

Supporting Information

¹³C and ¹⁵N isotope analysis of desphenylchloridazon by liquid chromatography isotope ratio mass spectrometry (LC-IRMS) and derivatization-gas chromatography isotope ratio mass spectrometry (GC-IRMS)

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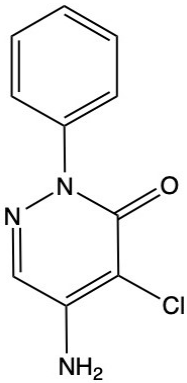
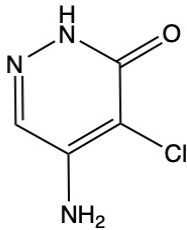
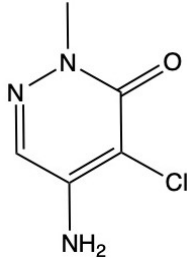
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I. General Information

Table S1: Properties of chloridazon and its metabolites desphenylchloridazon and methyl-desphenylchloridazon ¹⁻⁴. n.a. = not available

	Chloridazon	Desphenylchloridazon	Methyl-desphenylchloridazon
IUPAC Name	5-amino-4-chloro-2-phenylpyridazin-3(2H)-one	5-amino-4-chloro-3(2H)-pyridazine	5-amino-4-chloro-2-methyl-3(2H)-pyridazine
Chemical Structure			
Empirical formula	C ₁₀ H ₈ ClN ₃ O	C ₄ H ₄ ClN ₃ O	C ₅ H ₇ ClN ₃ O
Molecular Weight (g/mol)	221.6	145.55	159.58
Melting Point	205-206 °C	315 °C with sublimation	n.a.
Boiling Point	n.a.	n.a.	n.a.
Solubility in water (mg/L)	422	490	n.a.
pK _a	3.38	9.05	n.a.
GC suitability	Yes	After derivatization	Yes

II. Experimental / Methods

II.1. Peak Identification and Quantification with GC-qMS. The retention time of the target analyte was confirmed with a gas chromatography – quadrupole mass spectrometry (GC-qMS). A 7890A GC was coupled with a 5975C qMS (Agilent, Santa Clara, CA, US). 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 μ m. The instrument was operated with helium carrier gas (grade 5.0) at a flow of 1.4 mL/min. A volume of 1 μ L was injected with a CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland) in splitless mode (injection temperature 250 °C). The GC temperature program, adapted from Kuhlmann ², started at 100 °C and was held for 1 min. Subsequently, the temperature was ramped with 5 °C/min to its final temperature of 240 °C and held for 30 min. Via a heated transfer line of 250 °C, the analyte was transferred into the MS. Ions were generated using an electron impact ionization with an electron-accelerating voltage of 70 V. The MS was operated in scan mode (from m/z 40 to 550). For instrument control and data evaluation, the software ChemStation E.02.02.1431 was used. The data evaluation was carried out using m/z 145 and 159 as qualifier ions for MDPC. The m/z 159 was also used as a quantifier ion.

II.2. Concentration Measurements with UHPLC. Concentrations of CLZ and DPC were determined by ultra-high pressure liquid chromatography quadrupole time of flight mass spectrometry (UHPLC-QTOF-MS). A Synapt G2 Q-TOF mass spectrometer (Waters, Milford, MA, USA) equipped with an electrospray (ESI) probe and coupled to an Acquity UPLC™ system (Waters) was used. A detailed description of the method can be found in Torrentó et al. ⁵. Briefly, the mass spectrometer was operated in positive ionization mode using the MS full scan mode. An Acquity UPLC BEH C18 column (50 mm $\times$ 2.1 mm, 1.7 μ m, Waters) was used, at a flow rate of 0.4 mL/min in gradient mode. A guard column (5 mm $\times$ 2.1 mm, 1.7 μ m) with an identical phase was placed before the column. Water and formic acid 0.05% (solvent A) and acetonitrile and formic acid 0.05% (solvent B) were used as mobile phase, according to the following gradient: 2-65% B in 4.5 min, 65-100% B in 1 min, holding at 100% B for 1.5 min and re-equilibration at 2% B for 1.5 min.

Quantification was performed by the internal standard method, based on peak areas, using terbuthylazine as internal standard. The quantifier ions for CLZ, DPC and MDPC were 222.039, 146.012 and 160.028,

respectively. With this method, the limit of quantification was 28.1 µg/L for DPC, 9.0 µg/L for MDPC and 4.3 µg/L for CLZ.

II.3. Seepage Water Extraction Method Validation with Spiked Samples. The extraction method developed by Torrentó et al.⁵ was used. Briefly, 4 to 10 L water samples were extracted by solid phase extraction (SPE) using cartridges packed with 8 g of the hydrophobic Bakerbond SDB-1 (J.T. Baker) sorbent and 8 g of the hydrophilic Septra ZT (Phenomenex) sorbent. Cartridges were conditioned four times with 15 mL of ethyl acetate (EtAc) followed by four times 15 mL of ultrapure water. Samples were extracted at a flow rate of 5 mL/min. Afterwards, the cartridges were washed four times with 15 mL of ultrapure water and dried under vacuum overnight to remove the excess of water. The eluates were eluted eight times with 15 mL EtAc. The eluates were evaporated until dryness followed by reconstitution with the required volume of ultrapure water for LC-IRMS injections and methanol for derivatization prior to GC-IRMS injection.

Before extracting DPC-containing environmental seepage water samples, the SPE method was validated with 10 L seepage water samples spiked with 1 to 50 µg/L DPC⁵. As shown in Figure S1, an offset of carbon and nitrogen isotope values of the spiked samples from the EA-value is observed. As this offset is constant and also reflected in the standards, it can be corrected accordingly.

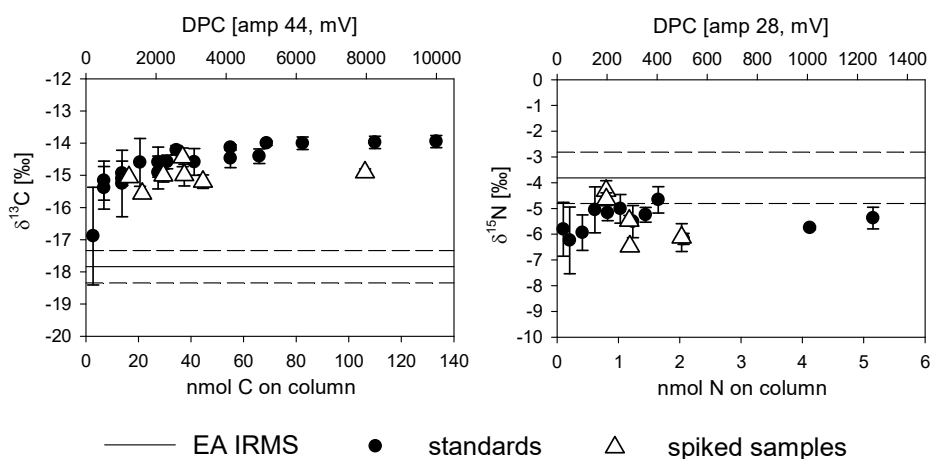


Figure S1: Validation of the SPE method for the determination of carbon (left panel) and nitrogen (right panel) isotope ratios of DPC in 10-L drainage water samples (black circles) spiked with 1 to 50 µg/L DPC. Results of analyzed standards (empty triangles) and the EA/IRMS values (black lines) are also shown. The error bars indicate the standard deviations of quadruplicate (carbon) or triplicate (nitrogen) measurements. The dashed lines represent the interval of the ratios measured by EA/IRMS $\pm 0.5\text{‰}$ for carbon and $\pm 1\text{‰}$ for nitrogen.

II.4. Fractionative HPLC –Sample Clean-Up Method for the Experiment: Evolution of Isotope Ratios of DPC from Different Chloridazon Sources. In contrast to the spiked samples for SPE method validation, the DPC-containing environmental seepage water samples that were spiked with CLZ showed co-eluting interferences in the derivatization GC-IRMS, so that an additional clean-up step was required. Thus, a fractionated HPLC was used after derivatization. Samples were reconstituted in 800 μ L MiliQ water/acetonitrile (90/10) and injected into a Shimadzu UHPLC-DAD (Nexera XR, LC-20AD XR) equipped with a Synergi 4 μ m Hydro-RP 80 $\text{\AA}$ (100 mm x 4.6 μ m; Phenomenex, Aschaffenburg, Deutschland). Thereto, a gradient of 0.1 mM KH_2PO_4 buffer at a pH of 7 and acetonitrile (ACN) was pumped at a flow rate of 1.0 mL/min. The method started at a percentage of 10 % ACN, held for 2 min and increased linearly to 20 % within 4 min. Subsequently, the gradient was increased to 50 % within 3 min and to 75 % within 9 min, held for 2 min, before the proportion of ACN was decreased to 10 % again (held for 5 min). The detector was operated at an absorbance of 210 nm. The derivatized DPC eluted at a retention time of 3 min. Thus, the fraction with the target analyte was collected from 1.75 to 4.10 min. Subsequently, the solvents of both standards and samples were evaporated by freeze-drying and reconstituted in 30 μ L acetone. As shown in Figure S2, no isotope fractionation was induced.

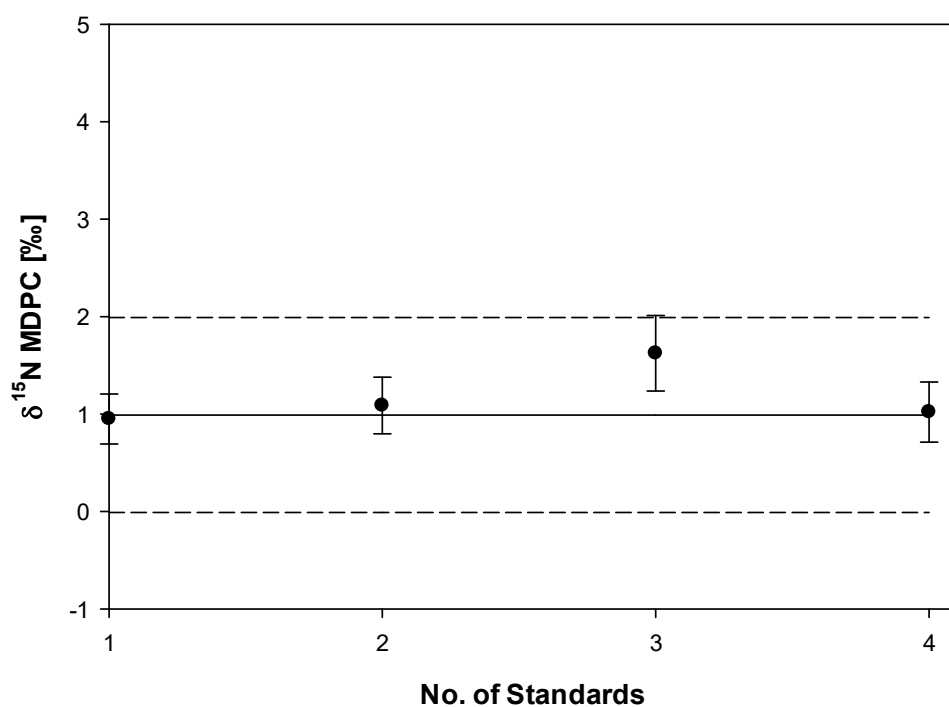


Figure S2: MDPC standards, which were enriched with fractionative HPLC prior GC-IRMS. The black line indicates the referencing value of the standard determined with EA-IRMS, the dashed line shows the limits within the acceptable standard deviation ($\pm 1\%$).

II.5. Fractionative HPLC –Separation of DPC from MDPC in Environmental Samples prior Derivatization.

Within the presented feasibility study, the influence of MDPC was negligible. Thus, the following method has not been applied. However, for samples in which the ratio of DPC to MDPC is greater 10%, fractionative HPLC needs to be used prior to derivatization to separate the two analytes. As the method described previously in II.4. does not separate DPC and MDPC, a new method had to be developed. Therefore, both standards and samples were reconstituted in 800 µL MiliQ water/ACN (99/1) and injected into a Shimadzu UHPLC-DAD (Nexera XR, LC-20AD XR). For peak separation, a Synergi 4 µm Hydro-RP 80 Å (100 mm x 4.6 µm; Phenomenex, Aschaffenburg, Deutschland) was used at a temperature of 35 °C. The mobile phase consisted of a 0.5 mM KH₂PO₄ buffer at a pH of 7 and ACN and pumped at a flow rate of 1.0 mL/min. A gradient method was used starting at a percentage of 1 % ACN, held for 2 min. Then, the ACN was increased linearly to 10 % within 4 min. The gradient was then increased to 50 % within 3 min. Finally, the ACN was increased linearly to 75 % within 9 min, held for 2 min. Before the next run, the proportion of ACN was decreased to 1 % again (held for 5 min). The absorbance of the detector was set to 210 nm. DPC eluted at a retention time of 4.2 min, so its fraction was collected from 1.8 min to 7.0 min. MDPC was retarded for 7.7 min. Thus, the fraction containing MDPC was collected from 7.0 min to 11.0 min. Afterwards, the ACN/water mixture of these fractions were evaporated by freeze-drying. The fractions containing MDPC were reconstituted in 50 µL acetone, while the fractions containing DPC, were dissolved in 1 mL methanol as preparation for the derivatization procedure. The standard measurements of DPC and MDPC (Figure S3) show that no isotope fractionation was induced.

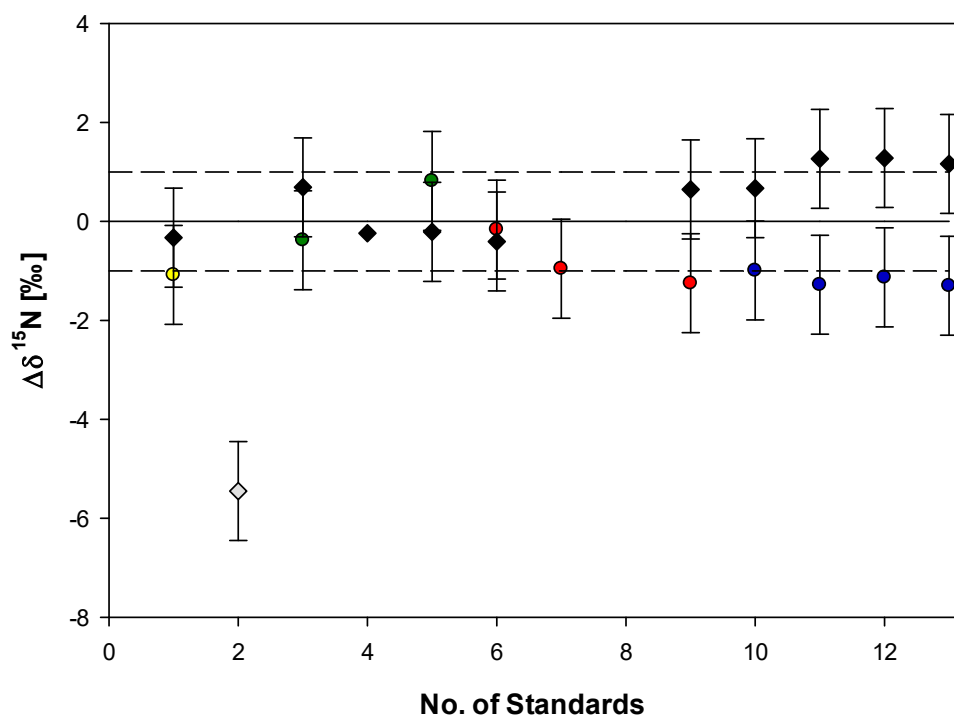


Figure S3: Derivatized DPC standards (circles) and MDPC standards (black diamonds), which were enriched with fractionative HPLC prior derivatization GC-IRMS. The different colors of circles represent different derivatization and measurement days. The black line indicates the referencing value of the standard determined with EA-IRMS, the dashed line shows the limits within the acceptable standard deviation ($\pm 1\%$)

III. Results and Discussion

Table S2: Isotope Ratios of ^{13}C and ^{15}N of selected compounds used for isotope correction determined by EA-IRMS

Standard	$\delta^{13}\text{C} \pm \text{SD} [\text{‰}]$ n = 5	$\delta^{15}\text{N} \pm \text{SD} [\text{‰}]$ n = 5
Desphenylchloridazon	-17.84 ± 0.05	-3.81 ± 0.04
Methyldesphenylchloridazon	-21.17 ± 0.06	$+0.99 \pm 0.12$
Desethylatrazine	-32.08 ± 0.09	-9.42 ± 0.08
Acetochlor	-25.00 ± 0.06	$+0.46 \pm 0.09$

Table S3: Round Robin Test of Isotope Ratios of DPC determined by EA-IRMS

Standard	$\delta^{13}\text{C} \pm \text{SD} [\text{‰}]$ n = 5	$\delta^{15}\text{N} \pm \text{SD} [\text{‰}]$ n = 5
HMGU Laboratory 1	-17.84 ± 0.05	-3.81 ± 0.04
HMGU Laboratory 2	-17.93 ± 0.09	-3.78 ± 0.12
ETH Zurich	n.a.	-3.64 ± 0.27

III.1. LC-IRMS Chromatogram of DPC

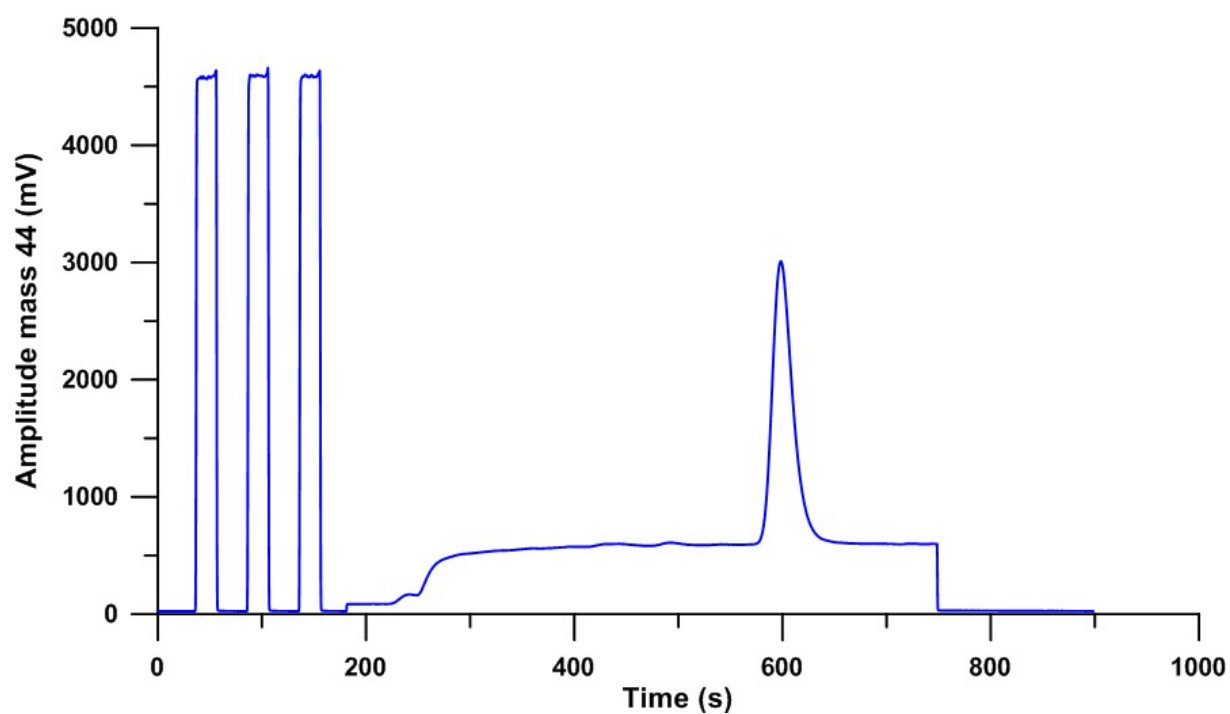


Figure S4: LC-IRMS chromatogram of a DPC standard (27.5 nmol C on column) showing the trace for mass 44.

III.2. Temperature optimization during DPC derivatization. Figure S5 shows the application of TMSD excess applied to 250 mg/L DPC solved in methanol in relation to the resulting peak area ratio (PAR) at a temperature of 50 °C and 70 °C. BAM (250 mg/L) dissolved in methanol was used as an internal standard. The PAR is calculated by applying the following equation:

$$\text{PAR} = \frac{\text{Peak Area (Target Analyte)}}{\text{Peak Area (Internal Standard)}} \quad (\text{S1})$$

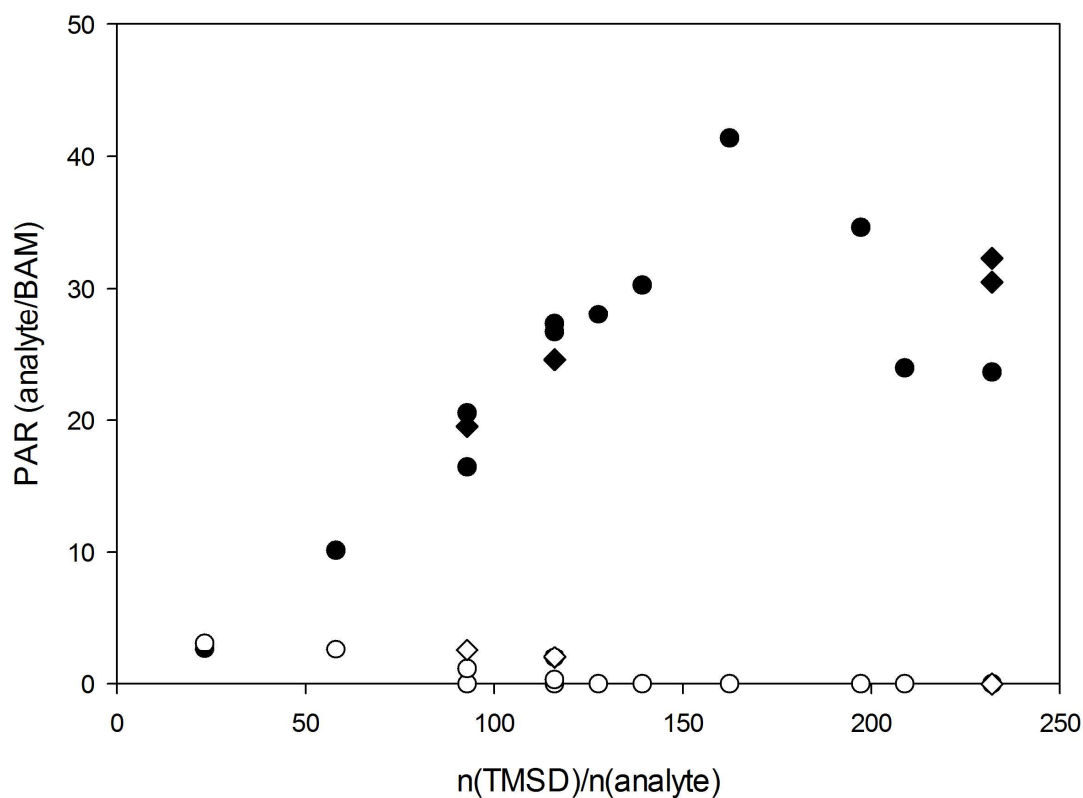


Figure S5: Derivatization of 250 mg/L DPC with varying excess of TMSD at 50 °C (diamonds) and 70 °C (circles). The black symbols shows the PAR of derivatized DPC in relation to BAM (internal standard), while the white symbols represent the PAR of the remaining fraction of DPC in relation to BAM

185 **Table S4: Isotope Ratios of ^{13}C and ^{15}N of commercially available chloridazon products determined by EA-IRMS**

Producer	$\delta^{13}\text{C} \pm \text{SD} [\text{‰}]$ n = 5	$\delta^{15}\text{N} \pm \text{SD} [\text{‰}]$ n = 5
Dr. Ehrenstorfer	-24.65 $\pm$ 0.04	-22.22 $\pm$ 0.03
Sigma Aldrich	-23.37 $\pm$ 0.03	-32.04 $\pm$ 0.05
Neochema	-24.67 $\pm$ 0.04	-22.17 $\pm$ 0.05
Oskar Tropitzsch	-27.43 $\pm$ 0.02	-5.70 $\pm$ 0.03
Chemos	-21.82 $\pm$ 0.03	-31.49 $\pm$ 0.99

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187 **Table S5: Initial composition of seepage water used for the experiment: Evolution of Isotope Ratios of DPC from Different**
188 **Chloridazon sources**

Compound	Concentration [$\mu\text{g/L}$]
CLZ (source A)	< 0.02
DPC (from degraded CLZ A)	10.5
MDPC	< 0.05
Atrazine	10
Desethylatrazine	10
Acetochlor	10
Metolachlor	10
2,6 Dichlorobenzamid	10

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190 **Table S6: Concentration analytes in the seepage water used for the two-source mixing model**

Time after CLZ application	Sample ID	Concentration CLZ [nmol/L]	Concentration DPC [nmol/L]	Concentration MDPC [nmol/L]
0 months	t_0	136.0	81.7	<0.3
7 months	t_1	88.7	120.9	0.5
11 months	t_2	0.0	163.5	0.5

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192 IV. References Supporting Information

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