already undergone intensive testing about potential harmful effects on humans. However, the facts that sucralose is a chlorinated compound and proved to be rather persistent seem to push the research compared to former years, when eco-toxicologists were always years behind after analytical chemists identified new emerging contaminants. In fact, there seems to be little evidence of acute adverse effects on the aquatic environment so far. The chronic exposure at ecotoxicologically relevant concentrations led to an increased mortality of juvenile gammarideans (Adolfsson-Erici et al., 2009) and sucralose was also identified to inhibit the sucrose transport in sugarcane (Reinders et al., 2006). For acesulfame ecotoxicological studies are still missing in the scientific literature.

Consequently the question arises if merely the pronounced persistence of a compound, without a bioaccumulative or toxic potential, is reason enough to ban it or to regulate its use. In this context artificial sweeteners are simply another class of contaminants for which the tenth principle of green chemistry (the breaking down into harmless degradation products after usage) (Anastas and Warner, 1998) was not taken into account before their market launch. The precautionary protection of the environment, water resources and consequently the human health should be strongly considered in the future for newly designed chemicals, because aquatic organisms, WWTPs, and drinking water treatment facilities already have to deal with countless organic trace pollutants awaiting their discovery.

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Ich versichere, dass ich die eingereichte Dissertation „Artificial sweeteners - Studies of their environmental fate, drinking water relevance, use as anthropogenic markers, and ozonation products“ selbständig und ohne unerlaubte Hilfsmittel verfasst habe. Anderer als der von mir angegebenen Hilfsmittel und Schriften habe ich mich nicht bedient. Alle wörtlich oder sinngemäß den Schriften anderer Autorinnen oder Autoren entnommenen Stellen habe ich kenntlich gemacht.

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Marco Scheurer Karlsruhe, 03.02.2012