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^{13}C- and ^{15}N-Isotope Analysis of Desphenylchloridazon by Liquid Chromatography−Isotope-Ratio Mass Spectrometry and Derivatization Gas Chromatography−Isotope-Ratio Mass Spectrometry

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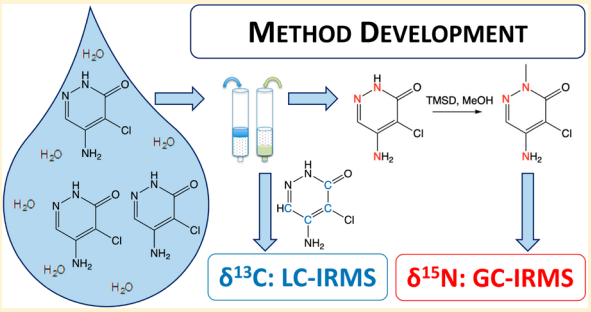
Supporting Information

ABSTRACT: The widespread application of herbicides impacts surface water and groundwater. Metabolites (e.g., desphenylchloridazon from chloridazon) may be persistent and even more polar than the parent herbicide, which increases the risk of groundwater contamination. When parent herbicides are still applied, metabolites are constantly formed and may also be degraded. Evaluating their degradation on the basis of concentration measurements is, therefore, difficult. This study presents compound-specific stable-isotope analysis (CSIA) of nitrogen- and carbon-isotope ratios at natural abundances as an alternative analytical approach to track the origin, formation, and degradation of desphenylchloridazon (DPC), the major degradation product of the herbicide chloridazon. Methods were developed and validated for carbon- and nitrogen-isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) of DPC by liquid chromatography−isotope-ratio mass spectrometry (LC-IRMS) and derivatization gas chromatography−IRMS (GC-IRMS), respectively. Injecting standards directly onto an Atlantis LC-column resulted in reproducible $\delta^{13}C$-isotope analysis (standard deviation <0.5‰) by LC-IRMS with a limit of precise analysis of 996 ng of DPC on-column. Accurate and reproducible $\delta^{15}N$ analysis with a standard deviation of <0.4‰ was achieved by GC-IRMS after derivatization of >100 ng of DPC with 160-fold excess of (trimethylsilyl)diazomethane. Application of the method to environmental-seepage water indicated that newly formed DPC could be distinguished from "old" DPC by the different isotopic signatures of the two DPC sources.



In many regions of the European Union, groundwater is our most important drinking-water resource and is therefore constantly screened for contaminants.[1,2] In recent years, there is growing concern about pollution by persistent and mobile organic contaminants such as polar compounds and their metabolites.[3−6] Metabolites are often more persistent and polar than the parent compounds, resulting in high leaching potentials with increased risks of contaminating groundwater.[7] For some of them, however, methods are lacking to demonstrate their origin, formation, and degradation. To evaluate their environmental fates, conventional models rely on parent-compound-to-metabolite ratios. However, as pesticides are still applied on the fields, there is constant formation of persistent metabolites. Thus, the evaluation of metabolite degradation with conventional models based on concentration measurements may lead to bias. Further bias is introduced when one contaminant is formed from at least two different sources (parent compounds).[8]

A representative compound for polar contaminants is desphenylchloridazon (DPC). It is among the most frequently detected micropollutants related to crop production, exceeding concentrations of 10 μg/L in natural water.[2,9−16] DPC is formed by microbial degradation of the selective systemic herbicide chloridazon (CLZ).[16−19] CLZ is applied in the agricultural production of mangold, beetroot, and sugar beet.[20] Consequently, there is the constant formation of DPC from newly applied CLZ. DPC can be transformed to methyl-desphenylchloridazon (MDPC).[9,21] Its transformation pathway and environmental fate, however, are still mostly unknown.

This study presents compound-specific stable-isotope analysis (CSIA) as an alternative approach for identifying a

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compound's origin and transformation by analyzing stable-isotope ratios at natural abundance.[22] As herbicides deriving from different manufacturers may differ in their $^{13}C/^{12}C$ or $^{15}N/^{14}N$ isotopic signatures, isotope analysis enables a distinction between different sources. In particular, DPC contains the same nitrogen atoms as its parent compound, CLZ, so it is expected to show the same nitrogen-isotope signature provided that the isotope ratio is not changed by isotope effects during degradation. In contrast, only some of the carbon atoms of CLZ are transferred to DPC because it is formed by cleavage of the phenyl-ring from the heterocyclic pyridazine-ring (see the structures in Table S1), so DPC may show a different carbon-isotope signature from that of CLZ. Carbon-isotope analysis, however, may still be particularly insightful, because changes in isotope ratios of DPC may be detectable by CSIA, delivering evidence about the formation and biodegradation of this persistent metabolite. Because molecules with light isotopes are usually degraded more rapidly than those with heavy isotopes, transformation leads to an enrichment of heavy isotopes in the fraction of the remaining pesticide.[8] This increase in the isotope ratio (e.g., $^{13}C/^{12}C$) can therefore give evidence of the degradation of the compound.[8] By combining both elements in the form of a dual-element isotope plot, further information about the reaction mechanism of a compound's degradation or its origin can be gained.[23]

Even though methods for carbon- and nitrogen-isotope analysis exist for several pesticides and their metabolites,[8,24−30] most CSIA methods for environmental-compound analysis have focused so far on GC-amenable compounds. CSIA is typically accomplished by coupling gas chromatography (GC) to isotope-ratio mass spectrometry (IRMS). Like most polar organic compounds, however, DPC is not amenable to GC as it decomposes before reaching a boiling point (see Table S1). To analyze the isotopic composition of such polar organic compounds, derivatization-GC-IRMS has been brought forward as alternative strategy.[24,25,31,32] This approach was chosen as the methylation of DPC enhances its GC suitability. Methylation of a compound using "mild" derivatization reagents (e.g., trimethyl sulfonium hydroxide (TMSH) or methanol/BF3) allows control over the isotope ratio of the methyl group that is introduced. Hence, the change in the $^{13}C/^{12}C$ composition of the target analyte caused by the introduction of an additional carbon atom can be corrected by equations stated in the literature.[31,33,34] However, these mild reagents fail to derivatize groups of low reactivity, such as amino-, amide-, and hydroxyl-groups.

Consequently, for compounds containing less-reactive groups, an alternative strategy must be followed. For $^{13}C/^{12}C$ isotope analysis, liquid chromatography is the method of choice.[35−38] LC-IRMS has the advantage that compounds can be analyzed directly without derivatization, but liquid chromatography presents the challenge that carbon-isotope measurements must be conducted without organic eluents, which otherwise would be converted to CO_2 and interfere with the $^{13}C/^{12}C$ analysis of the analyte.[39,40] For nitrogen-isotope analysis, such sensitive LC-IRMS is not possible, but here GC-IRMS after derivatization by more reactive reagents is an option, because for $^{15}N/^{14}N$ analysis, control over carbon-isotope ratios is not required. To this end, the idea of Kuhlmann[41] is followed, where the methylation of DPC with diazomethane is described. Further adaptations described by Mogusu et al.[24] include the use (trimethylsilyl)diazomethane (TMSD), a less-explosive substitute compared with diazomethane, to methylate polar organic compounds.[42,43] For diazomethane and TMSD, the control over the isotope value of the additional carbon atom is lost because no reproducible isotope effects are expected.[31] As the methylation leaves the $^{15}N/^{14}N$ ratio unaffected, however, this approach is well-suited for nitrogen-isotope analysis.

Following these two approaches, this study demonstrates the feasibility of dual-element isotope analysis of a very polar and fairly ubiquitous environmental contaminant using the complementary methods LC-IRMS and GC-IRMS. The development of a precise and true method[44] for LC-IRMS and GC-IRMS to measure $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios of DPC is presented. The developed methods were optimized, and a feasibility study tested the applicability to environmental-seepage water to probe for the formation of DPC from different sources, simulating a typical field situation.

EXPERIMENTAL SECTION

Chemicals. Desphenylchloridazon (5-amino-4-chloro-3-pyridazinone, CAS no.: 6339-19-1) was obtained from BASF (99.8%, Limburgerhof, Germany). Methyl-desphenylchloridazon (5-amino-4-chloro-2-methyl-3(2*H*)-pyradizone, CAS no.: 17254-80-7) was purchased from LGC Standards GmbH (Wesel, Germany). Chloridazon (≥98%, CAS no.: 1698-60-8) and acetochlor (96.3%, CAS no.: 34256-82-1) were sourced from Chemos GmbH & Company KG (Regenstauf, Germany). Desethylatrazine (purity not available, CAS no.: 6190-65-4) was produced by Synchem (Felsberg, Germany). (Trimethylsilyl)diazomethane (2.0 M dissolved in diethyl ether, CAS no.: 18107-18-1, acutely toxic and hazardous to health), sodium persulfate (≥99.9%, CAS no.: 7775-27-1), and phosphoric acid (≥85%, CAS no.: 7664-38-2) were supplied by Sigma-Aldrich (Merck KGaA, Darmstadt, Germany), whereas methanol (≥99.9%, CAS no.: 67-56-1) and acetone (≥99.9%, CAS no.: 67-64-1) were received from Roth (Karlsruhe, Germany). Ultrapure water was derived from a Millipore DirectQ apparatus (Millipore, Bedford, MA).

EA-IRMS Measurement for Determination of Reference Values. The carbon and nitrogen isotope compositions of our in-house standards of CLZ, DPC, and MDPC were characterized by an elemental analyzer−isotope-ratio mass spectrometer (EA-IRMS) as described by Meyer et al.[45] A system consisting of a EuroEA (Euro Vector, Milano, Italy) was hyphenated to a Finnigan MAT 253 IRMS via a FinniganTM ConFlow III interface (Thermo Fisher Scientific, Bremen, Germany). The standards were calibrated against the organic referencing materials USG 40 (L-glutamic acid), USG 41 (L-glutamic acid), and IAEA 600 (caffeine) provided by the International Atomic Energy Agency (Vienna, Austria).

The carbon ($\delta^{13}C$)- and nitrogen ($\delta^{15}N$)-isotope values (‰) are reported relative to PeeDee Belemnite (V-PDB) and air, respectively, according to eqs 1 and 2:

$$\delta^{13}C = \frac{^{13}C/^{12}C_{sample} - ^{13}C/^{12}C_{reference}}{^{13}C/^{12}C_{reference}} \quad (1)$$

$$\delta^{15}N = \frac{^{15}N/^{14}N_{sample} - ^{15}N/^{14}N_{reference}}{^{15}N/^{14}N_{reference}} \quad (2)$$

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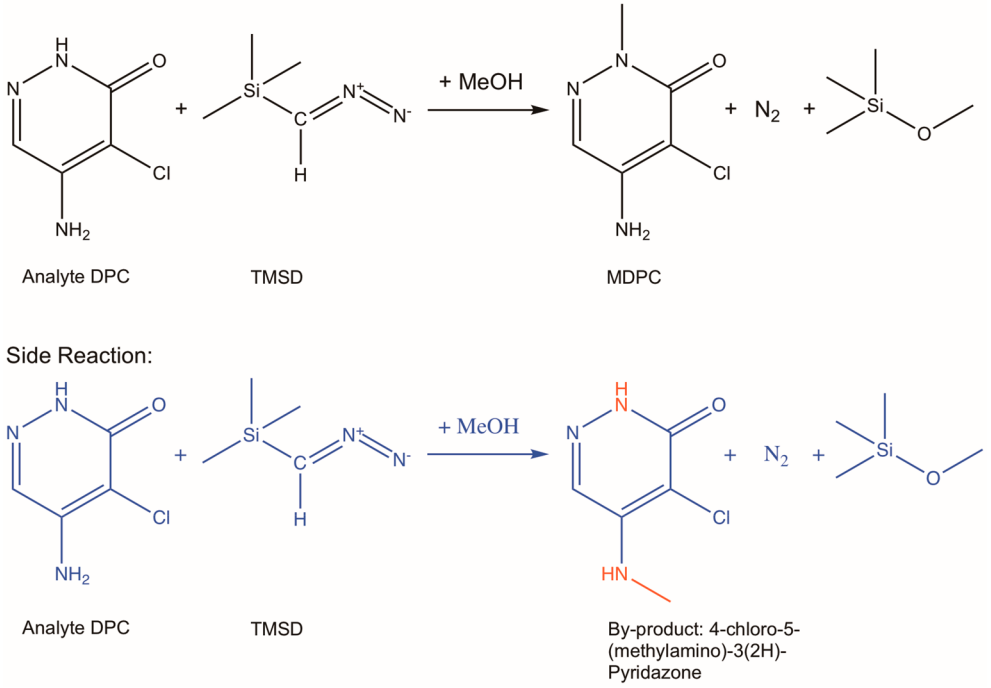
Scheme 1. Derivatization Reaction of DPC with TMSD with Methanol as a Catalytic Converter[a]



[a]The formation of the byproduct during derivatization is shown in blue. The difference in the methylation of the amino-groups is highlighted in red.

For carbon analysis by LC-IRMS, $\delta^{13}C$ values were determined relative to our laboratory CO_2 monitoring gas, which was introduced at the beginning and end of each analysis run. $\delta^{15}N$ values were determined analogously relative to our laboratory N_2 monitoring gas. Both gases were previously calibrated against RM8563 (CO_2) and NSVEC (N_2) supplied by the International Atomic Energy Agency (IAEA).

Isotope Analysis by LC-IRMS. High-performance liquid chromatography (HPLC) was carried out on a Dionex system consisting of an Ultimate 3000 HPLC pump and an Ultimate 3000 autosampler (Thermo Fisher Scientific). Chromatography was performed with an Atlantis T3 Sentry guard column (3 μm, 3.9 × 20 mm, 100 Å, Waters, Milford, MA) and an Atlantis T3 column (3 μm, 3 × 100 mm, 100 Å, Waters) operated isocratically at 500 μL/min with a pH 2 phosphoric acid solution at room temperature. Isotopic-ratio measurements were carried out on a Delta V Advantage IRMS coupled to the LC system by an Isolink interface (Thermo Fisher Scientific). The eluting compounds were quantitatively oxidized using oxidant (90 g/L $Na_2S_2O_8$) and phosphoric acid (1.5 M H_3PO_4), each introduced at a flow rate of 30 μL/min in the oxidation reactor held at 99.9 °C. Before use, the reagent solutions were degassed in an ultrasonic bath under vacuum for 30 min. To avoid reuptake of CO_2, all solutions were continuously sparged with helium during use. In order to avoid clogging in the system, an in-line filter with a pore size of 5 μm (Vici, Schenkon, Switzerland) was placed in front of the oxidation reactor of the LC-IsoLink interface. The ion source was held at 2×10^{-6} mbar, the accelerating voltage was 3 kV, and ions were generated by electron ionization at 124 eV. The injection volume ranged between 10 and 100 μL. Peak identification was based on retention times in comparison with external standards. The LC-IRMS system and data collection were controlled using Isodat 3.0 software (Thermo Fisher Scientific).

Derivatization Procedure with (Trimethylsilyl)-diazomethane (TMSD). Derivatization of DPC was accomplished on the basis of the method of Kuhlmann[41] using diazomethane, as previous attempts with TMSH and methanol/BF_3 had been unsuccessful (data not shown). However, because of the classification of diazomethane as toxic and explosive, here the more stable (trimethylsilyl)-diazomethane (TMSD) was tested as a less-explosive substitute. Reaction of the target analyte with TMSD forms diazomethane in situ, which subsequently methylates the analyte (see Scheme 1) to form MDPC. The derivatization of DPC with TMSD was carried out offline in 20 mL headspace vials. A 250 mg/L standard of DPC dissolved in methanol was used for method development. Derivatization of the target analyte was evaluated at different temperatures (50 and 70 °C, Figure S5) by varying reaction times (data not shown) and with different TMSD-to-analyte ratios. TMSD-to-analyte ratios varied between 90 and 230, which correspond to 80−200 μL of a 2 M TMSD solution in diethyl ether added to 1 mL of a 250 mg/L DPC solution. After addition of the TMSD, the vial was tightly crimped and placed for 2 h in a heated water bath. Afterward, the methanol was evaporated until complete dryness using a gentle stream of nitrogen. As it was tested with standards, no nitrogen-isotope fractionation was introduced during evaporation. The residue was reconstituted three times with acetone and transferred into a GC vial with a 200 μL insert. The final reconstitution volume for isotope measurements was 200 μL. The limit of precise isotope analysis and the method's trueness were determined using varying concentrations of the DPC standard (5 to 1000 mg/L).

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GC-IRMS Conditions for Nitrogen-Isotope Analysis. For the analysis of $\delta^{15}N$ isotope ratios, a GC-IRMS system consisting of a TRACE GC Ultra gas chromatograph (Thermo Fisher Scientific) coupled with a Finnigan MAT 253 isotope-ratio mass spectrometer (IRMS, Thermo Fisher Scientific) was used. Both instruments were linked via a Finnigan Combustion III interface (Thermo Fisher Scientific). The IRMS was operated in a vacuum of 2.1×10^{-6} mbar with an accelerating potential of 9 kV and an emission energy of 2 mA. For combustion of the target analyte, a NiO tube CuO−NiO reactor (Thermo Fisher Scientific) was used at a temperature of 1030 °C. The gas chromatograph was equipped with a DB-1701 column (J&W Scientific, Santa Clara, CA) with a length of 30 m, an inner diameter of 0.25 mm, and a film thickness of 1 μm. The instrument was operated with helium carrier gas (grade 5.0) at a flow rate of 1.4 mL/min. Splitless injection was performed into a splitless liner at 250 °C (Thermo Fischer Scientific). The GC temperature program started at 100 °C and was held for 1 min; this was followed by a temperature ramp of 25 °C/min to 240 °C, followed by another temperature ramp of 10 °C/min until the final temperature of 280 °C was reached and held for 5 min. In contrast, for on-column injection, the flow and injector temperature were controlled by an Optic 3 device (ATAS, GL Science, Eindhoven, Netherlands) equipped with a custom-made glass on-column liner. Samples were injected using a PAL autosampler (CTC Analytics AG, Zwingen, Switzerland). The ATAS injector had an initial temperature of 50 °C, which was held for 300 s, and then the temperature was ramped at 4 °C/s to 250 °C. The split flow started at 14 mL/min. After injection, the split flow was set to 0 mL/min for 120 s and finally set to its initial value of 14 mL/min. Simultaneously, the flow rate started at 0.3 mL/min, was held for 120 s, and then was increased to 1.4 mL/min within 120 s. Meanwhile, the initial temperature of the GC oven was set to 40 °C, which was held for 1 min; the temperature was then ramped at 25 °C/min to 240 °C, held there for 0 min, ramped to 10 °C, and held there for 5 min. The injection volume ranged between 1 and 3 μL for splitless injection and 1 and 4 μL for on-column injection. To control the system and verify the method, retention times and isotope values were constantly monitored by bracketing samples with in-house standards of desethylatrazine (DEA), acetochlor (ACETO), and MDPC.

Isotope-Value-Correction Procedure. All reported isotope ratios are expressed as arithmetic means of three replicate measurements with their respective standard deviations ($\pm\sigma$). For LC-IRMS, calibration was performed using in-house standards and monitoring gas peaks allocated throughout the chromatograms. Trueness of the LC-IRMS system was achieved by correction with a bracketing method using a DPC standard (Table S2) whose signature had previously been determined by EA-IRMS.

For correction of $\delta^{15}N$-isotope values, two approaches were applied. In the first measurement campaign, as there was no MDPC standard within the required concentration range commercially available, a correction based on the comparison with DEA and ACETO was used to test for the trueness of the isotope values after conversion to N_2 in the combustion furnace. The EA-IRMS values (Table S2) of these standards were plotted against the measured GC-IRMS values. The differences were used to correct values of the derivatized DPC analyte. DPC was measured by three laboratories (Table S3) to increase the accuracy and thus reduce measurement errors deriving from other analytical methods. In the second measurement campaign, authentic MDPC synthesized by LGC Standards GmbH was used so that the principle of identical treatment by Werner and Brand[46] could be applied, and drifts during measurements as well as differences within the combustion efficiencies were corrected directly.

Peak Identification and Quantification with GC-qMS. Gas chromatography−quadrupole mass spectrometry (GC-qMS) measurements were carried out to identify MDPC and any coproducts generated during derivatization. The instrumental setup is described within Supporting Information Section II.1. One microliter of a derivatized 250 mg/L solution was injected and measured in scan mode. MDPC was identified using the presence of mass-to-charge ratios 159 and 145 as qualifier ions. Additionally, the retention times and spectra were confirmed by measuring the nonderivatized authentic standard of MDPC.

Isotope Ratios of Commercially Available Chloridazon Products: Source Fingerprinting. Carbon- and nitrogen-isotope ratios of CLZ standards from different suppliers (see Table S4) were analyzed to check whether CLZ standards deriving from different suppliers show different isotopic signatures as a result of industrial production. All samples were measured with the EA-IRMS method already described.

Evolution of Isotope Ratios Deriving from Different Chloridazon Sources. The developed method was applied to investigate whether it is possible to track DPC deriving from different CLZ sources in seepage water (collected from a lysimeter site, described in detail by Torrentó et al.[47]). Therefore, 30 μg/L CLZ ($\delta^{15}N = -31.5 \pm 1.0$‰) was spiked into 10 L of seepage water that contained 10 μg/L DPC ($\delta^{15}N = -15.1 \pm 1.0$‰) originating from another CLZ source from previous experiments. The samples were then stored at 13 °C in the dark over various periods of time (0 to 11 months). Subsequently, the concentrations of CLZ, DPC, and MDPC was measured with ultrahigh-performance liquid chromatography (UHPLC, see Supporting Information Section II.2.). The nitrogen-isotope values of DPC were determined with derivatization-GC-IRMS. To this end, samples were concentrated using the solid-phase-extraction procedure by Torrentó et al.[48] (see Supporting Information Section II.3 and Figure S1). Prior to GC-IRMS analysis, preparative HPLC was required as an additional cleanup step. Method details are described in Supporting Information Section II.4 and Figure S2.

RESULTS AND DISCUSSION

DPC-Carbon-Isotope Analysis. To determine the limit of precise isotope analysis of the LC-IRMS method, a DPC standard was injected at concentrations between 2.8 and 133 nmol of C on-column (Figure 1). A chromatogram is shown in Figure S4. The limit of precise isotope analysis was determined with the moving-mean procedure described by Jochmann et al.[49] using an uncertainty interval of ±0.5‰. The limit obtained for carbon-isotope analysis of DPC by LC-IRMS was 27.5 nmol of C on-column (996 ng of DPC on-column), which corresponds to an injection of 50 μL of a 0.14 mM (20 mg/L) solution of DPC. This value is within the range of detection limits previously determined for other compounds analyzed by LC-IRMS.[25,50]

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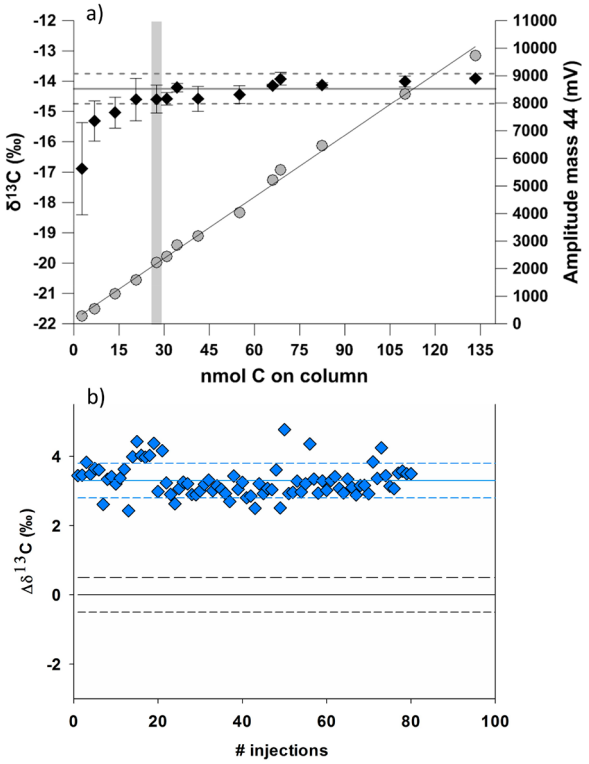

Figure 1. (a) Correlation of amount-dependency tests for carbon-isotope values as well as the amplitudes by LC-IRMS. Gray circles represent the average intensities for each amount on-column, and black diamonds represent the average corresponding δ values of replicate measurements. The limit of precise isotope analysis was determined following the procedure described by Jochmann et al.[49] and is shown by the gray rectangle. The gray horizontal line stands for the mean of all values with intensities above the gray rectangle. (b) Reproducibility of carbon-isotope values (blue diamonds) of DPC with LC-IRMS. The results are stated as the deviations of the measured values from the values determined by EA-IRMS ($\Delta\delta^{13}$C). The blue line shows the average carbon-isotope values ± 0.5‰ (dashed lines), and the black line represents the EA δ^{13}C values of DPC ± 0.5‰ (dashed lines).

The method showed good reproducibility of δ^{13}C values, with a mean value of −14.6 ± 0.5‰ for 80 individual injections of 27.5 nmol of C of DPC on-column comprising different measurement sequences over a time of 3.5 months (Figure 1b). A mean absolute offset of +3.3‰ between the average value determined by LC-IRMS and the EA value was measured. Such a difference between LC-IRMS values and EA-IRMS values has been previously observed for amino acids,[50,51] caffeine and ethanol,[52] pharmaceuticals,[53] and bentazone.[31] Several analyses in flow-injection-analysis (FIA) mode (i.e., bypassing the LC column) resulted in the same offset between EA values and FIA-IRMS values (data not shown). This observation suggests incomplete wet oxidation of DPC rather than a chromatography-related issue as a reason for this offset. Attempts to optimize oxidation conditions led to neither a reduced offset nor a higher intensity of the DPC peak. As the δ^{13}C values obtained by LC-IRMS were reproducible, the resulting offset was constant and could be corrected accordingly.

Derivatization of DPC: Nitrogen-Isotope Analysis. As shown in Figure 2, DPC derivatization resulted in MDPC and its isomer 4-chloro-5-(methylamino)-3(2*H*)-pyridazone as major byproduct; as well as a minor byproduct deriving from the reaction of TMSD with itself. Both products were identified by GC-qMS. Additionally, MDPC was verified using an authentic standard. For method-development and -optimization purposes, the yield of derivatized DPC was tested by GC-qMS for two temperatures, 50 and 70 °C, maintaining the same TMSD-to-analyte ratio expressed as a molar ratio (n(TMSD)/n(analyte) ratio). The temperature dependence was minor, indicating robustness of the method. A slightly higher yield of the target analyte (derivatized DPC) was achieved at a temperature of 70 °C (Supporting Information Section III.2, Figure S5); thus, method validation with GC-IRMS continued to use this temperature for derivatization. The ratio of the isomer to the target analyte remained at approximately 1/10 and was unaffected by the temperature. The recovery of derivatized DPC at 70 °C was approximately 65%, which was quantified using an authentic standard at different concentrations (R^2 > 0.99, data not shown).

Figure 3a shows the measured δ^{15}N-isotope values of 250 mg/L DPC derivatized with increasing excess of the derivatization reagent TMSD. A plateau of the δ^{15}N-isotope value is reached at an excess of TMSD of greater than 150 n(TMSD)/n(analyte), indicating optimum transformation of DPC to MDPC at this proportion. Following the approaches of Reinnicke et al.[31] and Mogusu et al.,[24] further method validation was carried out with an excess of 160 n(TMSD)/n(analyte) as a conservative approach. The δ^{15}N-isotope values

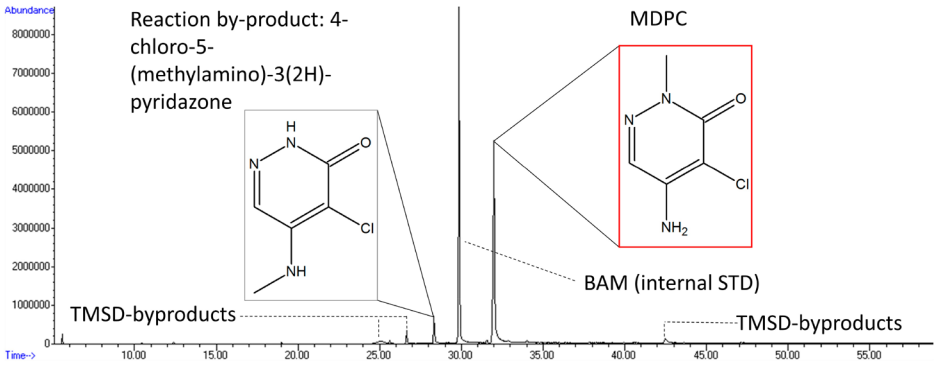

Figure 2. Chromatogram of DPC derivatized with TMSD showing the derivatization product MDPC (red box) and the reaction byproduct 4-chloro-5-(methylamino)-3(2*H*)-pyridazone (gray box). 2,6-Dichlorobenzamide (BAM) was used as an internal standard. An authentic standard of MDPC was applied for peak identification.

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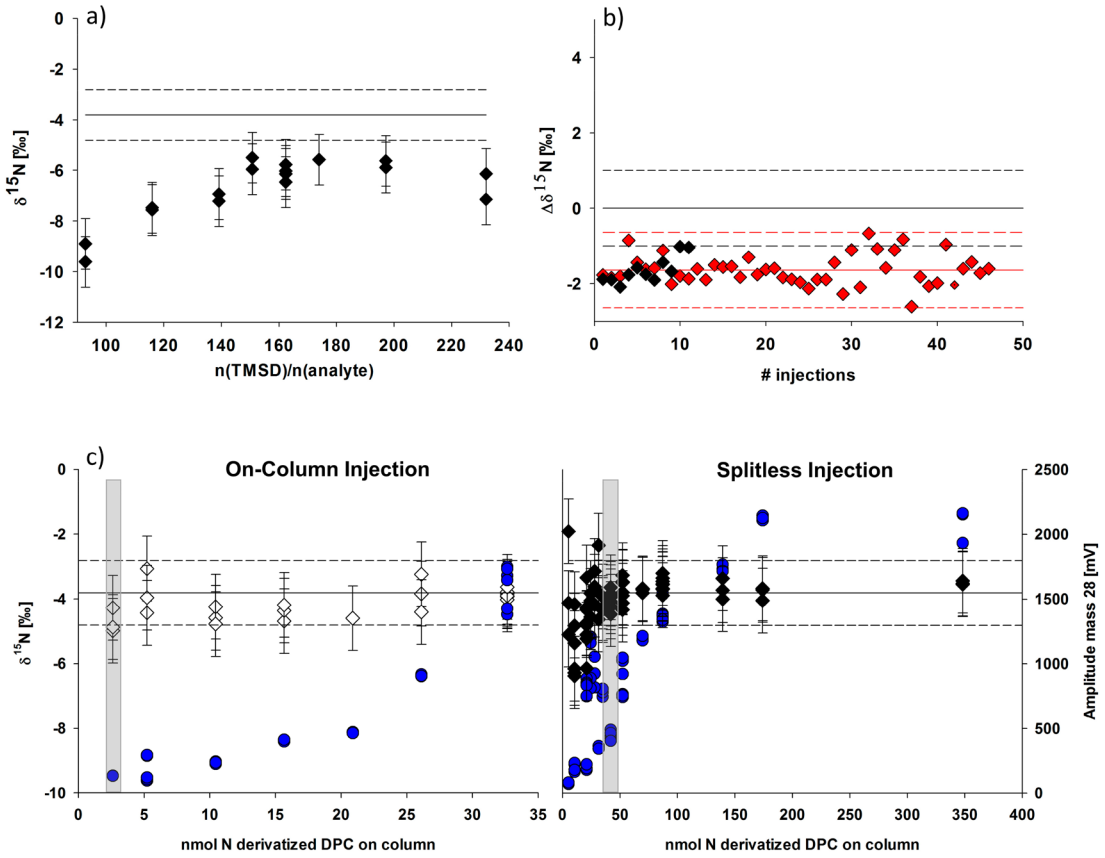

Figure 3. (a) δ^{15}N values from DPC showing dependence on the excess of TMSD used for the derivatization procedure, (b) reproducibility of δ^{15}N values of derivatized DPC (black diamonds) and MDPC (red diamonds) measured with GC-IRMS, and (c) δ^{15}N values from DPC and the amplitudes (blue circles) showing dependence on the amount of nitrogen from derivatized DPC injected onto the column for determining the limit of precision. The amount of derivatized DPC equals the initial amount of DPC used for derivatization. Black diamonds show the δ^{15}N-isotope values using splitless injection, whereas the white diamonds show the precision gained with on-column injections. Data were corrected for the offset caused by the combustion efficiency. The gray rectangles mark the limits of precise nitrogen-isotope analysis. Results in panel (b) are stated as the deviation of the measured value from the value determined by EA-IRMS ($\Delta\delta^{15}$N). The red line shows the average δ^{15}N-isotope value and its tolerated standard deviation of ±1‰ (red dashed line); the solid lines shows the target-isotope values determined with EA, whereas the dashed lines indicate the tolerated standard deviations of ±1‰.

show a deviation from the EA-IRMS value ($\Delta\delta^{15}$N) of −1.6 ± 0.4‰ (black markers in Figure 3b), which can be corrected for. Because the pure nonderivatized standard of MDPC shows a similar offset (red markers in Figure 3b), we conclude that this deviation results from incomplete combustion of the target analyte rather than from isotopically sensitive branching due to the formation of the major byproduct during derivatization.

Figure 3c shows the nitrogen-isotope values of DPC derivatized with an excess of TMSD greater than n(TMSD)/n(analyte) = 150 (140 μL of a 2 M TMSD solution in 1 mL of a 5−1000 mg/L analyte solution) injected with two different injection techniques. All values were corrected for the offset due to incomplete combustion. For comparison, the EA-IRMS reference value is shown as a black line. The limit of precise nitrogen-isotope analysis of DPC is, as expected, amplitude-dependent. For splitless injection, this limit is equal to 31 nmol of N from injected derivatized DPC, corresponding to an injection of 1.2 μg of nonderivatized DPC. Additionally, on-column injection was tested as a more sensitive method. In accordance with the findings of Schreglmann et al. for sensitive isotope analysis of atrazine,[54] on-column injections of the derivatized DPC resulted in a decrease of the limit of precise isotope analysis by a factor of 10, as shown in Figure 3b. Thus, 2.06 nmol of N from derivatized DPC on-column (100 ng of DPC on-column) were sufficient for accurate results, which corresponded to an injection of 1 μL of a 0.69 mM DPC solution.

Isotope Ratios of Commercially Available Chloridazon Products: Source Fingerprinting. δ^{13}C and δ^{15}N EA-IRMS measurements of commercially available CLZ products were used to investigate the possibility of distinguishing among different sources. The results are shown as a dual-element isotope plot in Figure 4. There is significant variability for both elements. δ^{15}N-isotope values ranging from −5.7 to −32.0‰ were measured (Table S4). As both CLZ and DPC contain identical N atoms, the metabolite can be related to the parent on the basis of their nitrogen-isotope compositions. This highlights the potential of using the δ^{15}N value of DPC as a fingerprint to retrace the parent compound, CLZ.

In contrast to nitrogen-isotope values of CLZ, the detected variability of its δ^{13}C values cannot directly be used to draw conclusions on the carbon-isotope signature of DPC because cleavage of the phenyl-ring may cause differences in the isotopic signatures of the parent compound and metabolite.

Evolution of Isotope Ratios of DPC from Different Chloridazon Sources. The developed method was applied to DPC-containing environmental-seepage water spiked with CLZ. Its original composition is listed in the Supporting

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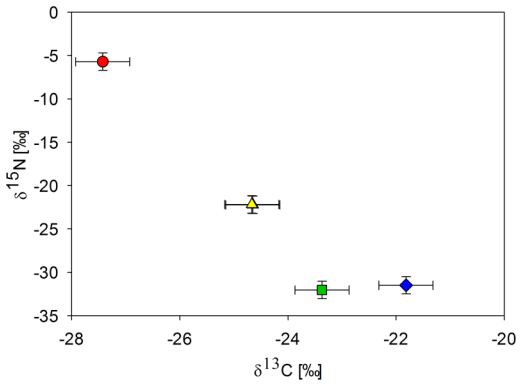

Figure 4. Dual-isotope plot of chloridazon standards derived from different suppliers.

Information (Table S5). The spiked seepage water was used to test whether a mixture of the nitrogen-isotope value of DPC deriving from the spiked CLZ and the DPC already present in the water could be observed over a defined time period, simulating a typical field situation.

Concentration measurements of CLZ, DPC, and MDPC in the seepage water (Figure 5, upper panel, and Table S6 in the Supporting Information) showed a significant decrease in CLZ concentration (white) after 7 months (t_1) and concentrations below the limit of detection after 11 months (t_2).

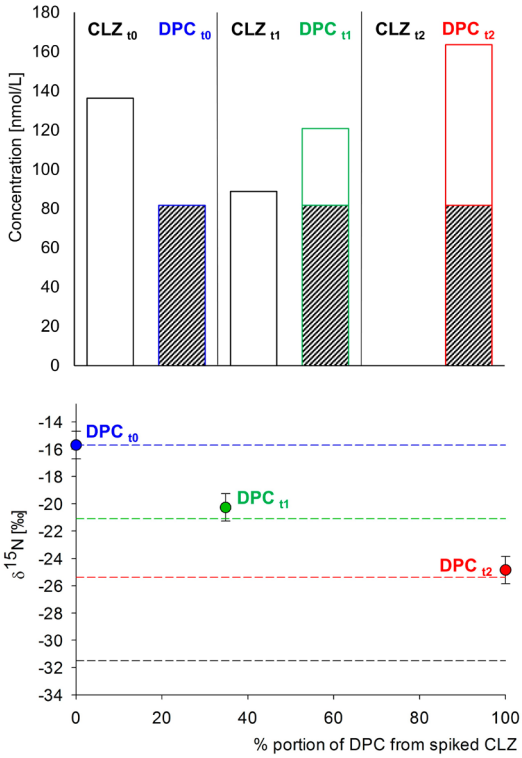

Figure 5. Degradation of CLZ to DPC over time and the resulting change of the δ^{15}N value of DPC due to two different sources of CLZ. Measured δ^{15}N values are shown as circles, whereas the dashed lines are the corresponding δ^{15}N values, calculated on the basis of the mixing of the two CLZ sources originating from the initial δ^{15}N of DPC (t_0) and the spiked CLZ (the initial δ^{15}N is shown as a black dashed line). It is assumed that CLZ is degraded completely to DPC. Samples were taken directly after spiking with CLZ (t_0) and after storage for 7 months (t_1) and 11 months (t_2).

Simultaneously, the DPC concentration increased over time, consisting of the initial DPC (shaded gray) and newly formed DPC from degraded CLZ (white). After 8 months, the concentration of DPC remained constant (data not shown). The formation of DPC from CLZ agrees with the findings of Buttiglieri et al.[16] and Schuhmann et al.[21] in environmental samples, where CLZ was degraded within the first 8 to 12 weeks after application on an agricultural field.

The corresponding nitrogen-isotope values are shown in the panel below (Figure 5). Concomitant with the disappearance of CLZ by reaction, a shift in the δ^{15}N of DPC toward the isotopic composition of the added CLZ (-31.5 ± 1.0‰) was observed. Formation of MDPC was small (the ratio of MDPC to DPC was always smaller than 10%), so its influence on the DPC nitrogen isotopes and its contribution to the mass balance in the samples can be neglected. Also, the interference of MDPC with derivatized DPC on the nitrogen-isotope value remains within the uncertainty of the presented isotope analysis. In the case that this ratio is greater in environmental samples, fractionative HPLC can be used to separate the two analytes prior to derivatization-GC-IRMS (Supporting Information Section II.5).

As the initial nitrogen-isotope composition as well as the concentrations of both DPC and CLZ are known, a two-sources-mixing model, based on the weighted arithmetic mean of the isotope ratio, was applied to investigate whether DPC nitrogen-isotope values accurately reflect the relative contribution of either source. To this end, it was assumed that all additional DPC was formed from CLZ, and calculations were based on the EA-IRMS values of the CLZ that was applied. The differences between the measured points and the calculated isotope values (dashed lines) of Figure 5 (lower panel) were less than 1‰ and thus within the measurement uncertainty of the instrument. This indicates that nitrogen-isotope values of DPC did indeed reflect the relative contribution of the DPC from a different origin, and therefore, the approach holds promise for future source elucidation of CLZ metabolites in field samples.

We note that the mass balance does not close for DPC formation from CLZ (Figure 5). Possible explanations are either (a) that part of the CLZ was degraded without forming DPC (potentially producing biomass) or (b) that DPC was degraded via a so-far-unknown transformation pathway that did not entail nitrogen-isotope fractionation. Evidence against the second hypothesis, however, is given by our observation that after complete CLZ degradation, the concentration of DPC remained constant (data not shown). Although further investigations into this matter are beyond the scope of this feasibility test, the possibility of adding carbon-isotope analysis to the picture, as newly established in this contribution but not yet pursued in this feasibility test, provides added value for probing not only for formation of metabolites from different sources but also for their further degradation.

CONCLUSION AND OUTLOOK

With LC-IRMS and GC-IRMS, this study brings forward two complementary approaches to accomplish reproducible, precise, and true compound-specific carbon- and nitrogen-stable-isotope analysis of DPC in the micrograms-per-liter concentration range (996 and 100 ng of DPC on-column for carbon- and nitrogen-isotope analysis, respectively). Taking the reported DPC concentrations of 0.72−7.4 μg/L in surface and groundwater into account,[16] the combination of the

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presented methods with large-volume extraction as presented by Torrentó et al.[48] enables the isotopic analysis of DPC in environmental water samples. Thus, the application of the developed methods brings forward a basis for the analysis of environmental water samples from field surveys, and the combination of the developed methods gives access to dual-element isotope plots. Our study highlights the potential of such plots to distinguish different sources. Future DPC-degradation studies may use such dual-element isotope information to obtain additional information about the transformation pathways of DPC and the underlying mechanisms.[55] Until now, only the transformation to MDPC is known, which was, however, observed to occur on longer time scales than those in our experiment.[21] Additionally, as shown in the degradation experiment of chloridazon, these methods can be used to distinguish the source of DPC by measuring the nitrogen-isotope signature and to identify the mixing of DPC derived from different CLZ sources.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.8b04906.

Properties of chloridazon and its metabolites desphenyl-chloridazon and methyldesphenylchloridazon; supplementary methods including peak identification and quantification with GC-qMS, concentration measurements with UHPLC, seepage-water-extraction-method validation with spiked samples, and fractionative HPLC (sample-cleanup method and separation of DPC from MDPC in environmental samples prior to derivatization); LC-IRMS chromatogram of DPC; and temperature optimization during DPC derivatization (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) European Commission—Directorate-General for Environment. EU Publications: Brussels, 2008.

(2) Postigo, C.; Barceló, D. *Sci. Total Environ.* **2015**, *503*−*504*, 32−47.

(3) Richardson, S. D.; Ternes, T. A. *Anal. Chem.* **2011**, *83*, 4614−4648.

(4) Fenner, K.; Canonica, S.; Wackett, L. P.; Elsner, M. *Science* **2013**, *341*, 752−758.

(5) Schwarzenbach, R. P.; Escher, B. I.; Fenner, K.; Hofstetter, T. B.; Johnson, C. A.; von Gunten, U.; Wehrli, B. *Science* **2006**, *313*, 1072−1077.

(6) Reemtsma, T.; Berger, U.; Arp, H. P. H.; Gallard, H.; Knepper, T. P.; Neumann, M.; Quintana, J. B.; Voogt, P. d. *Environ. Sci. Technol.* **2016**, *50*, 10308−10315.

(7) Kolpin, D. W.; Thurman, E. M.; Linhart, S. M. *Environ. Sci. Technol.* **2001**, *35*, 1217−1222.

(8) Meyer, A. H.; Elsner, M. *Environ. Sci. Technol.* **2013**, *47*, 6884−6891.

(9) Weber, W. H.; Seitz, W.; Schulz, W.; Wagener, H.-A. *Vom Wasser* **2007**, *105*, 7−14.

(10) Reinhardt, M. K.; Kozel, R.; Hofacker, A.; Leu, C. *Aqua Gas* **2017**, *6*, 78−89.

(11) Sturm, S.; Kiefer, J.; Kollotzek, D.; Rogg, J.-M. *gwf Wasser | Abwasser* **2010**, *151*, 950−959.

(12) Grummt, T. P. *Gesundheitliche Orientierungswerte (GOW) für nicht relevante Metaboliten (nrM) von Wirkstoffen aus Pflanzenschutzmitteln (PSM)*; Umweltbundesamt, 2019. https://www.umweltbundesamt.de/dokument/gesundheitliche-orientierungswerte-gow-fuer-nicht.

(13) Umweltbundesamt (UBA), 2004.

(14) Bundesamt für Umwelt (BAFU); National Aquaculture Group (NAQUA). Switzerland, 2013.

(15) Loos, R.; Locoro, G.; Comero, S.; Contini, S.; Schwesig, D.; Werres, F.; Balsaa, P.; Gans, O.; Weiss, S.; Blaha, L.; Bolchi, M.; Gawlik, B. M. *Water Res.* **2010**, *44*, 4115−4126.

(16) Buttiglieri, G.; Peschka, M.; Frömel, T.; Müller, J.; Malpei, F.; Seel, P.; Knepper, T. P. *Water Res.* **2009**, *43*, 2865−2873.

(17) Lingens, F.; Blecher, R.; Blecher, H.; Blobel, F.; Eberspächer, J.; Fröhner, C.; Görisch, H.; Görisch, H.; Layh, G. *Int. J. Syst. Bacteriol.* **1985**, *35*, 26−39.

(18) Thier, H.-P.; Zeumer, H. *Manual of Pesticide Residue Analysis*; VCH Verlagsgesellschaft, 1987; Vol. *I*.

(19) Roberts, M. C., Croucher, L., Roberts, T. R., Hutson, D. H., Lee, P. W., Nicholls, P. H., Plimmer, J. R., Eds.; *Metabolic Pathways of Agrochemicals: Part 1: Herbicides and Plant Growth Regulators*; Royal Society of Chemistry, 2007.

(20) Pesticide Properties DataBase, 2009. *University of Hertfordshire*. https://sitem.herts.ac.uk/aeru/ppdb/en/ (accessed Jan 2019).

(21) Schuhmann, A.; Gans, O.; Weiss, S.; Fank, J.; Klammler, G.; Haberhauer, G.; Gerzabek, M. *J. Soils Sediments* **2016**, *16*, 1032−1045.

(22) Schmidt, T. C.; Zwank, L.; Elsner, M.; Berg, M.; Meckenstock, R. U.; Haderlein, S. B. *Anal. Bioanal. Chem.* **2004**, *378*, 283−300.

(23) Elsner, M.; Imfeld, G. *Curr. Opin. Biotechnol.* **2016**, *41*, 60−72.

(24) Mogusu, E. O.; Wolbert, J. B.; Kujawinski, D. M.; Jochmann, M. A.; Elsner, M. *Anal. Bioanal. Chem.* **2015**, *407*, 5249−5260.

(25) Kujawinski, D. M.; Wolbert, J. B.; Zhang, L.; Jochmann, M. A.; Widory, D.; Baran, N.; Schmidt, T. C. *Anal. Bioanal. Chem.* **2013**, *405*, 2869−2878.

(26) Elsayed, O. F.; Maillard, E.; Vuilleumier, S.; Nijenhuis, I.; Richnow, H. H.; Imfeld, G. *Chemosphere* **2014**, *99*, 89−95.

(27) Alvarez-Zaldívar, P.; Payraudeau, S.; Meite, F.; Masbou, J.; Imfeld, G. *Water Res.* **2018**, *139*, 198−207.

(28) Schürner, H. K. V.; Seffernick, J. L.; Grzybkowska, A.; Dybala-Defratyka, A.; Wackett, L. P.; Elsner, M. *Environ. Sci. Technol.* **2015**, *49*, 3490−3498.

(29) Wu, L.; Yao, J.; Trebse, P.; Zhang, N.; Richnow, H. H. *Chemosphere* **2014**, *111*, 458−463.

DOI: 10.1021/acs.analchem.8b04906
Anal. Chem. 2019, 91, 3412−3420

(30) Reinnicke, S.; Simonsen, A.; Sørensen, S. R.; Aamand, J.; Elsner, M. *Environ. Sci. Technol.* **2012**, *46*, 1447−1454.
(31) Reinnicke, S.; Bernstein, A.; Elsner, M. *Anal. Chem.* **2010**, *82*, 2013−2019.
(32) Maier, M. P.; De Corte, S.; Nitsche, S.; Spaett, T.; Boon, N.; Elsner, M. *Environ. Sci. Technol.* **2014**, *48*, 2312−2320.
(33) Maier, M. P.; Qiu, S.; Elsner, M. *Anal. Bioanal. Chem.* **2013**, *405*, 2825−2831.
(34) Silfer, J. A.; Engel, M. H.; Macko, S. A.; Jumeau, E. J. *Anal. Chem.* **1991**, *63*, 370−374.
(35) Kowal, S.; Balsaa, P.; Werres, F.; Schmidt, T. C. *Anal. Bioanal. Chem.* **2012**, *403*, 1707−1717.
(36) Reemtsma, T.; Alder, L.; Banasiak, U. *J. Chromatogr. A* **2013**, *1271*, 95−104.
(37) Fuhrmann, A.; Gans, O.; Weiss, S.; Haberhauer, G.; Gerzabek, M. H. *Water, Air, Soil Pollut.* **2014**, *225*, 1944.
(38) Elsner, M.; Jochmann, M. A.; Hofstetter, T. B.; Hunkeler, D.; Bernstein, A.; Schmidt, T. C.; Schimmelmann, A. *Anal. Bioanal. Chem.* **2012**, *403*, 2471−2491.
(39) Godin, J.-P.; Fay, L.-B.; Hopfgartner, G. *Mass Spectrom. Rev.* **2007**, *26*, 751−774.
(40) Godin, J. P.; McCullagh, J. S. O. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 3019−3028.
(41) Kuhlmann, F. *Z. Lebensm.-Unters. Forsch.* **1981**, *173*, 35−39.
(42) Presser, A.; Hüfner, A. *Monatsh. Chem.* **2004**, *135*, 1015−1022.
(43) Ranz, A.; Korpecka, J.; Lankmayr, E. *J. Sep. Sci.* **2008**, *31*, 746−752.
(44) *Accuracy (trueness and precision) of measurement methods and results—Part 1: General principles and definitions*; ISO 5725-1; International Organization for Standardization: Geneva, 1994.
(45) Meyer, A. H.; Penning, H.; Lowag, H.; Elsner, M. *Environ. Sci. Technol.* **2008**, *42*, 7757−7763.
(46) Werner, R. A.; Brand, W. A. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 501−519.
(47) Torrentó, C.; Prasuhn, V.; Spiess, E.; Ponsin, V.; Melsbach, A.; Lihl, C.; Glauser, G.; Hofstetter, T. B.; Elsner, M.; Hunkeler, D. *Vadose Zone J.* **2018**, *17*, No. 170033.
(48) Torrentó, C.; Bakkour, R.; Gaétan, G.; Melsbach, A.; Ponsin, V.; Hofstetter, T. B.; Elsner, M.; Hunkeler, D. *Analyst*, submitted for publication, 2018.
(49) Jochmann, M. A.; Blessing, M.; Haderlein, S. B.; Schmidt, T. C. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 3639−3648.
(50) Smith, C. I.; Fuller, B. T.; Choy, K.; Richards, M. P. *Anal. Biochem.* **2009**, *390*, 165−172.
(51) McCullagh, J. S. O. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 483−494.
(52) Zhang, L.; Kujawinski, D. M.; Jochmann, M. A.; Schmidt, T. C. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 2971−2980.
(53) Kujawinski, D. M.; Zhang, L.; Schmidt, T. C.; Jochmann, M. A. *Anal. Chem.* **2012**, *84*, 7656−7663.
(54) Schreglmann, K.; Hoeche, M.; Steinbeiss, S.; Reinnicke, S.; Elsner, M. *Anal. Bioanal. Chem.* **2013**, *405*, 2857−2867.
(55) Hofstetter, T. B.; Berg, M. *TrAC, Trends Anal. Chem.* **2011**, *30*, 618−627.

DOI: 10.1021/acs.analchem.8b04906
Anal. Chem. 2019, 91, 3412−3420