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Removal of the Iodinated X-ray Contrast Medium Diatrizoate by Anaerobic Transformation

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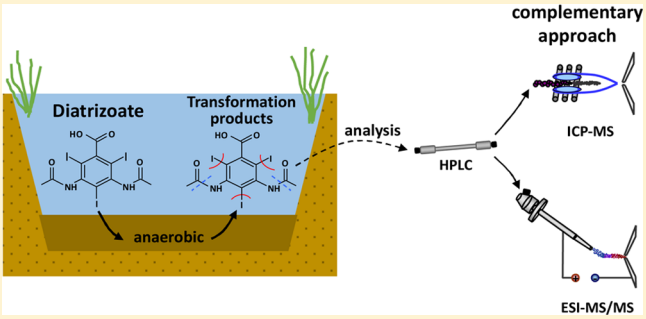
Supporting Information

ABSTRACT: The iodinated X-ray contrast medium diatrizoate is known to be very persistent in current wastewater treatment as well as in environmental compartments. In this study, the potential of anaerobic processes in soils, sediments, and during wastewater treatment to remove and transform diatrizoate was investigated. In anaerobic batch experiments with soil and sediment seven biologically formed transformation products (TPs) as well as the corresponding transformation pathway were identified. The TPs resulted from successive deiodinations and deacetylations. The final TP 3,5-diaminobenzoic acid (DABA) was stable under anaerobic conditions. However, DABA was further transformed under air atmosphere, indicating the potential for the mineralization of diatrizoate by combining anaerobic and aerobic conditions. With the development of a methodology using complementary liquid chromatography−electrospray ionization−tandem mass spectrometry and liquid chromatography−inductively coupled plasma−mass spectrometry techniques, all identified TPs were quantified and the mass balance could be closed without having authentic standards for four of the TPs available. The detection and quantification of diatrizoate TPs in groundwater, in technical wetlands with anaerobic zones, and in a pilot wastewater treatment plant established for anaerobic treatment highlights the transferability and up-scaling of the results attained by laboratory experiments to environmental conditions.



INTRODUCTION

Diatrizoate is an iodinated X-ray contrast medium (ICM) used for the imaging of soft tissues by enhancing the absorption of X-rays due to the iodine atoms in its basic structure. Diatrizoate is typically administered in elevated doses of up to >100 g per application and excreted mainly nonmetabolized.[1] Consequently, diatrizoate is present in wastewater at concentrations of several μg/L.[2,3] In wastewater treatment plants (WWTPs), it is known to be persistent under aerobic conditions. Because of its high polarity, removal of diatrizoate by sorption to excess sludge is also negligible. Therefore, diatrizoate has been detected at elevated concentrations in the effluents of WWTPs, surface waters, groundwater, and even in finished drinking water.[3−8]

Owing to the persistence of diatrizoate during conventional biological wastewater treatment and its ubiquitous occurrence in the water cycle, several strategies for the removal of diatrizoate by advanced wastewater treatment and polishing drinking water treatment have been investigated. In processes such as ozonation,[9] γ-irradiation of air-equilibrated or NO_2-saturated solutions,[10] and photocatalysis in oxic TiO_2 suspensions[11] only limited quantities of diatrizoate were oxidized. A transformation of diatrizoate by both oxidative and reductive processes was found by applying Fe^0 at pH 3 in the presence of oxygen.[12] A deiodination of diatrizoate was achieved by a reductive catalysis with Pd on Al_2O_3 catalysts[13] or microbially produced Pd nanoparticles,[14−16] electrochemical reduction,[17] and photocatalysis with anoxic TiO_2 suspensions.[11] Hence, diatrizoate seems to be susceptible to a reductive dehalogenation by various abiotic treatment options.

However, studies focusing on the biological transformation of diatrizoate are scarce. Incubation with the white rot fungus *Tramtes versicolor* led to a partial deiodination.[18] Two other studies reported the formation of transformation products (TPs) by deacetylation in aerobic batch experiments with sediment−water or activated sludge.[19,20] In a recent study with a moving bed biofilm reactor system with subsequent denitrification and nitrification, a reduction of the diatrizoate concentrations up to 73% was observed involving the formation of several TPs.[21]

To the best of our knowledge no information is currently available about the fate of diatrizoate in strictly anaerobic environmental zones or during anaerobic wastewater treatment. Therefore, the aim of this study was to elucidate the potential of different environmental matrices (soil, sediment, and sewage sludge) for an anaerobic transformation of diatrizoate. Furthermore, the transformation pathway was elucidated by

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Table 1. Overview of Batch Experiments Prepared for the Investigation of the Anaerobic Transformation of Diatrizoate

ID	compound, added concentration	matrix, solid/liquid ratio	additional information	objective
1.1.a	diatrizoate, 100 μg/L (=first spike)	soil + GW,[a] 1/4	no addition	investigation of transformation potential
1.1.b			addition of acetate (10 mM)	
1.1.c			addition of Fe(III) (10 mM) +acetate (1.25 mM)	
1.2	diatrizoate, 10 mg/L (=second spike, respike of 1.1.c)	soil + GW, 1/4	-	identification of TPs, elucidation of transformation pathway, investigation of mass balance
1.3.a	diatrizoate, 10 mg/L (=third spike, respike of 1.2)	soil + GW, 1/4	nonautoclaved	differentiation between abiotic and microbial process
1.3.b			autoclaved	
2.a	diatrizoate, 10 mg/L	soil + GW, 1/4	nonautoclaved	reproducibility of mass balance, confirmation of microbial transformation
2.b			autoclaved	
3	TP 236, 10 mg/L	soil + GW,1/4	-	elucidation of transformation pathway
4.a	TP 236, 10 mg/L	soil + GW,1/4	incubated under air atmosphere	test for aerobic stability
4.b	DABA, 10 mg/L		incubated under air atmosphere	
4.c	DABA, 10 mg/L		-	test for anaerobic stability
5	diatrizoate, 5 μg/L	sediment + surface water, 1/5.5	-	investigation of the transformation at environmental concentrations

[a]GW = groundwater.

identifying the formed TPs. In addition, a major focus was set on the development of an analytical approach enabling the quantification of TPs without the need for authentic reference standards by the complementary use of liquid chromatography−electrospray ionization−tandem mass spectrometry (LC−ESI−MS/MS) and liquid chromatography−inductively coupled plasma−mass spectrometry (LC−ICP−MS) techniques. The latter is a powerful tool for the quantification of iodinated compounds due to its species-unspecific response.[22,23] The environmental relevance of the identified TPs and the transferability of the results obtained from lab-scale studies to the field-scale were assessed by quantifying the diatrizoate TPs formed under anaerobic conditions in environmental compartments and a pilot plant designed for anaerobic wastewater treatment.

MATERIALS AND METHODS

Chemicals, Standards, and Solvents. Information on the chemicals, standards and solvents used for this study is provided in the Supporting Information.

Soil, Sediment, Water. The soil used for the batch experiments was from the Ap horizon layer of an agricultural field in Braunschweig, Germany, exhibiting a low organic matter content of approximately 0.9% and a sand content of more than 90%. The iron content of the soil was 0.46%. This field has been irrigated with secondary treated wastewater and sludge for more than 50 years. A summary of the soil characteristics is provided by Ternes et al.[24] The sediment for the batch experiments was taken from a sulfate reducing zone of a polishing pond that is fed by treated wastewater (8 L/s) from a conventional municipal WWTP with nitrification and denitrification (hydraulic retention time (HRT) (biology), 24 h; solid retention time (SRT), 20 d) for several years. From this sediment 10 cm cores were taken by a cylindric tube. The tube was immediately closed airtight and transported to the lab, where it was processed in a glovebox under an argon atmosphere.

The groundwater used in the batch systems was collected from a deep well in the district Arenberg of Koblenz, Germany. Characteristics of this groundwater are documented by Kormos et al.[25] Surface water was taken from the river Rhine in Koblenz (km 590.3), Germany.

Batch Experiments. *General Preparation.* The anaerobic laboratory systems consisted of 500 mL amber glass screw cap bottles closed with butyl/PTFE septa or of 100 mL glass serum bottles closed with butyl stoppers, respectively, to keep the systems free of oxygen. To avoid contact with atmospheric oxygen, all working steps concerning the preparation, treatment, and sampling of the batch experiments were conducted in a glovebox under argon atmosphere. The anaerobic conditions in the batch systems were confirmed by measuring the redox potentials as well as concentrations of Fe(II), sulfate, and/or sulfide during the experiments. A variety of anaerobic batch experiments were prepared using different solid and aqueous matrices (Table 1). To selected experiments acetate and Fe(III) were added as additional carbon source and/or terminal electron acceptor (TEA) to facilitate the establishment of Fe(III) reducing conditions. These were chosen because in the long term it is foreseen that Fe(III)-reducing conditions in a pilot wastewater treatment plant will be established. This is part of the ERC project ATHENE, in which different anaerobic reactors are combined with aerobic ones to enable a more complete micropollutant removal. Hence, Fe(III) reducing conditions were chosen to show whether the planned establishment of an Fe(III) reducing reactor at the pilot plant might be beneficial for the removal of iodinated contrast media such as diatrizoate. An oxygen-free atmosphere was guaranteed by purging argon through the aqueous phases prior to preparing the batch systems and repeating this step after preparation. Depending on the specific objective of the respective experiment, diatrizoate was spiked at different

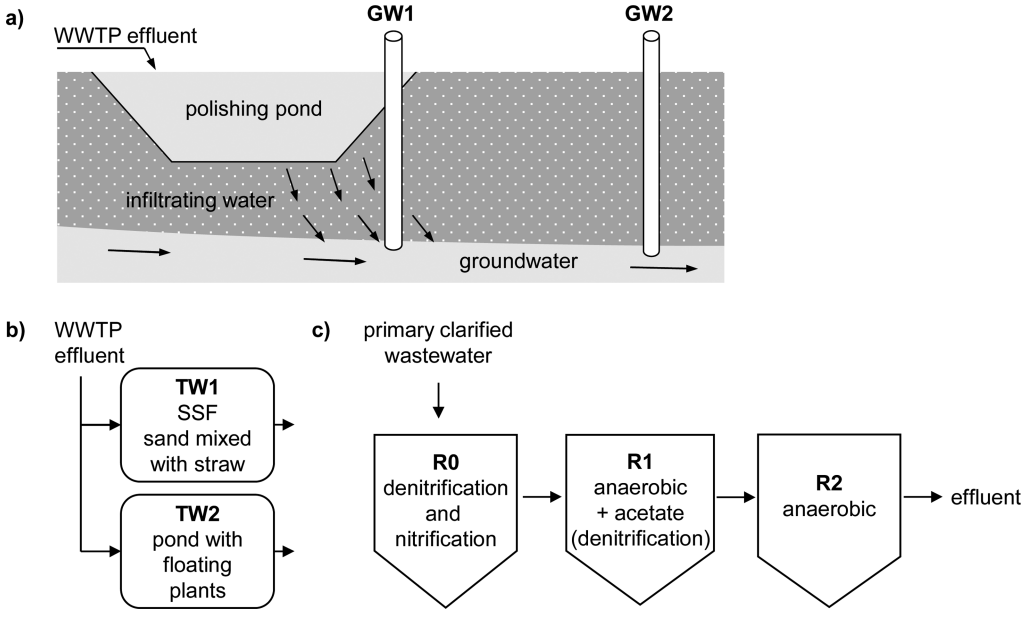

Figure 1. Schematic illustration of the systems from which the environmental samples were taken; (a) groundwater wells, (b) technical wetlands and (c) reactors of a pilot WWTP.

concentration levels (5 μg/L to 10 mg/L) as an aqueous solution that was deoxygenized by purging with argon. After preparation, the batch systems were removed from the glovebox and the bottles were thoroughly shaken by hand and were then placed on a shaking device (100 rpm) for up to 200 days. They were always incubated in the dark at room temperature (22 ± 1 °C).

Investigation of Diatrizoate Transformation. The first batch experiments were conducted to investigate whether a transformation of diatrizoate is occurring in anaerobic soil–water systems as well as to identify the TPs.

To investigate the potential for a transformation, duplicate batch experiments were set up from 100 g (fresh weight) of soil and 400 mL of groundwater (experiment 1.1.a, Table 1). Additional systems were prepared by adding 10 mM of sodium acetate (experiment 1.1.b) or 10 mM of an amorphous Fe(III)oxyhydroxide (synthesized according to Lovley and Phillips[26]) and 10 mM of sodium acetate (experiment 1.1.c), each in duplicate. Diatrizoate was spiked to all batch systems attaining a concentration of 100 μg/L.

For the identification of TPs and elucidation of the mass balance, after complete dissipation of diatrizoate in experiment 1.1 (100 d) an elevated concentration of 10 mg/L diatrizoate was respiked to the batch system 1.1.c, resulting in experiment 1.2. To elucidate whether the transformation depends on microbial activity, experiment 1.2 was after complete dissipation of diatrizoate (another 50 d) divided into two aliquots (experiments 1.3.a, 1.3.b). The batch system 1.3.b was sterilized by autoclaving (20 min at 120 °C, 120 kPa). Diatrizoate was then added to both aliquots via sterile cellulose acetate syringe filters (0.2 μm, GE Healthcare, Buckinghamshire, UK) from a deoxygenized aqueous solution.

To investigate the reproducibility of the mass balance and to confirm the microbial transformation, two further batch systems were designed with 100 g of soil and 400 mL of groundwater. They were spiked with 10 mg/L of diatrizoate (experiment 2.a) and respiked after diatrizoate was completely dissipated. Two autoclaved controls were prepared and operated in parallel (experiment 2.b).

Furthermore, to confirm parts of the proposed transformation pathway, duplicate anaerobic batch systems prepared from 100 g of soil and 400 mL of groundwater as described above were spiked with 10 mg/L of the commercially available diatrizoate TP 236 (experiment 3).

Batch Experiments under Air Atmosphere. The aerobic stability of TP 236 and of the final TP DABA, which was rather stable under anaerobic conditions, was tested in duplicate batch systems under air atmosphere (experiments 4.a, 4.b). Groundwater (400 mL) was added to 100 g of soil and spiked with 10 mg/L of TP 236 or DABA, respectively. After shaking thoroughly by hand, the systems were closed only with a layer of tissue each to prevent from sample contamination, while also allowing for throughout the experiment a continuous diffusion of atmospheric oxygen into the system.

Transformation at Environmental Relevant Concentrations. To investigate the transferability of the results of the batch experiments described above, an experimental setup was designed (experiment 5) including (i) much lower diatrizoate concentrations (5 μg/L) and (ii) using 10 g of an originally anaerobic sediment from a sulfate-reducing zone of a polishing pond and (iii) 55 mL of Rhine water taken at Koblenz. In total, 19 separate batch systems were prepared. Three of them were sampled per time point.

Sampling. In experiments 1 to 4, samples (1–3 mL) were passed through 0.45 μm regenerated cellulose syringe filters (C. Roth, Karlsruhe, Germany) into 1.5 mL HPLC vials. The vials were stored in airtight glass tubes under an argon atmosphere at 4 °C until analysis. In experiment 5, samples were taken by centrifuging and passing the entire aqueous phase (ca. 50 mL) through 0.45 μm regenerated cellulose syringe filters (C. Roth, Karlsruhe, Germany) into 60 mL vials, which were closed with butyl/PTFE septa and stored at −18 °C.

Environmental Samples. Groundwater (grab samples) from different depths, including Fe(III) and Mn(IV) reducing zones, was sampled from two wells that are influenced by

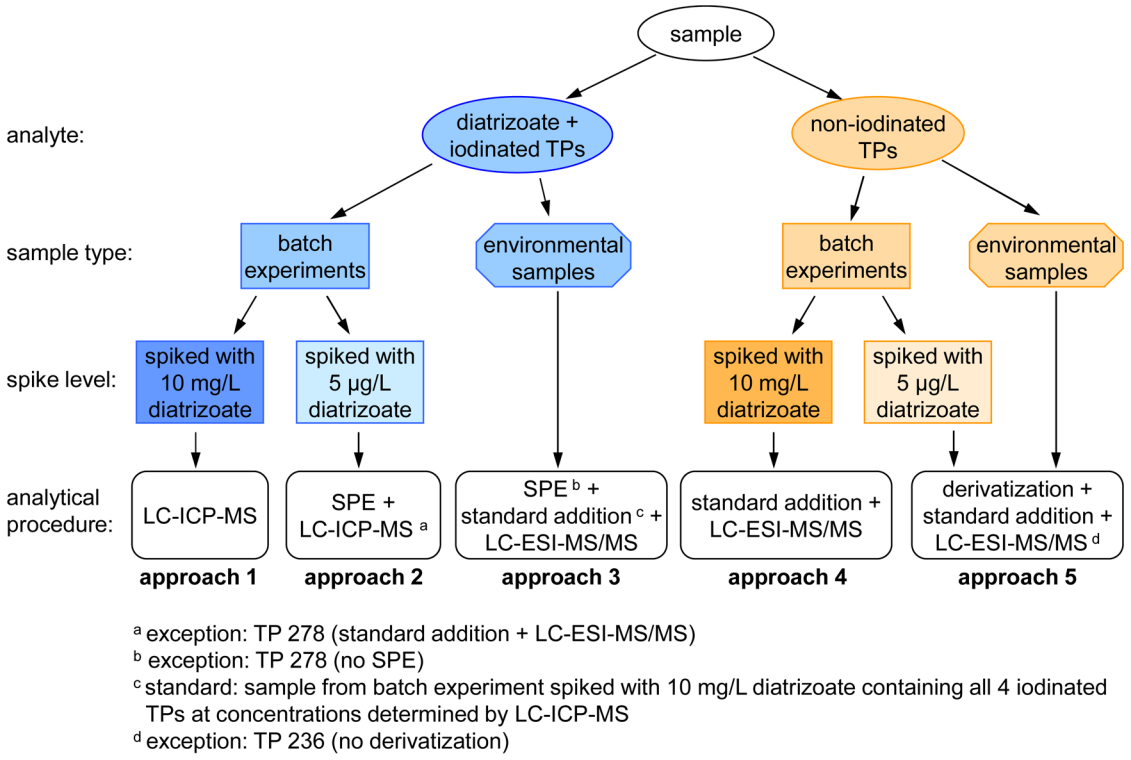

Figure 2. Methodology for the quantification of diatrizoate and its anaerobic TPs by complementary LC−ESI−MS/MS and LC−ICP−MS measurements.

infiltrating water from a polishing pond receiving the effluent of a conventional WWTP (Figure 1a). Further grab samples were taken from the effluents of two technical wetlands (TW) fed by the WWTP effluent previously mentioned (Figure 1b). In addition, 40 sets of samples were taken over a period of 5 months from three consecutive reactors of a pilot WWTP (Figure 1c): a conventional denitrifying and nitrifying reactor (R0, HRT 12 h, 3 d composite samples from the effluent) and two anaerobic reactors (R1, HRT 5.75 d, grab samples from the aqueous phase in the reactor; R2, HRT 5.75 d, 3 d composite samples from the effluent). Further details are listed in the Supporting Information.

Analytical Methods. *Identification of Transformation Products.* Identification of the TPs was performed with an Accela liquid chromatography system (Accela pump and autosampler, Thermo Fisher Scientific, Bremen, Germany) coupled with a high resolution LTQ-Orbitrap-MS (LTQ Orbitrap Velos, Thermo Fisher Scientific). Mobile phases were 0.1% formic acid in ultrapure water and 0.1% formic acid in acetonitrile. Samples from the batch experiments were screened for peaks emerging over the course of time of the incubation experiments. The ESI source was operated in positive ionization mode. Elemental compositions of the TPs were determined by full scan measurements using the undiluted samples. Chemical structures of the TPs were assigned by MS^n experiments determining elemental compositions of the fragment ions. Further information on the chromatographic conditions, MS source parameters, and the data-dependent acquisition parameters are provided in the Supporting Information.

Quantitative Analysis of Samples. Since analytical reference standards were not available for all diatrizoate TPs, different strategies were applied to quantify diatrizoate and its anaerobic TPs in batch experiments and environmental samples (Figure 2).

Quantification of Diatrizoate, Iodinated TPs and Inorganic Iodine. For the quantification of diatrizoate, the four iodinated TPs and the released inorganic iodine a complementary approach based on LC−ICP−MS for quantification and LC−ESI−MS/MS for peak assignment was applied (approach 1). The molar concentrations of the analytes were calculated on the basis of the concentrations of the isotope ^{127}I determined by LC−ICP−MS. For quantification, an external calibration was prepared by spiking diatrizoate into ultrapure water. Peak assignment was achieved by means of complementary LC−ESI−MS/MS measurements of selected samples containing all four iodinated TPs via a comparison of retention times.

To improve sensitivity, samples from the batch experiments spiked with 5 µg/L diatrizoate and environmental samples were enriched via SPE prior to quantification (approach 2).

Because of the coelution of further iodinated organic compounds the direct application of LC−ICP−MS for environmental samples is limited. Therefore, environmental concentrations of diatrizoate and the iodinated TPs were determined by LC−ESI−MS/MS measurements in MRM mode. HPLC conditions were chosen as described later for the noniodinated TPs. Quantification was done applying a standard addition approach. In this case samples from batch experiments containing all identified iodinated TPs at concentrations previously determined by LC−ICP−MS as described above were used as the required standard solution (approach 3).

Quantification of Noniodinated TPs. The three noniodinated TPs, for which authentic reference standards are available, were determined via LC−ESI−MS/MS. Owing to low absolute recoveries (<30%) the SPE was not suitable for two of the three noniodinated TPs. To increase the detection

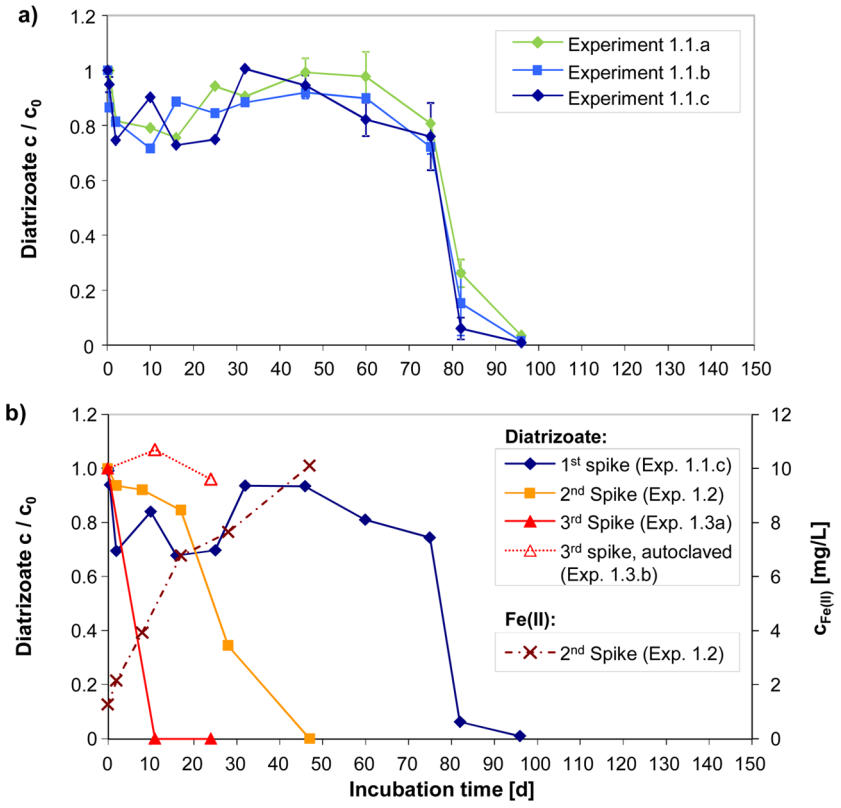

Figure 3. (a) Time series of diatrizoate concentrations (c_0 = 100 μg/L) in anaerobic soil−water batch experiments spiked with sodium acetate (experiment 1.1.b, Table 1), amorphous Fe(III)oxyhydroxide and sodium acetate (experiment 1.1.c) or none of both (experiment 1.1.a) (each n = 2). Symbols and error bars represent arithmetic means, minimum and maximum values. (b) Time series of diatrizoate concentrations in one of those batch systems after repeated additions of diatrizoate at 100 μg/L (first spike, experiment 1.1.c) and 10 mg/L (second and third spike, experiments 1.2 and 1.3.a) as well as Fe(II) concentrations in experiment 1.2. C_0 is the measured initial concentration.

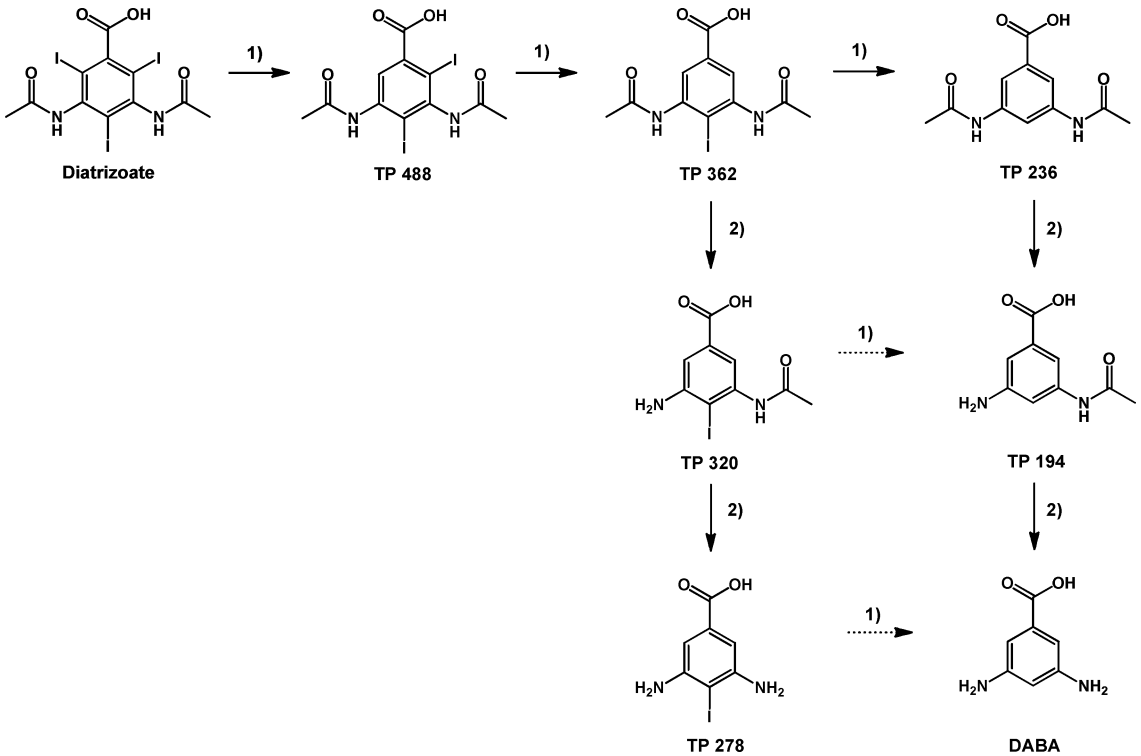

Figure 4. Proposed transformation pathway of diatrizoate: (1) deiodination, (2) deacetylation. The exact positions of deiodination and deacetylation could not be determined. The transformation steps with the dashed arrows are proposed, but have not been confirmed yet.

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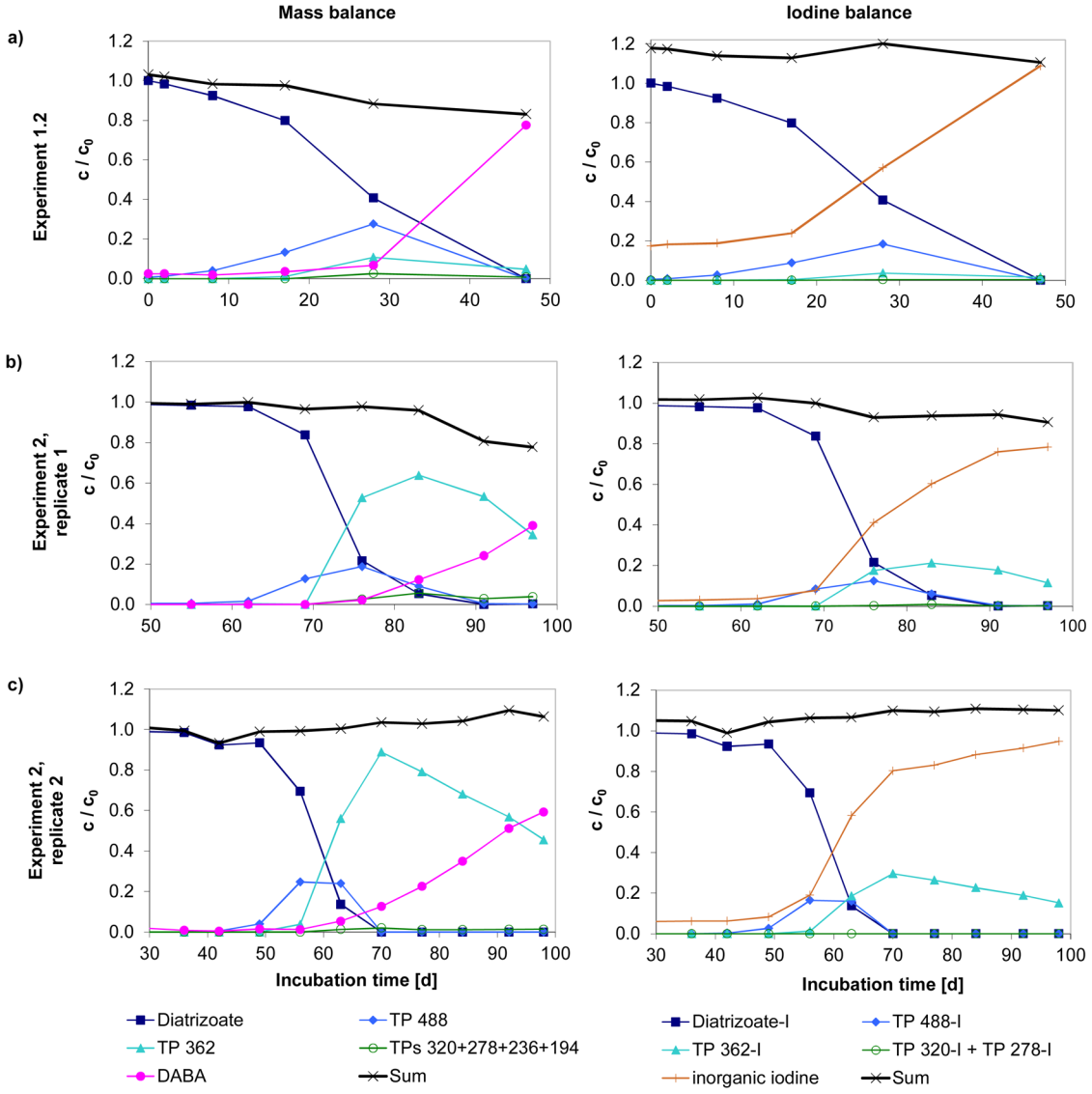

Figure 5. Mass balance (left diagrams) and iodine balance (right diagrams) of diatrizoate and the identified TPs in batch experiment 1.2 and both replicates of the batch experiment 2. C_0 is the measured initial concentration of diatrizoate or diatrizoate−iodine, respectively. The relatively high initial concentration of inorganic iodine in experiment 1.2 results from the first addition of diatrizoate to the batch system.

sensitivity, samples from batch experiment 5 (spiked with 5 μg/L diatrizoate) and environmental samples were derivatized with dansyl chloride prior to quantification of DABA and TP 194 (Supporting Information, Scheme S1) (approach 5). TP 236 as well as the derivatized TPs DABA and TP194 were quantified using the standard addition method (approaches 4 and 5).

Information on sample preparation with SPE and derivatization, on chromatographic conditions, and ICP-MS and ESI-MS/MS parameters as well as on method validation is provided in the Supporting Information.

RESULTS AND DISCUSSION

Transformation of Diatrizoate in Anaerobic Soil-and-Water Batch Systems. In all soil−water batch experiments spiked with (i) sodium acetate, (ii) 10 mM of an amorphous Fe(III)oxyhydroxide and sodium acetate, or (iii) none of both (experiment 1.1.b, c and a, see Table 1), diatrizoate (spiked with 100 μg/L) dissipated after a lag phase of about 80 d (Figure 3a). In the different batch systems, the diatrizoate concentrations decreased to <5% of the initial concentration until day 100 of the experiment.

After diatrizoate was respiked twice to one replicate of the batch experiment 1.1.c with 10 mg/L, the lag phases decreased to 10−20 d and <11 d (second and third spikes, experiments 1.2 and 1.3.a, Figure 3b). Anaerobic conditions in this experiment were confirmed by increasing Fe(II) concentrations.

Prior to the third addition one aliquot of the batch system was autoclaved. In this aliquot (experiment 1.3.b) the concentrations of diatrizoate remained constant for 24 d, while in the nonautoclaved batch system 1.3.a it decreased below the LOQ within 11 d. The decrease of the lag phase by repeatedly spiking diatrizoate as well as the inhibited transformation of diatrizoate in the autoclaved systems (Figure 3b; experiment 2, Supporting Information, Figure S1), strongly underlined the microbial transformation of diatrizoate. The observed lag phases and their reduction by respiking diatrizoate are an indication for an adaptation of the microbial community

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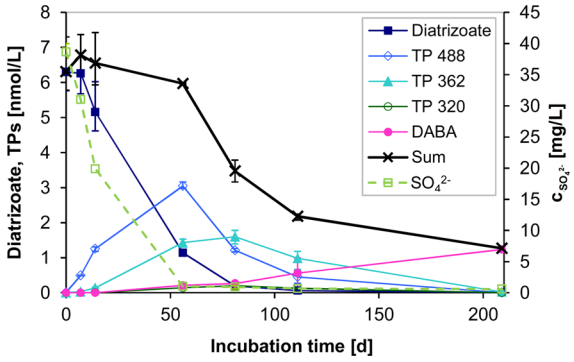

Figure 6. Mass balance of diatrizoate and the identified TPs over time, as well as sulfate concentrations in batch experiment 5. Symbols and error bars of diatrizoate and its TPs represent arithmetic means and ranges of three parallel experiments per time point (exceptions: 7 d (n = 2), 209 d (n = 1)). Symbols and error bars of SO_4^{2-} concentrations at 0 and 81 d represent arithmetic means and ranges (n = 3), symbols at the other time points represent concentrations in single batch systems.

to the anaerobic conditions and the use of diatrizoate as electron acceptor and/or C-source.

Identification of Transformation Products. Seven TPs of diatrizoate were identified via LC−LTQ-Orbitrap-MS measurements in samples from batch experiment 1.2 (see Table 1) (Figure 4). These TPs differed from diatrizoate in the number of iodines and acetyl groups. By MS^2 and MS^3 fragmentation experiments the number of iodines and acetyl groups present in the respective TP was confirmed by the loss of iodine and acetyl moieties (Supporting Information, Table S3, Figure S2). With these analyses and the literature results on fragmentation pathways of ICMs,[10,25,27] the chemical structures could be assigned to the fragments and the TPs. The fragmentations of all TPs as well as an exemplary fragmentation pathway of TP 362 are documented in the Supporting Information (Table S3, Figure S2).

Transformation Pathway. The anaerobic transformation pathway of diatrizoate (Figure 4) was determined from the structures of TPs identified by the LC−LTQ-Orbitrap MS^n experiments and from the sequence of their formation and dissipation in the batch experiments (Figure 5), which is discussed in detail in the following sections.

The first TP observed in all batch experiments was TP 488, followed by TP 362, then by TP 236 and/or partly and completely deacetylated TPs, and finally by the completely deiodinated and completely deacetylated DABA (Figure 5). The TPs 320, 278, 236, and 194 were each detected only in one or two of the batch systems and only at minor concentrations (data not shown explicitly, but as sum in Figure 5), so that a clear sequence of their formation was not found. However, the complete deacetylation of the noniodinated TP 236 to DABA via TP 194 was confirmed in experiment 3 (Supporting Information, Figure S3a). The same deacetylations were also observed during incubation of TP 236 under an air atmosphere (experiment 4.a, Figure S3b). The deiodination of TP 362 to TP 236 and the deacetylation of TP 362 via TP 320 to TP 278 were concluded from the proposed structures, since these are the only plausible pathways for the formation of TP 236 and TP 278 involving the identified TPs. The deiodinations of TP 320 to TP 194 and of TP 278 to DABA are also reasonable, but could not be experimentally confirmed or excluded owing to a lack of authentic reference standards. Hence, the transformation of diatrizoate is initiated by a sequential cleavage of at least two of the iodines, followed by deacetylations of the amide groups.

Reductive dehalogenations are common biological transformations of halogenated aliphatic and aromatic pollutants under anaerobic conditions. They have been studied with mainly chlorinated or brominated phenols, benzoates, and anilines.[28−30] Also 5-amino-2,4,6-triiodoisophthalic acid, which is a precursor in the synthesis and also the core structure of different ICMs, was deiodinated under anaerobic conditions.[31]

The hydrolysis of amides under aerobic conditions is a very common and often described biological transformation process catalyzed by hydrolases.[32,33] It is however not observed, or only at lower rates, when structural features of the substrate hinder sterically the enzyme−substrate interactions.[32,34] Even though oxygen is not directly involved, there are much less reports about amide hydrolyses under anaerobic conditions.[35] The results of most studies confirmed that diatrizoate is rather persistent under aerobic conditions and that similar to other ICMs an amide hydrolysis is unlikely under environmentally

Table 2. Concentrations and LOQs of Diatrizoate and Its TPs in Groundwater Wells, Technical Wetlands and Effluents of Two Consecutive Anaerobic Reactors from a Pilot WWTP. Values Are Given in μg/L and in Brackets in nmol/L

	diatrizoate	TP 488	TP 362	TP 320	TP 278	TP 236	TP 194	DABA
				groundwater wells				
GW 1	0.96 (1.57)	0.14 (0.29)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
GW 2	1.02 (1.67)	0.10 (0.21)	0.26 (0.72)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
LOQ	0.04	0.07	0.09	0.05	0.10	0.20	0.07	0.02
				technical wetlands				
Influent	2.52 (4.10)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
TW 1	2.42 (3.94)	0.34 (0.70)	0.16 (0.44)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
TW 2	1.32 (2.15)	0.33 (0.67)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
LOQ	0.05	0.15	0.10	0.15	0.20	0.40	0.07	0.05
				reactors from pilot WWTP				
R0	12.40 ± 4.55[a] (20.2 ± 7.41)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R1	5.93 ± 2.18[a] (9.65 ± 3.54)	2.32 (4.76)	0.87 (2.39)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R2	1.54 ± 0.69[a] (2.50 ± 0.12)	1.74 (3.56)	3.14 (8.69)	0.20 (0.62)	0.44 (1.57)	<LOQ	<LOQ	0.11 (0.70)
LOQ	0.05	0.15	0.10	0.15	0.35	0.40	0.07	0.05

[a]Arithmetic means and standard deviations (n = 40) from 3 d composite samples during five months.

relevant conditions.[2,3,25] It can be concluded from the results of this study that both under aerobic and under anaerobic conditions the amide hydrolysis of diatrizoate and probably also of other ICMs is hindered by the triiodinated aromatic structure, which might be due to the steric hindrance of the enzymes by the large iodines.[32] The results of this study indicate that at least two iodines need to be cleaved prior to an amide hydrolysis.

Furthermore, the final TP 3,5-diamino benzoic acid (DABA) was stable under anaerobic conditions. However, DABA was dissipated in batch systems incubated under an air atmosphere (Supporting Information, Figure S3c). However, no TPs could be detected. It might be mineralized or incorporated into the microbial biomass. However, it cannot be excluded that very polar compounds with low molecular weights were formed that were not amenable to the chromatographic methods used or to the detection by ESI-MS. The transformation of diatrizoate to DABA under anaerobic conditions and the consecutive dissipation of DABA under air atmosphere highlights that the combination of strictly anaerobic and aerobic conditions can be beneficial for a more complete degradation of rather persistent organic compounds.

Methodology to Close the Mass Balances. Complementary LC−ICP−MS and LC−ESI−MS/MS measurements (approach 1 in Figure 2) allowed for a successful peak assignment of diatrizoate and the iodinated TPs (Supporting Information, Figure S4). During method validation the total iodine concentrations obtained by flow injection into ICP−MS were comparable to the sum of concentrations of iodinated species obtained by LC−ICP−MS (95 ± 4%, n = 17). Thus, this approach enabled the accurate quantification of the iodinated TPs without authentic (species-specific) reference standards.

By this, diatrizoate and the iodinated TPs could be quantified in the batch experiments with LOQs down to 0.2 μg/L (Supporting Information, Table S4), corresponding to ≪1% of the spiked diatrizoate concentration of 10 mg/L. However, relative to the concentration of 5 μg/L diatrizoate spiked to batch experiment 5, these LOQs are relatively high (up to 10% regarding the molar concentrations, Table S4). Except for that of TP 278, the LOQs were reduced by a factor of 10 down to 0.02 μg/L by using SPE (approach 2), with absolute recoveries ranging between 78% and 97% (Table S5). The LOQ of TP 278 was reduced to 0.06 μg/L by direct injection into LC−ESI−MS/MS and with the use of the standard addition approach applied for the environmental samples (approach 3).

By detection of diatrizoate and the iodinated TPs in environmental samples with LC−ESI−MS/MS, the selectivity for these analytes could be increased considerably compared to that from LC−ICP−MS detection. Thereby, the analytes were quantified without the interference of further iodinated compounds.

For the noniodinated TPs 194 and DABA, by a direct injection into LC−ESI−MS/MS (API 5500) LOQs of 0.1 μg/L and 0.5 μg/L were determined, respectively. By the derivatization with dansyl chloride (approach 5) these LOQs could be reduced down to 0.07 μg/L and 0.02 μg/L, respectively (Supporting Information, Table S4). TP 236 could be sensitively quantified (LOQ: 0.03 μg/L) by direct injection without further sample preparation.

By combining LC−ESI−MS/MS and LC−ICP−MS, all TPs were quantified and the mass balance could be measured, although authentic reference standards of the four iodinated TPs were not available.

Mass Balance. The mass balance of the transformation was assessed in batch experiment 1.2 (first respike of the initial experiment 1.1.c, Table 1) to verify whether the quantitative relevant TPs have been identified. Reproducibility of the mass balance was investigated in both replicates of batch experiment 2 (Figure 5).

In all three batch systems diatrizoate was transformed to DABA via the other identified TPs. The mass balance was in all three systems mainly closed by considering diatrizoate and the TPs 488, 362, and DABA, while the other TPs occurred only at minor portions. The elevated portions of TP 488 and TP 362 indicate that the first and second deiodination are the rate-limiting steps in the transformation pathway. The transformation was more rapid, and a higher portion of DABA was formed in experiment 1.2 than in the batch systems of experiment 2. This can be explained by the respiking of experiment 1.2 and an adaptation of the microbial community. In both replicates of experiment 2 the dissipation of diatrizoate with the formation of TPs was nearly parallel, although the lag phase was slightly shorter in replicate 2. However, the transformation of diatrizoate was qualitatively comparable in all three batch systems.

The total sum of diatrizoate and its TPs remained nearly constant throughout the experiments. The concentration at the end of the experiments ranged between 80% and 110% of the initial concentration (Figure 5, left diagrams). The amount of inorganic iodine released in the course of the experiments was also in accordance to the proposed complete deiodination of diatrizoate (Figure 5, right diagrams). This is also apparent from the sum of all iodine species (diatrizoate, iodinated TPs, and inorganic iodine) which remained fairly constant throughout the experiments. The (almost) closed mass balances at all time points over the course of the experiment further demonstrated that the quantitatively relevant TPs had been identified and were quantified correctly using the set of different quantification techniques.

Transformation under Environmental Conditions. *Batch Experiment at Environmental Concentrations.* Diatrizoate, spiked at 5 μg/L, was transformed without a lag phase down to <1% of the initial concentration within 111 d (Figure 6). After 14 d with a slower decrease, the diatrizoate concentration decreased in an exponential course typical for first order reactions (k = 0.047 ± 0.006, $t_{1/2}$ = 15 d ± 1.6 d). Sulfate reducing conditions in the system were confirmed by decreasing sulfate concentrations. Ferrous iron concentrations remained below 0.23 mg/L throughout the experimental duration.

The short lag phase indicates that not much time was needed to enable the present microbial community to transform diatrizoate. This might be due to the permanent contact of the sediment with treated wastewater in the polishing pond. Parallel to the transformation of diatrizoate, the predominant TPs (TP 488, 362, and DABA) of the experiments 1.2 and 2 (Figure 5) were formed also in this experiment conducted at lower concentrations. The detection of these TPs confirmed that diatrizoate can also be transformed at environmentally relevant concentrations to DABA according to the proposed transformation pathway. The concentrations of most of the analytes differed at the same time points between the individual batch systems by less than 20%. This indicates the excellent reproducibility of the batch experiments.

However, in contrast to the previous batch experiments the portion of DABA was relatively low. This led to an appreciable decrease of the sum of diatrizoate and the TPs after 56 days. Even though DABA was persistent in the anaerobic soil−water batch experiments (see above), the microbial community of the pre-exposed sediment used in this experiment seems to be capable of transforming DABA. Further plausible explanations are an elevated loss of DABA due to physical sorption or covalent binding of the reactive amino groups to electrophilic sites in the sediment matrix.[36,37] Assuming a limited number of sorption/binding sites, it is plausible that these processes only influenced the mass balance in this batch experiment with the significantly lower initial concentrations of diatrizoate. However, for a definite elucidation of the DABA losses, additional experiments, in particular with ^{14}C-labeled compounds, are necessary. Apart from losses of DABA, also an alternative transformation pathway cannot be totally excluded; however, because of the steric hindrance alternative transformations are unlikely until two of the iodine atoms are cleaved.

Environmental Occurrence. The anaerobic TPs were detected (i) in groundwater which is influenced by infiltrating water from a polishing pond, (ii) effluents of technical wetlands fed with WWTP effluent, and (iii) anaerobic reactors from a pilot plant (Table 2).

Similar to the batch experiments, TP 488 and TP 362 were present at the highest concentrations. TPs 320, 278, and DABA were detected at lower concentration levels in a pilot WWTP, but not in groundwater or technical wetlands.

Groundwater and Technical Wetlands. In the samples both from groundwater and from technical wetlands, TP 488 and, in one sample of each type, also TP 362 was detected. The sum of both TPs was up to 0.36 μg/L (0.93 nmol/L) in the groundwater samples and up to 0.50 μg/L (1.14 nmol/L) in the technical wetland samples, which accounts for 36% and 22%, respectively, of the total amount of diatrizoate and TPs in those samples. This indicates that a relevant percentage of diatrizoate is transformed to those TPs which have also been the quantitatively most important ones in the batch experiments. Since both TPs could not be detected in the influent of the technical wetlands (WWTP effluent), it can be concluded that they were formed during passage through the two wetland systems.

Wastewater. In the effluent samples of both anaerobic reactors of the pilot WWTP the TPs 488 and 362 were detected, which were not present in the influent. The concentration of TP 488 decreased from the first to the second anaerobic reactor, whereas the concentration of TP 362 increased. In addition, the TPs 320, 278, and DABA were detected in the second anaerobic reactor. Hence, the sequence of the formation of the TPs as well as the increase and decrease of their concentrations were in agreement with the proposed transformation pathway. The compiled concentrations of the detected TPs corresponded to approximately 80−90% of the average decrease of diatrizoate in the anaerobic reactors. This mass balance was in the same range as observed in the laboratory batch experiments. Thus, it can be stated that a significant proportion of diatrizoate was transformed in the anaerobic reactors according to the transformation pathway suggested.

The occurrence and pattern of the identified TPs in samples from the different environmental matrices indicate that the anaerobic transformation of diatrizoate observed in the batch experiments is a common process if diatrizoate reaches anaerobic environmental compartments.

Hence, the results of the lab-scale experiments can be at least qualitatively transferred to real natural systems such as wetlands as long as anaerobic zones occur. Predominantly, those TPs are found that were formed by a release of one or two iodines. The third deiodination and deacetylation seem to be of minor importance but were observed in a technical pilot plant reactor. It can be concluded that independent of the matrix (i.e., soil, sediment), specific redox conditions (Fe(III) reducing, SO_4^{2-} reducing), or the presence of additional C sources (acetate), diatrizoate can be transformed by microorganisms under anaerobic conditions.

■ ASSOCIATED CONTENT

Supporting Information

Additional experimental details and results including tables and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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