# Transformation of the X-ray Contrast Medium Iopromide In Soil and Biological Wastewater Treatment

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In water/soil systems, the iodinated contrast medium iopromide was quantitatively biotransformed into several transformation products (TPs). Twelve TPs were identified via HPLC-UV and LC tandem MS. The chemical structures of the TPs were elucidated via fragmentation in $MS^2$ and $MS^3$ of LC tandem MS with a linear ion trap and $^1H$ and $^{13}C$ NMR analyses. All TPs exhibited transformations at the side chains containing either carboxylic moieties and/or primary and secondary amide moieties, while the triiodoisophthalic acid structure remained unaltered. A transformation pathway was proposed based on the sequence of TP formation in aerobic batch experiments. Additionally, the occurrence of iopromide TPs was investigated in native water samples. All TPs identified were found in municipal WWTP effluents because of their formation during biological wastewater treatment with maximum concentrations of up to 3.7 ± 0.9 $\mu$g/L (TP 819). Predominantly, those TPs were present at higher concentrations in WWTP effluents which were formed at the beginning of the transformation pathway. Furthermore, four TPs formed at the end of the transformation pathway (TP 759, 701A/B, and 643) were also found in bank filtrate up to 0.050 $\mu$g/L and in groundwater of an wastewater irrigation area up to 4.6 $\mu$g/L.

## Introduction

Many pharmaceuticals such as iodinated contrast media (ICM), lipid regulators, antiphlogistics, $\beta$-blockers, antiepileptics, antibiotics, and illicit drugs have been identified in wastewater and the aquatic environment within the last 5−10 years. In Germany, approximately 500 t/a of X-ray contrast media are applied, with iopromide accounting for about 130 t/a (*1*). Since ICM are administered at high daily doses (up to 200 g/d) and excreted mainly nonmetabolized (>95%) (*2, 3*), they are frequently detected in wastewater at elevated concentrations (sometimes >10 $\mu$g/L). Gartiser et al. (1996) reported that X-ray contrast media are the main contributors to the burden of total organic halogens in clinical wastewater (*4*). Due to their incomplete removal in wastewater treatment plants (WWTPs), ICM were found at elevated concentrations in rivers and streams (*5–10*). In general, ICM are known to be present in groundwater and drinking water because they are to some extent recalcitrant during soil-aquifer passage and are not even completely removed by activated carbon filtration and ozonation (*6, 8, 11–15*). However, few studies have reported that photodegradation was an efficient removal process for some ICM (*16–18*). Monitoring studies at full-scale WWTPs have indicated that iopromide is removed at a high percentage, probably due to biodegradation (*19–21*). Reasons for the variability in removal rates ranging from negligible to more than 80% are currently not known. Kalsch (1999) observed two unidentified transformation products (TPs) of iopromide in laboratory scale experiments with activated sludge (*22*). For diatrizoate, the author was able to identify deacetyl-diatrizoate and 3,5-diamino-2,4,6-triiodobenzoic acid as the two major TPs. In a modified Zahn−Wellens test (OECD Guideline 302 B on inherent aerobic biodegradability of organic substances with sludge taken from municipal WWTPs) 3,5-diamino-2,4,6 triiodobenzoic acid was also identified as TP (*23*). Using LC ion trap MS and H/D exchange, Pérez et al. (2006) identified three iopromide TPs (TP 805A, 805B, 819) in a laboratory biodegradation study with conventional activated sludge (*5*). Additionally, they found another TP in nitrifying sludge (dehydroxylated iopromide at side chains A and B). However, the authors did not find these TPs at appreciable concentrations in municipal wastewater samples (*19*). Putschew et al. (2000) reported that iopromide dissipated significantly during bank filtration, while diatrizoate was not significantly removed (*8*). A German case study revealed the removal of iopromide to be below the limit of quantification (LOQ) after passing through soil (*20*). In water/sediment studies and in soil column studies iopromide was transformed to at least four unknown TPs (*24, 25*). The transformation of iopromide and other ICM in WWTPs has been investigated, but no research has been reported about the (bio)transformation of these compounds in contact with soils and sediments.

The objective of the current study was to identify the iopromide TPs formed in contact with a biological active soil of a wastewater irrigation area, to propose a transformation pathway, and to determine whether the formed TPs are present in groundwater and bank filtrates as well as in raw and treated wastewater.

## Materials and Methods

**Identification and Determination of TPs in Water/Soil Systems.** *Water/Soil Systems.* The transformation pathway of iopromide was investigated in laboratory-scale batch experiments. The batch experiments consisted of 100 g of soil and 500 mL of groundwater and were incubated in the dark at room temperature (20−24 °C) for 103 d. In order to obtain a sufficient TP quantity for NMR analysis, elevated concentrations up to 1 g/L were used. The soil used for the batch experiments was an Ap horizon of a podzolic brown soil taken from a wastewater irrigation area in Braunschweig, Germany, which was air dried and stored at 4 °C prior to the experiments. The sandy soil consisted of 2.9% clay, 6.1% silt, 91.3% sand, and 0.61% organic matter. Groundwater was taken from a deep well in Arenberg, a district of Koblenz, Germany. Iopromide, CAS No. 73334-07-3, and the surrogate standard desmethoxyiopromide (DMI), CAS No. 76350-28-2, were provided by Bayer-Schering AG, Berlin, Germany. To investigate whether the transformation of iopromide was microbial-derived, batch experiments with 2 g of soil, 20 mL of iopromide solution (1 g/L), and 2 mL of formaldehyde (37%, v/v) were carried out. Blank laboratory-scale experiments without iopromide were always performed in parallel

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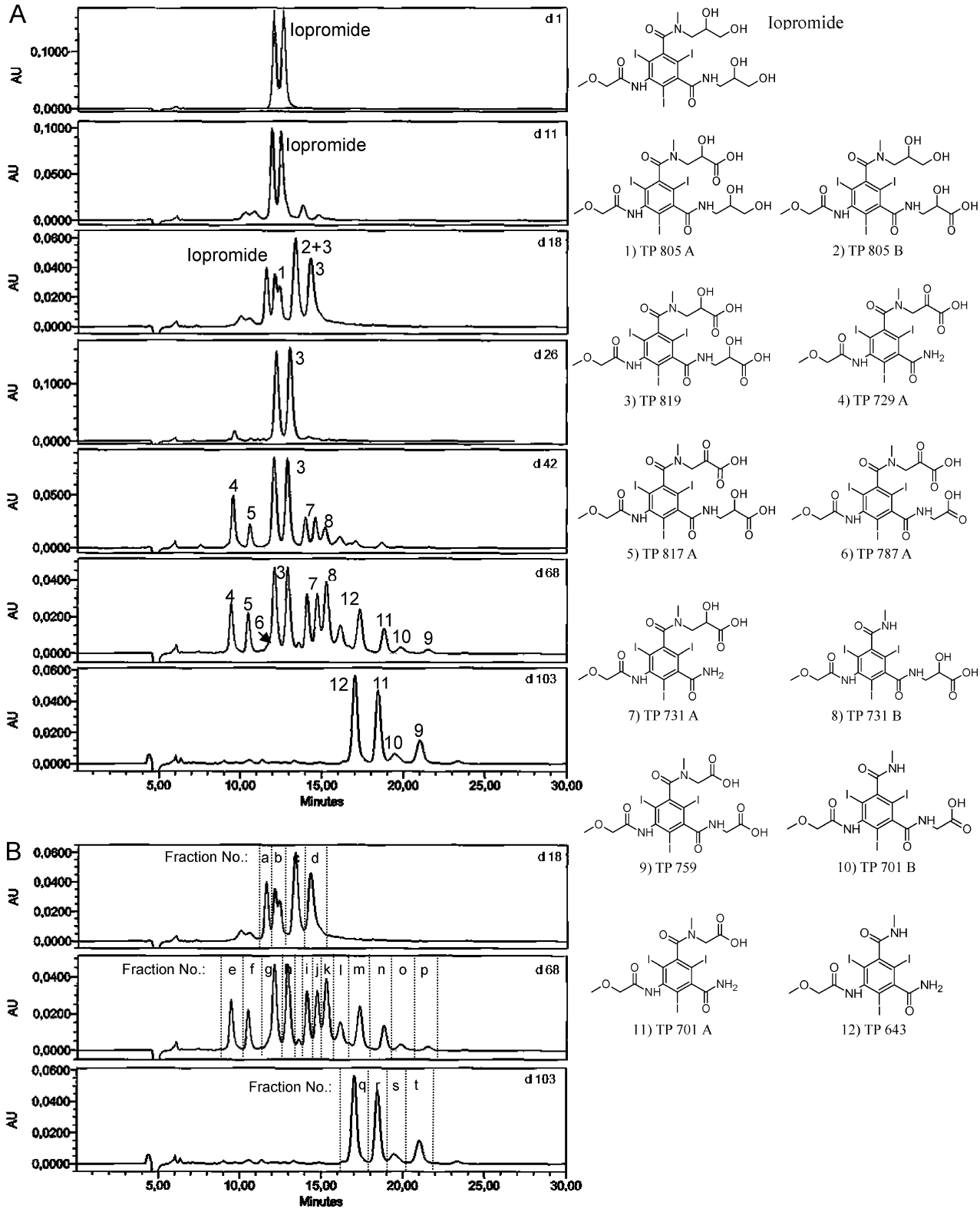


**FIGURE 1. (A) Iopromide transformation observed via fractionated HPLC-UV and (B) fractions taken to isolate TPs.**

to exclude compounds not related to iopromide. Aliquots of the water phase were repeatedly sampled from 0 to 103 d and analyzed by HPLC/UV and LC tandem MS as described below. Aliquots of the water phase were also taken to isolate TPs after preconcentration by freeze-drying, cleanup via solid-phase extraction (SPE), and separation via semi-preparative HPLC to obtain a sufficient quantity for MS fragmentation studies, NMR analysis, and the calibration to quantify TPs in native samples.

*Preconcentration of TPs by Freeze-Drying and Cleanup.* After 18, 68, and 103 d, approximately 125 mL of the aqueous phase from two water/soil systems were taken, combined, and filtered through a 0.2 $\mu$m cellulose acetate filter (Sartorius, Germany), freeze-dried at 0.14 mbar, and redissolved in 25 mL of Milli-Q water. For cleanup, SPE was used to remove absorbable organic matrix impurities. For this, aqueous aliquots of 2.5 mL were cleaned using 3 mL SPE cartridges filled with 400 mg of $C_{18}$ec sorbent (Bulk Isolute sorbent $C_{18}$ec,

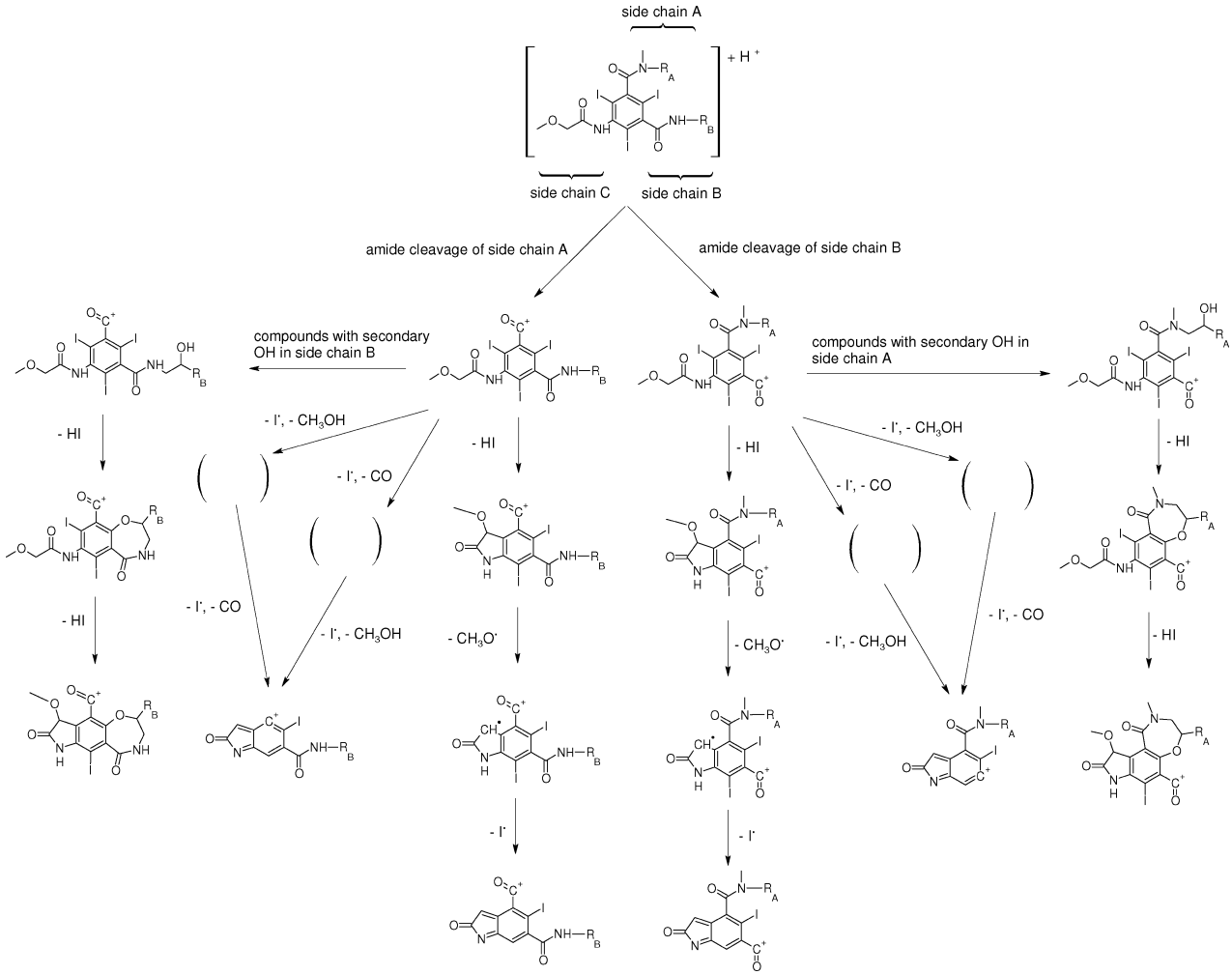


**FIGURE 2. General proposed fragmentation pathway of iopromide and its TPs. ( ): intermediates with one cleaved iodine atom (two alternatives based on position of the cleaved iodine).**

International Sorbent Technololgy, Hengoed Mid Glam, UK). The cartridges were preconditioned using 2 mL of n-heptane, 2 mL of acetone, 4 × 2 mL of methanol, and 4 × 2 mL of Milli-Q water. After passing the samples through the cartridges at a flow rate of approximately 2 mL/min, the water (neutral pH 6.8) was collected for the semipreparative isolation of TPs.

*Semipreparative Isolation of TPs.* TPs and iopromide were separated by semipreparative HPLC using a Synergy Polar RP column (10 mm i.d., 250 mm, 4 $\mu$m, Phenomenex, Aschaffenburg, Germany) at a flow rate of 2 mL/min and an injection volume of up to 1 mL. All other HPLC conditions were the same as described below. Fractions of the eluate were then collected using an automated sample collector (Advantec SF-2120 Super Fraction Collector, Techlab GmbH, Erkerode, Germany). Afterwards, freeze-drying was used to obtain TPs as solid substance.

*HPLC/UV Method.* The aqueous phase (60 $\mu$L) was sampled from the water/soil systems and diluted to 2 mL with Milli-Q water. The analytes were separated with an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA) using two coupled Synergi Polar RP columns, 3 mm i.d., 150 mm, 4 $\mu$m (Phenomenex, Aschaffenburg, Germany). An isocratic eluent (pH 2.6) consisting of 0.1% aqueous formic acid and 10% acetonitrile was used at a flow rate of 0.4 mL/min. Column temperature was 50 °C and injection volume was 50 $\mu$L. The UV system was operated at 242 nm.

*LC Tandem MS Method.* The aqueous phase (10 $\mu$L) was sampled from the water/soil systems, diluted to 2 mL with Milli-Q water, and spiked with 10 $\mu$L of the surrogate standard DMI (20 $\mu$g/mL). A stock solution was prepared by dissolving 1 mg of iopromide and 1 mg of each isolated TP (TP 819, 731 A, 731 B, 729 A, 759, 701 A, 701 B, and 643) (Table S1 in Supporting Information) in 10 mL of Milli-Q water. Calibration samples for analysis of the batch experiments were prepared by diluting the stock solution with Milli-Q water and then spiking with 10 $\mu$L of DMI (20 $\mu$g/mL). The external calibration of iopromide and TPs ranged from 1 to 6000 ng/mL, and analyses were performed via LC tandem MS as described below. The HPLC conditions have already been described above for the HPLC/UV method. The compound and source-dependent parameter settings of the LC tandem MS (4000 Q-Trap, Applied Biosystems, Langen, Germany) are listed in Table S3 and S4. (chapter 3 of Supporting Information).

*Determination of Molecular Weights and Fragmentations by Mass Spectrometry.* The molecular weights of the isolated TPs were determined by Q1 scans using the Q Trap ESI tandem MS. For structural elucidation, the MS fragmentation pathway of all TPs was studied by performing product ion scans and recording $MS^3$ spectra of all major product ions observed using the linear ion trap of the LC tandem MS. Further details are reported in Table S2 (chapter 3 of Supporting Information).

*pH Dependency of LC Retention Time.* The influence of pH on the retention times of the isolated TPs was studied to determine whether the TPs contained acidic moieties such as carboxylic groups. For this, aqueous aliquots of the water/soil systems from 18 and 68 d were analyzed by LC tandem

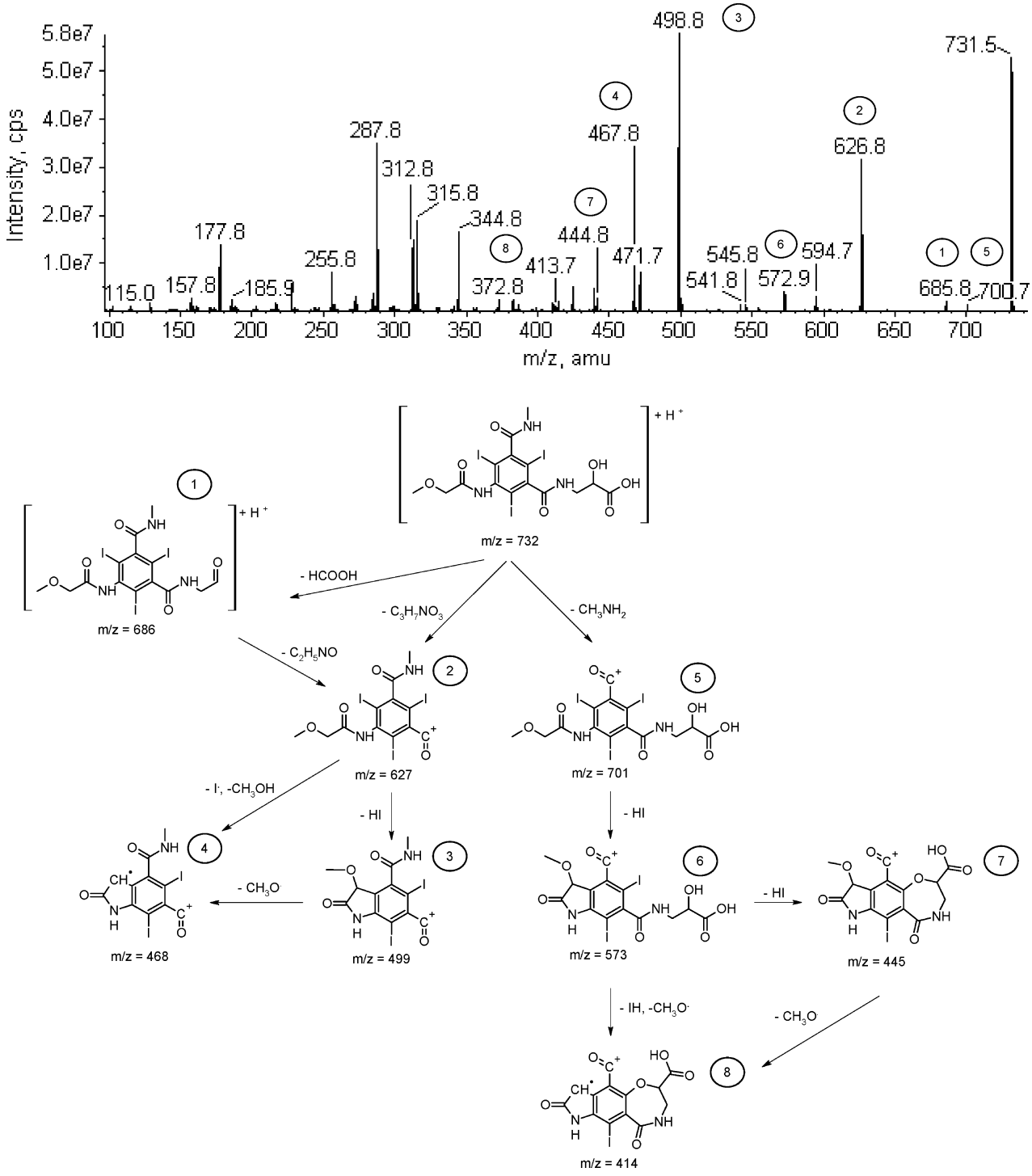


**FIGURE 3. Product ion spectrum ($MS^2$) and proposed fragmentation pathway of TP 731 B received by stepwise fragmentation of the product ions in $MS^3$.**

MS using an acidic (pH 2.6) as well as a neutral eluent consisting of Milli-Q water and acetonitrile (90/10, v/v).

*NMR Identification of Iopromide TPs.* Approximately 10−20 mg of the TPs and iopromide were dissolved in 0.8 mL of DMSO-$d_6$ and analyzed by NMR (Bruker NMR DRX 700 Avance and DRX 500 instrument, Rheinstetten, Germany). $^1$H NMR spectra were measured at 500 and 700 MHz, and $^{13}$C NMR spectra were measured at 176 MHz. Detailed information on the NMR experiments can be found in the Supporting Information.

**Sampling and Analysis of Iopromide and Its TPs in Native Water Samples.** For the analysis of iopromide TPs in native water samples, an analytical method was established based on the sample preparation described earlier (*6*). The detection of iopromide and its TPs via Q Trap LC tandem MS in MRM mode was carried out as described above. Procedures for sample collection, as well as the treatment processes at the three municipal WWTPs, are summarized in Supporting Information (chapter 2). Native water samples were filtered through glass fiber filters (GF 6, Schleicher and Schuell, Dassel, Germany). One liter of groundwater or bank filtrate, 200 mL of WWTP effluent, or 100 mL of WWTP influent were adjusted to pH 2.8 by adding sulfuric acid (3.5 mol/L). All samples were spiked with 10 $\mu$L of the internal standard DMI (20 $\mu$g/mL). SPE was carried out using ENV+ cartridges (3 mL, 200 mg, IST, Hengoed, UK). The analytes were eluted

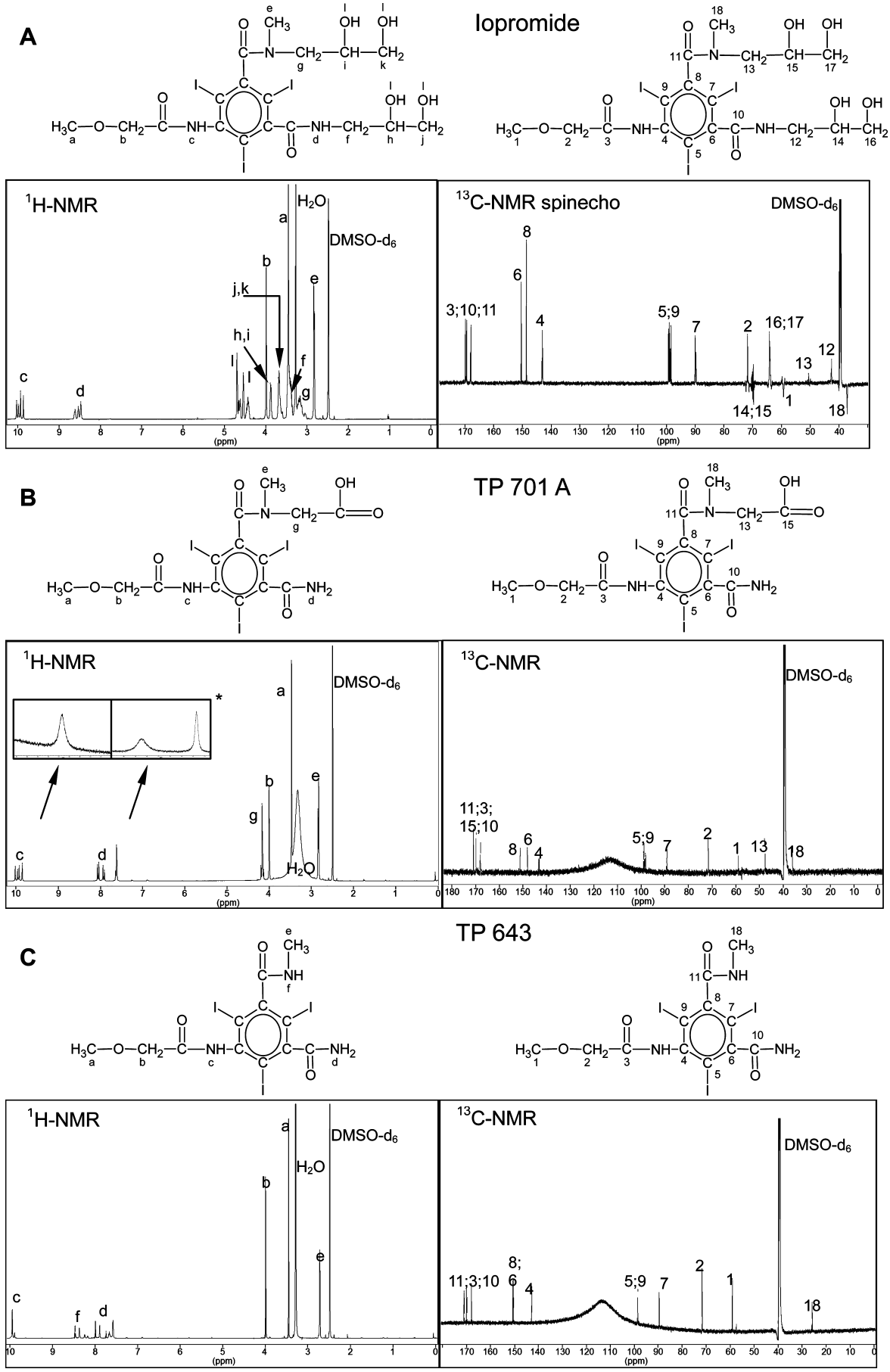


**FIGURE 4. NMR Spectra: A) $^1$H-NMR and $^{13}$C-NMR spinecho of iopromide, B) $^1$H-NMR and $^{13}$C-NMR of TP 701 A at 306K. *Signals c and d at 393K and C) $^1$H-NMR and $^{13}$C-NMR of TP 643.**

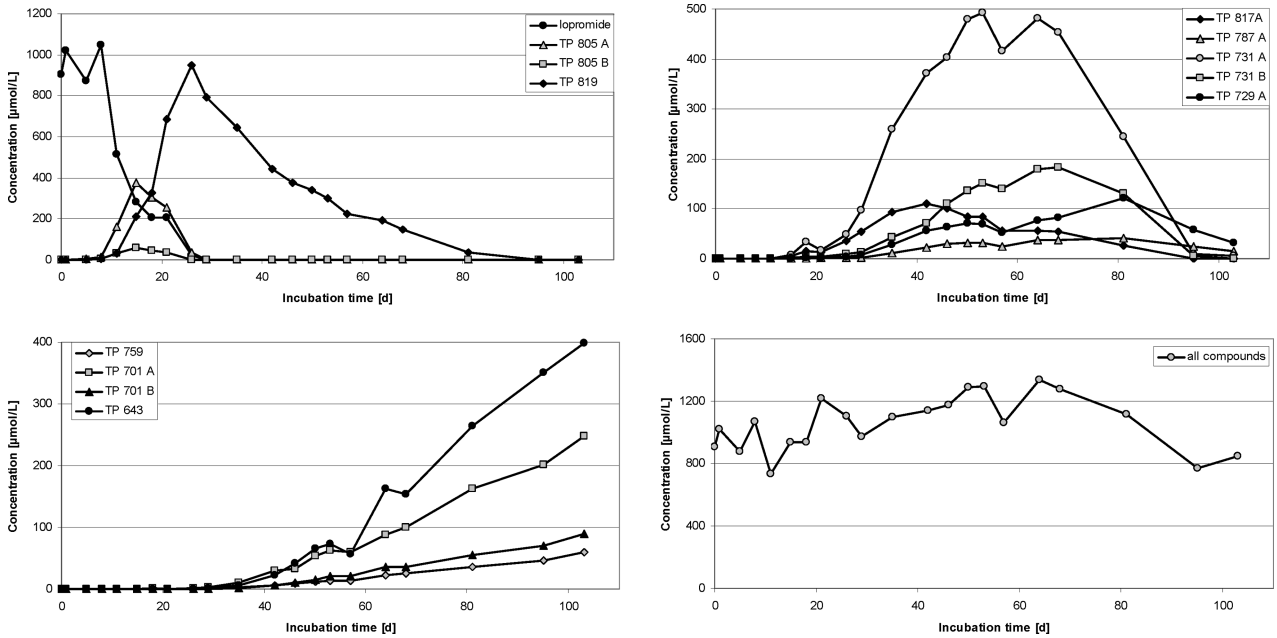


**FIGURE 5. Formation of TPs during incubation of iopromide in the water/soil system.**

with 8 mL of methanol. The extracts were concentrated to 100 $\mu$L and filled up to 1 mL with Milli-Q Water. The concentrations of the calibration samples ranged from 1 to 6000 ng/L and were prepared by spiking 1 L of pristine groundwater (pH of 2.8) with the analytes and 10 $\mu$L of the internal standard DMI (20 $\mu$g/mL).

Relative recoveries for TPs and iopromide in treated wastewater and groundwater always exceeded 90% (Table S6, chapter 5 of Supporting Information). The limit of quantification (LOQ) of iopromide is referred to the second lowest calibration point in the linear regression as long as the calculated signal/noise ratio of the analytes in the native samples extracts was at least 10. Because of external calibration, missing standards (TP 805A, TP 805B, TP 817A), and the use of only one surrogate standard, an additional safety factor of 3−5 was used for the LOQs calculation, always enabling a signal/noise ratio of higher than 10.

## Results and Discussion

**Degradation of Iopromide in the Water/Soil Systems.** As reported by several authors (*8, 19, 24, 25*), iopromide is subject to transformation processes in the environment, despite its stability during human metabolism. The concentration of iopromide in the water/soil system began to decrease, after a lag-phase of approximately 10 d (Figure 5). After 40 d, iopromide was no longer detected in the water/soil system, but several new peaks of TPs appeared in the HPLC/UV chromatogram. In the blank samples, no peaks were found at similar retention times. The relative abundance of the TPs changed in the water/soil systems over the experimental period. Ten of these TPs reached maximum signal intensities after approximately 70 d, and four were still present after 103 d. Even after 267 d, TP 643 was still present. A degradation of TP 643 was never observed under oxic conditions. In total, 12 TP could be identified as illustrated in Figure 1. During the entire experiment (up to 103 d), the mass balance of iopromide and its TPs always exceeded 81%. However, it has to be noted that all TPs, with a chiral center in the $\beta$-position to the methylated amide-nitrogen of side chain A, showed double peaks in the LC chromatograms. Because of the large iodine atoms, the free rotation of the three side chains seems to be hindered causing rotational isomers (see double peaks of Iopromide, TP 819 and TP 731 A in Figure 1). It might be possible that the hindered rotation and the chiral center in the $\beta$-position to the methylated amide-nitrogen are responsible for the double peaks in HPLC.

**Determination of Molecular Weights and the Presence of Acidic Moieties of the TPs.** The molecular weights of the isolated TPs were determined via Q1 scans in positive and negative ion mode (Table S2). The nominal masses were found to be odd, which suggested an uneven number of nitrogens in the structures of all TPs. Therefore, the three amide moieties of iopromide were not cleaved during transformation. The presence of acidic groups was elucidated by determining the shift of the retention times observed in an acidic and a neutral HPLC eluent. In comparison with the acidic eluent, the retention times of all TPs were strongly reduced in the neutral eluent, except for TP 643 which eluted at approximately 15 min under both conditions (Table S4). Therefore, it is assumed that TP 643 contains no carboxyl moieties, whereas at least the presence of at least one carboxyl group can be presumed for all other TPs.

**Mass Fragmentation via Tandem MS with a Linear Ion Trap.** TPs were purified and collected by fractionated HPLC to attain sufficient quantities for mass fragmentation.

*General Fragmentation Pathway of Iopromide and Its TPs.* All observed TPs showed a neutral loss of 128 amu in positive ion mode and a fragment at $m/z$ 127 in the negative ion mode, indicating the loss of HI or of the ion $I^-$, respectively. This confirmed that the isolated TPs derived from iopromide since it was the sole source of iodine in the water/soil systems.

The product ion spectra of the protonated TPs showed signal patterns with a mass shift corresponding to the difference in the molecular weight of side chain A and B. This was the result of the difference in the substituents $R_A$/$R_B$ (Figure 2) and the exclusive methylation of the amide nitrogen in side chain A, which is of special importance for structural elucidation. As a consequence, the characteristic cleavage of C−N-amide bonds in TPs with the same substitution ($R_A = R_B$) resulted in different fragments, depending on whether side chain A or B were cleaved, e.g. for iopromide and TP 819. For details, see Figure 2 and chapter 3.2 of the Supporting Information.

All TPs exhibited similar MS fragmentation pathways in the positive ion mode (Figure 2). The pathways are a result of the cleavage of the C−N-amide bond of side chains A and B, a neutral loss of HI, a cleavage of the $CH_3O$ moiety and a neutral loss of I·. The cleavage of the C−N bonds were

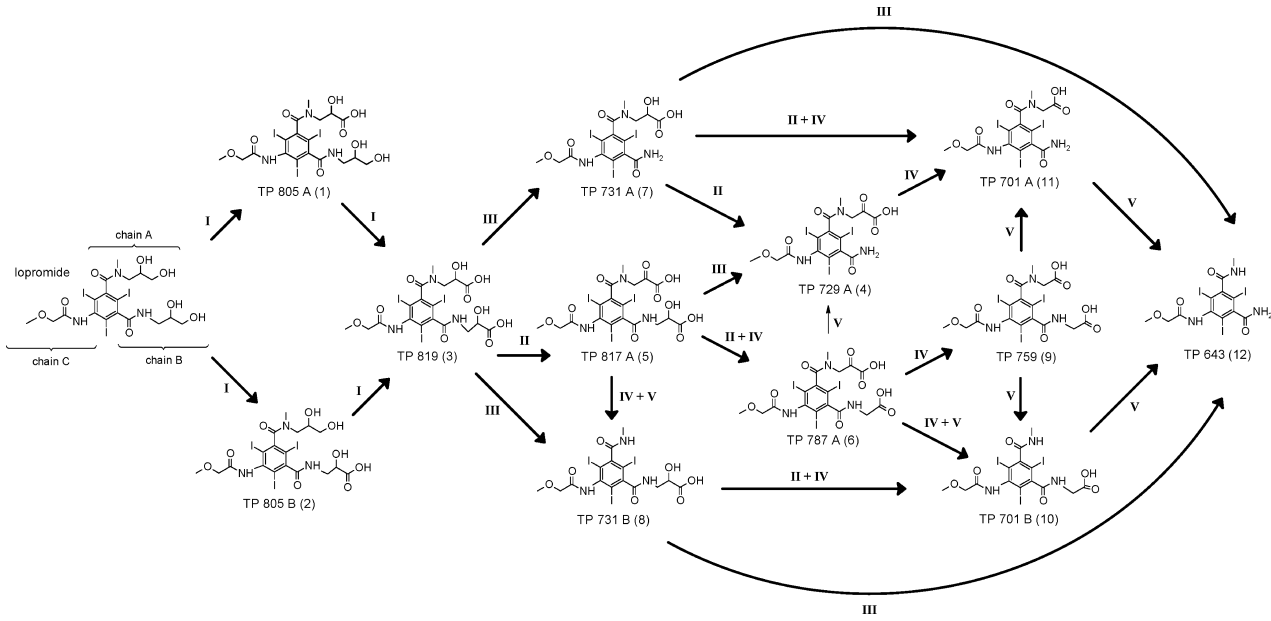


**FIGURE 6. Proposed transformation pathway of iopromide. I: oxidation of primary hydroxy groups, II: oxidation of secondary hydroxy groups, III: removal of α-hydroxypropionic acid side chains: direct cleavage of the amide−methylene bonds at side chains A and B, IV: oxidative decarboxylation of the α-ketopropionic side chain, V: deacetylation: cleavage of the amide−methylene bonds at side chains A and B of TPs containing *N*-acetate moieties.**

found to occur in a single step or after several reactions at side chain A and B involving the elimination of HCOOH, $H_2O$, and $CO_2$, depending on their chemical structure. Elimination of HCOOH, $H_2O$, and $CO_2$ was also found to occur at later stages of the fragmentation pathway involving the side chain remaining after C−N cleavage. In addition, the loss of methanol, I•, and CO was frequently observed, cleavage of C−N bond of side chain C was never observed.

The elimination of HI resulted in the formation of a ring structure (five- or seven-membered ring). This reaction was found for all TPs, even for TP 643, whose substituents $R_A/R_B$ were cleaved. Therefore, it is assumed that side chain C is involved in this reaction as proposed in Figure 2.

A second elimination of HI was observed for TPs containing a secondary hydroxyl group and is likely due to the formation of a seven-membered ring, which was proposed earlier by Perez et al. in 2006 (*5*). It should be considered that different structures could be formed depending on which iodine atom was involved in the HI elimination reaction. The fragmentation patterns also confirmed whether peaks with the same molecular mass were caused by a hindered rotation (similar to iopromide) or different species.

*Proposed Fragmentation Pathway of TP 731 B.* As an example, the fragmentation pathway of TP 731 B is illustrated in Figure 3, whereas those for the other TPs are presented in the Supporting Information. The main fragmentation pathway of TP 731 B begins with the cleavage of the C−N bond at side chain B (fragment 2, $m/z = 627$) followed by the elimination of HI resulting in the formation of a five-membered ring structure (fragment 3, $m/z = 499$). The cleavage of $CH_3O$ of fragment 3 results in fragment 4 ($m/z = 468$). In addition, another pathway was found with HCOOH being cleaved from side chain B of TP 731 B, resulting in fragment 1 with a $m/z$ of 686. This fragment could then undergo a cleavage of the C−N bond at side chain B, resulting in fragment 2 ($m/z = 627$). Direct cleavage of the C−N bond in side chain A yielded fragment 5 ($m/z = 701$), followed twice by the elimination of HI and cleavage of $CH_3O$ leading to the fragments 6, 7, and 8 as shown below.

**NMR Identification of TPs.** For seven TPs (TP 701A, 701 B, 759, 729A, 819, 731B, and 643), quantities (several mg) could be purified which were sufficient for NMR analyses. Similar to the MS fragmentation, all NMR spectra of the selected TPs confirmed that side chain C and the triiodo-isophthalic acid remained unaltered and that the two amide moieties of chain A and B were still present (Figure 2). According to NMR spectra all identified TPs, except TP 643, contained at least one CO group, additionally to the amide groups in their side chains. The oxidation of the side chains resulted in a shift of the methylene protons (Table S5, chapter 4 of Supporting Information) which are deshielded due to the close proximity of the formed carbonyl or carboxyl groups (see CO signals at around 170 ppm in $^{13}C$ NMR).

The $^1H$ NMR (700 MHz) and $^{13}C$ NMR (176 MHz) spectra of iopromide, TP 701 A, and TP 643 are shown in Figure 4. The chemical shifts of side chain C are still present in the TPs as observed for iopromide in NMR spectra as well as the chemical shifts of the $CH_3$ group of the amide moiety at side chain A. However, at around 170 ppm, three CO groups were identified for TP 643 and four CO signals were seen for TP 701A. In side chain A of TP 643 only the methyl protons and one amide proton occurred. Hence, the alkyl group originally bound to the amide group was altered by the transformation processes. The multiplets of the amide protons are probably caused by rotational isomers (see $^1H$-NMR of TP 701 A in Figure 4). By increasing the temperature to 393 K, the multiplet splitting of the amide protons disappeared, indicating that free rotation of the amide protons was possible due to the enhanced kinetic energy. Furthermore, a NMR diffusion experiment confirmed that either one compound or at least compounds with similar sizes were present (*26, 27*). Due to the high affinity of the TPs to water, $^1H$ NMR spectra frequently showed broad water peaks hiding individual NMR signals.

Taking into account the MS fragmentation and the NMR spectra, the chemical structures of all 12 TPs were confirmed (see Figure 2, Table S1 and chapter 4.3 in the Supporting Information).

**Formation of TPs during Incubation of Iopromide in Water/Soil Systems.** The samples taken from the water/soil systems were analyzed by LC-tandem MS, using the calibration prepared with isolated TPs. As shown in Figure 5, after 26 d iopromide was quantitatively transformed into TP 819. Afterward TP 819 was transformed into the TPs 817 A,

**TABLE 1. Mean Concentrations of Iopromide and its TPs in $\mu$g/L in Raw and Treated Wastewater of Three German WWTPs[a]**

| | iopro-mide | TP 805 A[b] | TP 805 B[b] | TP 817 A[b] | TP 819 | TP 787 A | TP 731 A | TP 731 B | TP 729 A | TP 759 | TP 701 A | TP 701 B | TP 643 | Sum TPs in µg/L | percentage sum TP to dissipated Iopromide in %[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LOQ[c] influent | 0.010 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | -- | |
| LOQ[c] effluent | 0.010 | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.030 | 0.030 | 0.030 | -- | |
| WWTP 1 | | | | | | | | | | | | | | | |
| influent | 3.7<br><3.6> | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | 0 |
| effluent | 1.2<br><1.0> | 0.56<br><0.55> | 0.23<br><0.21> | <LOQ | 0.060 | <LOQ | 0.29 | <LOQ | 0.09 | 0.06 | 0.21<br><0.23> | 0.032<br><0.041> | 0.042<br><0.042> | 1.6 | 60 |
| WWTP 2 | | | | | | | | | | | | | | | |
| Influent | 31<br><22> | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | 0 |
| effluent | 7.0<br><6.7> | 2.5<br><2.2> | 0.85<br><0.79> | 0.06 | 0.80 | 0.12 | 0.30 | 0.014 | 0.085 | 0.16 | 0.11<br><0.10> | <LOQ | <LOQ | 5.0 | 21 |
| WWTP 3: grab samples | | | | | | | | | | | | | | | |
| effluent | 5.0<br><4.9> | 2.9 | 0.90±0.10 | 0.32±0.04 | 3.7±0.9<br>(4.4) | 0.22±0.01 | 1.7±0.3<br>(2.1) | 0.09±0.02<br>(0.11) | 1.1±0.1<br>(1.3) | 0.15±0.02<br>(0.18) | 0.50±0.04<br><0.44±0.04><br>(0.6) | 0.080±0.02<br><0.09±0.03><br>(0.12) | 0.25±0.06<br><0.18±0.06><br>(0.28) | 11.9 | |

[a] < LOQ: concentration below the limit of quantification. Statistical errors: confidence interval, $n = 3$, $P = 95\%$. Concentrations without brackets were determined by external calibration < >: concentrations confirmed by a calibration over the whole analytical method. ( ) : concentration confirmed by standard addition. [b] No reference standard available; for calibration see Table S5. [c] LOQ of the TPs was set at 0.010 $\mu$g/L using a safety factor of 3 for TP 701A, 701B and 643 and a factor of 5 for the other TPs. [d] Calculated by sum TP/($IP_{influent} - IP_{effluent}$) × 100 (sum TP: concentration of the sum of TPs in mol/L; IP: concentration of iopromide in mol/L).

**TABLE 2. Mean Concentrations in $\mu$g/L and Confidence Intervals ($n = 4$, $P = 95\%$) of Iopromide and its TPs in Bank Filtrate Samples and Two Groundwater Wells[a]**

| | iopromide | TP 805 A[b] | TP 805 B[b] | TP 819[b] | TP 731 A | TP 759 | TP 701 A | TP 701 B | TP 643 |
|---|---|---|---|---|---|---|---|---|---|
| **LOQ[c]** | 0.002 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 |
| **Bank Filtrates** | | | | | | | | | |
| I | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | 0.045±0.004 | 0.044±0.007<br><0.050±0.008> | 0.011±0.0008<br><0.013±0.001> | 0.013±0.001<br><0.011±0.001> |
| II | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | 0.030±0.002 | 0.040±0.003<br><0.045±0.004> | 0.011±0.0005<br><0.015±0.0007> | 0.014±0.002<br><0.012±0.001> |
| III | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | 0.027±0.002 | 0.040±0.003<br><0.045±0.003> | <LOQ<br><0.012 ± 0.001> | 0.013±0.001<br><0.011±0.001> |
| **Ground Water** | | | | | | | | | |
| Well 1 | 0.075±0.01<br><0.070±0.01> | 0.050±0.008 | 0.028±0.002 | 0.023±0.005 | 1.3±0.4<br>(1.5) | 3.6±0.4<br>(3.3) | 4.3±0.3<br>(3.2)<br><4.6±0.5> | 0.18±0.02<br><0.19 ± 0.02> | 0.55±0.02<br>(0.44)<br><0.46 ± 0.01> |
| Well 4 | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | 0.12±0.03 | 4.6±0.6<br>(4.0)<br><5.0 ± 0.9> | 0.21±0.03<br>(0.24)<br><0.28±0.04> | 0.50±0.07<br>(0.55)<br><0.39 ± 0.05> |

[a] Concentrations without brackets were determined by external calibration ( ): concentrations confirmed by direct injection, < >: concentrations confirmed by a calibration over the whole analytical method. TP 817, TP 729, TP 787A, TP 731B were never found above LOQ of 0.010 $\mu$g/L. < LOQ: concentration below the limit of quantification. [b] No reference standard available; for calibration see Table S5. [c] LOQ of the TPs was set at 0.010 $\mu$g/L using a safety factor of 5.

787 A, 731 A, 731 B, and 729 A which reached a maximum concentration between 40−70 d. Finally, at about 40 d the TPs 759, 701 A, 701 B, and 643 started to be formed and showed increasing concentrations even after 103 d. The sum of the concentrations ($\mu$mol/L) of iopromide and the 12 TPs exhibited that the mass balance was mainly complete over the whole experiment, indicating that the 12 TPs represents the quantitatively relevant TPs.

**Transformation Pathway.** A transformation pathway (Figure 6) could be proposed based on the sequence of TP formation (Figure 1 and 5) in aerobic batch experiments and microbial biochemical reactions reported in the literature. Since no transformation was observed in the batch system with formaldehyde, it can be assumed that microbial transformation of iopromide took place in the water/soil systems. In total, four to five different reactions could be envisioned: (I) oxidation of the primary hydroxyl groups, (II) oxidation of the secondary hydroxyl groups, (III) removal of α-hydroxypropionic acid side chains: direct cleavage of the amide−methylene bonds at side chains A and B (might be caused by subsequent IV and V reactions), (IV) oxidative decarboxylation of the α-ketopropionic side chain, and (V) deacetylation: cleavage of the amide−methylene bonds at side chains A and B of TPs containing *N*-acetate moieties.

Oxidation of primary and secondary hydroxyl groups (reaction I and II) are common reactions catalyzed by alcohol dehydrogenases or alcohol oxidases (*28*). A deacetylation (reaction V) has already been described as a crucial step in the degradation of NTA (*29*). After hydroxylation of the acetate methylene moiety by the NTA monooxygenase enzyme, the putative metabolite α-hydroxyl-NTA is spontaneously cleaved into glyoxylate and iminodiacetate. Oxidative decarboxylations (reaction IV) are known for α-keto acid-dependent dioxygenases. These enzymes are coupled with the hydroxylation/oxidation reactions of substrates with an oxidative decarboxylation of α-ketoglutarate (*30, 31*). Whether the iopromide TP containing α-keto carboxylic moieties could substitute for α-ketoglutarate remains unclear and needs to be addressed in further studies. Furthermore, it cannot be concluded if the removal of α-hydroxypropionic acid side chains is caused by a direct cleavage of the amide−methylene bonds (reaction III) at side chains A and B or by subsequent oxidative decarboxylation and deacetylation (reaction IV and V). However, TPs which resulted from the oxidative decarboxylation at side chain B were either not present or found at very low quantities in the degradation experiments.

The initial transformation step appears to be the oxidation of the primary hydroxyl groups of side chains A and B by alcohol dehydrogenases or alcohol oxidases which results in the formation of carboxylic moieties. After 26 d, iopromide was quantitatively transformed into TP 819 (see Figure 1) which was further transformed into several products (TP 731 A, 817 A, 729 A), presumably by oxidation of the secondary hydroxyl group at chain A and/or by cleavage of the amide−methylene bond of chain B. Subsequently, the α-oxopropionic group of chain A was oxidatively decarboxylated forming TP 701 A which could be further deacetylated to TP 643. TP 731 B could be formed either by a direct cleavage of the amide−methylene bond (chain A) of TP 819 or by a decarboxylation and deacetylation of side chain A of TP 817 A. TPs containing an α-ketopropionic group at side chain B were not detected, while TPs with a *N*-acetyl amide at side chain B were found (TP 787 A, 759, and 701 B). However, their abundance was much smaller than that for TPs with similar changes at chain A. Obviously, the amide−methylene bond of side chain B is cleaved more rapidly than that of chain A or the intermediates are less stable. It can be assumed that the missing methyl group at the amide moiety of chain B played a key role in the cleavage of this chain. However, further studies are needed to elucidate the biochemical reactions in detail and to confirm the suggested reactions. During the entire experiment, transformation of chain C was never observed.

**Occurrence in Groundwater, Bank Filtrate, and Wastewater.** The concentrations determined via the external calibration were confirmed either by a direct injection method for concentrations of TPs above 0.4 $\mu$g/L or by standard addition to the final extracts in case the isolated TPs could be used as reference compounds. In addition, a calibration was performed over the entire method for TP 701 A, TP 701 B, and TP 643. The concentrations detected in the environmental samples are summarized in Table 1 and 2.

*Wastewater Samples.* Iopromide was detected at 3.1 and 3.7 $\mu$g/L in wastewater influents from two municipal WWTPs, but none of the TPs were detected above the LOQ of 0.10 $\mu$g/L (Table 1). However, in the effluent of three WWTPs the 12 TPs were detected at least once with a sum of up to 11.9 $\mu$g/L. Maximum concentrations were found for TP 805 A and TP 819. The TPs detected at higher concentrations were compounds formed at the beginning of the transformation pathway (e.g., TP 805 A, 805 B, 819) or those which had already lost their α-hydroxypropionic acid side chain by cleavage of the amide−methylene bond (e.g., TP 731 A, 729 A, 701 A). Previously, Pérez et al. (2006) have reported the formation of TP 805 A, 805 B, and TP 819 in batch experiments with nitrifying sludge (*5*). The sum of the identified TPs accounts in our study for up to 60% of the iopromide dissipated in WWTP 1, whereas in WWTP 2 it was only about 21%. It appears that major contributors of the identified TPs are TP 805 A, TP 805 B, TP 819, and TP 731 A (see Figure S35 in chapter 6 of Supporting Information).

*Groundwater and Bank Filtrate.* In bank filtrates, only those TPs formed at the end of the proposed transformation pathway (Figure 6) were found. Maximum concentrations in bank filtrate (up to 0.05 $\mu$g/L) were detected for TP 759 and TP 701 A (Table 2). From the other TPs, only TP 701 B and TP 643 were present above the LOQ. A similar TP pattern was observed at a much higher concentration level detected in two groundwater wells of an area where wastewater irrigation took place for more than 50 years. For instance, TP 759 and TP 701 A were found in the groundwater wells up to 3.6 $\mu$g/L and 4.6 $\mu$g/L, respectively. The pattern of TPs in groundwater and bank filtrate samples was found to be different from that detected in WWTP effluents (see Figure S35 in chapter 6 of Supporting Information). Obviously, the contact with soil allows for the transformation to proceed and resulted in the formation of TPs at the end of the proposed transformation pathway. These findings confirm that the TPs identified in laboratory-scale experiments are formed during soil and subsoil passage as well as in WWTPs.

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## Supporting Information Available

Experimental details. This information is available free of charge via the Internet at http://pubs.acs.org.

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