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Spatial Distribution and Characterization of Long-Term Aged ^{14}C-Labeled Atrazine Residues in Soil

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The long-term behavior of the herbicide atrazine and its metabolites in the environment is of continued interest in terms of risk assessment and soil quality monitoring. Aqueous desorption, detection, and quantification of atrazine and its metabolites from an agriculturally used soil were performed 22 years after the last atrazine application. A lysimeter soil containing long-term aged atrazine for >20 years was subdivided into 10 and 5 cm layers (at the lysimeter bottom: soil 0−50 and 50−55 cm; fine gravel 55−60 cm depth, implemented for drainage purposes) to identify the qualitative and quantitative differences of aged ^{14}C-labeled atrazine residues depending on the soil profile and chemico-physical conditions of the individual soil layers. Deionized water was used for nonexhaustive cold water shaking extraction of the soil. With increasing soil depth, the amount of previously applied ^{14}C activity decreased significantly from 8.8% to 0.7% at 55−60 cm depth whereas the percentage of desorbed ^{14}C residues in each soil layer increased from 2% to 6% of the total ^{14}C activity in the sample. The only metabolite detectable by means of LC-MS/MS was 2-hydroxyatrazine while most of the residual ^{14}C activity was bound to the soil and was not desorbed. The amount of desorbed 2-hydroxyatrazine decreased with increasing soil depth from 21% to 10% of the total desorbed ^{14}C residue fraction. The amount of ^{14}C residues in the soil layers correlated well with the carbon content in the soil and in the aqueous soil extracts (p value = 0.99 and 0.97, respectively), which may provide evidence of the binding behavior of the aged atrazine residues on soil carbon. The lowest coarse layer (55−60 cm) showed increased residual ^{14}C activity leading to the assumption that most ^{14}C residues were leached from the soil column over time.

KEYWORDS: Atrazine; hydroxyatrazine; metabolites; LC-MS/MS; aged residues; desorption; organic carbon

INTRODUCTION

Since its introduction in 1958, and because of its worldwide use, the herbicide atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] (**Figure 1A**) has become relevant for investigations concerning soil and groundwater contamination (*1*). Even though its use has been banned in several countries, atrazine has been estimated to be the most heavily used pesticide, particularly in the USA, where 32 000 to 34 000 t were applied for agricultural purposes in 1993 (*2*).

Numerous investigations show that atrazine and/or its metabolites are widely present in groundwater, rivers, and sediments (*3, 4*). This finding led to approaches to decontaminate these environments by bioremediation using atrazine-degrading organisms (*5*−*8*).

After application, atrazine is subject to several dissipation and physical and chemical degradation pathways. Most losses are due to runoff, infiltration, adsorption, and biological

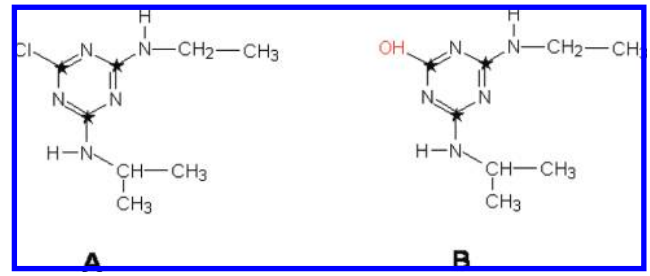

Figure 1. Structural formula of atrazine **(A)** and 2-hydroxyatrazine **(B)**. ^{14}C-labeled atrazine was uniformly ring-labeled (as indicated).

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Table 1. Residual ^{14}C-Atrazine Activity in the Soil[a]

soil layer depth (cm)	^{14}C activity (Bq g^{-1})	C_{org} (%)	C_{anorg} (%)	C_{total} (%)	Al (%)	Fe (%)	K (%)	Mg (%)	Ca (%)	Na (%)
0−10	136.95 ± 5.31	1.450 ± 0.008	0.032 ± 0.001	1.482	4.68	2.26	1.44	0.49	0.45	0.70
10−20	85.63 ± 3.96	0.924 ± 0.004	0.023 ± 0.005	0.947	4.75	2.28	1.41	0.50	0.45	0.69
20−30	31.42 ± 1.55	0.626 ± 0.005	0.010 ± 0.002	0.635	5.44	2.68	1.51	0.60	0.50	0.70
30−40	18.27 ± 0.87	0.424 ± 0.005	0.006 ± 0.001	0.430	6.20	3.26	1.59	0.72	0.49	0.66
40−50	13.42 ± 0.79	0.357 ± 0.002	0.007 ± 0.001	0.364	6.23	3.29	1.69	0.82	0.89	0.73
50−55	11.14 ± 0.78	0.350 ± 0.002	0.020 ± 0.002	0.371	6.23	3.27	1.65	0.74	0.53	0.72
55−60	93.25 ± 5.61	1.137 ± 0.012	7.017 ± 0.005	8.153	2.12	1.63	0.56	4.02	16.18	0.25

[a] Activity ± mean standard deviation ($n = 3$). Values for carbon, aluminum, iron, potassium, magnesium, calcium, and sodium are in %. ± mean standard deviation ($n = 3$). The mean standard deviation of three replicates for Al, Fe, K, Mg, Ca and Na is ±3%.

degradation (*9*). One of the predominant atrazine metabolites is hydroxyatrazine [2-ethylamino-4-hydroxy-6-(isopropylamino)-*s*-triazine] (**Figure 1B**), resulting from abiotic and/or biotic degradation pathways via hydrolysis and/or microbial degradation in soils (*10, 11*). Hydroxyatrazine is thought to be less mobile than atrazine or other atrazine metabolites and is likely associated as a bound residue to the soil matrix (*12, 13*). As stated by Johnson et al. (*14*), contaminants like atrazine are not irreversibly bound, resulting in slow leaching of this substance to deeper soil and water layers. However, it is generally accepted that the formation of bound residues, generally resulting in less mobility or extractability of the pesticide in soil, is dependent on contact time (*15*−*17*). Soil constituents, particularly organic carbon, have an important influence on atrazine sorption processes (*18, 19*). Both atrazine and its metabolites represent potential hazards for groundwater contamination. As reported by USEPA in 1990 (*20*), the amount of metabolites detected in well waters was greater than that of the parent atrazine; the amount of metabolites exceeded the U.S. recommended health advisory limit of 3.0 μg L^{-1} in 75% of documented cases, which is considerably higher than the European advisory limit of 0.1 μg L^{-1} (*21*).

A noticeable number of investigations concerning atrazine and its metabolites have been undertaken, but as yet little is known about the long-term behavior of this widely applied herbicide. Therefore, investigations of the long-term behavior of atrazine are of continued importance because this herbicide is still widely used throughout the world. Since the present study deals with the desorption capability of ^{14}C-labeled atrazine residues naturally aged for a long period (>20 years), it may provide important additional information concerning soil and potentially groundwater risk assessment.

The overall objective of this research was to detect, characterize, and quantify water-desorbable atrazine residues in different layers of an agriculturally used soil after 22 years of natural aging. Characteristics of ^{14}C-labeled atrazine residues in soil aged under outdoor agricultural conditions have not been reported to date. A further aim was to develop an easy analytical methodology to detect these residues after nonexhaustive cold water shaking extraction as the experimental part of a soil aggregate fractionation via liquid chromatography tandem mass spectrometry (LC-MS/MS).

MATERIALS AND METHODS

Soil Characteristics and Analysis. The experimental soil originated from a long-term lysimeter study used for maize conducted at the Bavarian State Research Center for Agriculture (LfL), Munich, Germany. The soil was a gleyic cambisol (18% sand, 64.4% silt, 17.6% clay; organic carbon content, see **Table 1**) originating from Puch, Fürstenfeldbruck, in Bavaria, Germany. The plastic lysimeter (dimensions 49 × 49 × 73 cm, with a surface area of 0.24 m^2) was filled in 1979 in accordance with the natural soil layers of the field of origin. Since 1982, three consecutive applications of uniformly ^{14}C-ring-labeled atrazine and nonlabeled atrazine were performed. The time and amount of application are presented in **Table 2**. The amount of atrazine at each application was approximately 1.7 kg ha^{-1}, corresponding to the recommended agricultural application dose of 1.7−2.8 kg ha^{-1}. The atrazine and ^{14}C-ring-labeled atrazine with a chemical purity of 99.7% were purchased from the former Ciba-Geigy. The lysimeter soil was solely used for annual maize cultivation with minimum tillage until August 2005. No plowing simulation was performed. After maize harvesting, crop residues were removed manually.

Table 2. Time of Application, Applied Amounts of Nonradioactive and Radioactive Atrazine As Active Compound, and Applied Radioactivity[a]

time of application	applied atrazine (mg)	applied ^{14}C-atrazine (mg)	specific ^{14}C activity (kBq mg^{-1})	applied ^{14}C activity (MBq)
1983	38.931	4.382	4271.109	18.716
1984	41.283	4.327	4272.013	18.485
1985	39.924	4.447	4271.194	18.994
total (133.294 mg)	120.138	13.156		56.195
new specific ^{14}C activity		421.587 kBq mg^{-1}	atrazine	

[a] Table first presented in ref *23*.

Inductively coupled plasma optical emission spectroscopy (ICP-OES; TJA-IRIS-Intrepid spectrometer, Thermo) was applied for the physicochemical analysis of the soil minerals Al, Fe, K, Mg, Ca, and Na. Analysis of the soil carbon was conducted by radiofrequency heating in flowing oxygen and infrared absorption by a Leco RC-412 multiphase carbon determinator.

Soil Analysis of ^{14}C Residues. For the detection of residual ^{14}C activity, aliquots of the homogenized soil of each soil layer were freeze-dried using a Lyovac GT2, Steris, pulverized in a mortar, and combusted via a Biological Oxidizer OX500, from R. J. Harvey Instrument Corp. Emerging $^{14}CO_2$ was trapped in Oxysolve C-400 oxidizer scintillation cocktail, Zinsser Analytic. The samples were analyzed by a liquid scintillation counter using an LSC, 2500 TR, Tri-Carb, Packard liquid scintillation analyzer.

Liquid Sample Preparation and Analysis. Aqueous samples for analysis were obtained as a result of a gentle cold water soil-aggregate fractionation, previously described by Séquaris and Lewandowski (*22*).The soil-aggregate fractionation was applied in a previous study (*23*) resulting in an aqueous DOM fraction (DOM = dissolved organic matter) of each soil layer which was used for analysis. To obtain this aqueous fraction, triplicates of 100 g of dry soil equivalents from each soil layer were transferred into 1000 mL Duran glass bottles. In a first step, 200 g of organic-free deionized Millipore water was added (Milli-Q Plus 185, Millipore purification system) and the soil−water mixture was shaken for 6 h at 150 rpm (Horizontal Shaker SM 25, Edmund Büler). Subsequently, 600 g of deionized water was added to make a total amount of 800 g and the solution was additionally shaken for 1 min. After consecutive sedimentation steps, the remaining liquid was transferred into centrifuge tubes and was centrifuged at 10 000*g* for 90 min (Beckman J2−21, Rotor JA 14). Subsamples of 5 mL aqueous DOM fraction were analyzed for desorbed residual ^{14}C activity in triplicates via LSC using an external standard (2500 TR, Tri-Carb, Packard liquid scintillation analyzer). The dissolved total organic carbon (TOC) and nonpurgeable organic carbon (NPOC) content was measured

Table 3. Residual ^{14}C-Atrazine Activity, pH, Total Organic Carbon (TOC), and Nonpurgeable Organic Carbon (NPOC) Content in the DOM Fraction[a]

DOM fraction of soil layer depth (cm)	^{14}C activity (Bq mL^{-1})	pH	TOC (mg L^{-1})	NPOC (mg L^{-1})
0−10	0.33 ± 0.011	5.89 ± 0.04	4.80 ± 0.11	4.46 ± 0.23
10−20	0.21 ± 0.009	6.56 ± 0.37	3.30 ± 0.17	2.55 ± 0.17
20−30	0.09 ± 0.005	6.99 ± 0.06	2.56 ± 0.88	2.20 ± 0.79
30−40	0.09 ± 0.006	7.25 ± 0.11	2.67 ± 0.28	2.94 ± 0.21
40−50	0.07 ± 0.005	7.43 ± 0.00	1.90 ± 0.29	1.69 ± 0.12
50−55	0.07 ± 0.005	7.79 ± 0.01	2.68 ± 0.12	3.72 ± 1.21
55−60 soil particles	0.34 ± 0.010	8.88 ± 0.00	4.98 ± 0.47	1.93 ± 0.14
55−60 gravel	0.20 ± 0.009	8.69 ± 0.19	3.70 ± 0.39	1.14 ± 0.09

[a] ± mean standard deviation of nine replicates.

Table 4. Gradient Program Conditions Applied for the HPLC Associated with LC-MS/MS

time (min)	liquid gradient	
	eluent A 0.1 M ammonium acetate solution (%)	eluent B acetonitrile (%)
0	83	17
5	83	17
20	0	100
25	0	100
30	83	17
40	83	17

prior to further treatments (Shimadzu total organic carbon analyzer, TOC-5050A, Shimadzu, ASI-5000A auto sampler). Results for desorbed ^{14}C activity, pH, and carbon content are given in **Table 3**. The remaining DOM fraction was concentrated by freeze-drying to a residual volume of 1−3 mL prior to further LC-MS/MS analysis. Samples were transferred into 2 mL Eppendorf cups and centrifuged for sedimentation of the particles for 5 min at 15 000*g* (Hettich Mikro Rapid). Residual particles of the concentrated DOM fractions in the bottle used for freeze-drying were dissolved in 2 mL of pure methanol (HPLC grade) and treated as above.

LC-MS/MS Analysis. LC-MS/MS was the method of choice for characterizing and quantifying the nature of the desorbed atrazine residues since previous UV-HPLC (Dionex, pump M480, sampler Gina 50, UV detector UVD 3405) and radio-HPLC (Berthold Radioflow Detector LB 590, Jasco UVD 2075 detector, solid scintillation cell YG 150 U4, pump 1580, Gina 50 sampler) analysis showed no results. The limit of quantification for radio-HPLC was 40 Bq mL^{-1}, and for UV-HPLC 0.1 mg L^{-1}, respectively.

The LC-MS/MS analysis was performed using a Thermo Electron Model TSQ-Quantum 2002 equipped with CTC-HTC-PAL sampler, and HPLC (Agilent) with binary pump and thermostated column compartment (Agilent Serie 1100). Atrazine (chemical purity 97.4%) and its metabolites such as desethyldesisopropyl-2-hydroxyatrazine (98.5%), desisopropyl-2-hydroxyatrazine (99.0%), desethyldesisopropylatrazine (98.0%), desethyl-2-hydroxyatrazine (99.0%), desethylatrazine (99.9%), desisopropylatrazine (96.1%) and 2-hydroxyatrazine (96.0%) were purchased from Riedel-de Haën.

First, a compound separation was obtained by HPLC (**Table 4**). For lower detection limits the fragmentation of atrazine and its metabolites was studied and the most intensive fragmentation in each case was selected. All the transitions were measured in parallel, which is known as MRM (multiple reaction monitoring).

Since 2-hydroxyatrazine was the only metabolite to be detected, deuterated (D_5)-2-hydroxy-atrazine (Dr. Ehrenstorfer GmbH, Germany) with a concentration of 0.01 μg mL^{-1} was used as internal standard for quantification. From each sample 100 μL was mixed with 100 μL of D_5 STD standard solution resulting in 0.001 μg 100 μL^{-1} of injected sample. Perfect Sil Target ODS-3 3 μm was used as the solid phase 125 mm in length, and 2.1 mm inner diameter. An additional HPLC precolumn 1 cm in length (2.1 mm × 10 mm × 3 μm) was applied. As HPLC eluent, a mixture of acetonitrile (Riedel-de Haën, 99.9% purity) and 0.1 M ammonium acetate solution was used, in accordance with Takáts et al. (*11*) (**Table 4**). The flow rate was 0.15 mL min^{-1} at 25 °C column temperature. The injection was performed in triplicate and the total injection volume of each sample was 5 μL. For LC-MS/MS analysis the positive electrospray ionization mode (ESI+) was applied. All the settings for the analysis and the mass transfer of all possible atrazine metabolites are given in **Table 5**. The limit of detection was 0.125 ng mL^{-1} for atrazine and 2-hydroxyatrazine.

Table 5. Conditions Applied for LC-MS/MS Analysis of Associated ^{14}C-Labeled Atrazine Residues in Liquid Samples[a]

MS Conditions					
ESI	polarity ESI positive	MRM mode	scan width 0.2 (Da)	scan time 0.4 s	peak width (fwhm) 0.7

Tune File Parameters				
spray voltage	sheath gas	aux gas	ion transfer	collision cell
4500 (V)	pressure 45 (psi)	pressure 10 (arbitrary units)	capillary 230 °C	pressure 1 (mTorr)

parent mass (Da)	product mass (Da)	collision energy (V)	tube lens (V)	molecule	retention time (min)
128	86.2	20	155	desethyldesisopropyl-2-hydroxyatrazine	4.46
156	69.1	36	209	desisopropyl-2-hydroxyatrazine	4.75
146	104.0	24	186	desethyldesiso-propylatrazine	5.78
170	128.1	22	207	desethyl-2-hydroxyatrazine	5.45
188	146.1	24	180	desethylatrazine	17.19
174	104.1	28	178	desisopropylatrazine	10.46
198	156.1	24	198	2-hydroxyatrazine	15.48
216	174.1	24	189	atrazine	21.8
203	161.2	26	192	D5-standard 2-hydroxyatrazine	15.21

[a] Abbreviations: ESI, electrospray ionization; MRM, multiple reaction monitoring; Da, dalton; FWHM, full width at half-maximum; V, voltage.

RESULTS AND DISCUSSION

Chemicophysical and ^{14}C Activity Analysis: Soil. Results of the originally applied ^{14}C-ring-labeled atrazine and the physicochemical characteristics of each soil layer are given in **Table 1**.

In total, 25% of the applied ^{14}C activity is still present in the lysimeter soil. The overall loss of ^{14}C activity must be attributed to a combination of the processes of plant uptake, mineralization, volatilization, and leaching. The major fraction of the residual ^{14}C activity of the originally applied ^{14}C-labeled atrazine was detected in the upper 0−10 cm of the soil, representing 8.8% of the previously applied ^{14}C activity (**Figure 2**). These results correspond to previous studies where most residual atrazine was found in the upper soil layer, but after much shorter periods of aging time (*24*−*26*). With increasing soil depths, the residual ^{14}C activity decreased to 0.7% of that originally applied (soil layer 50−55 cm, see **Figure 2**). The deepest layer (55−60 cm), consisting of fine gravel (drainage purposes) and intruded soil and root detritus, showed an increased ^{14}C activity of 6% (**Figure 2**). The results of residual ^{14}C activity are highly correlated with the organic carbon content in the corresponding soil layer (p value = 0.99; for data see **Table 1**). These results correspond to previous studies giving clear indications that organic carbon in soil is a long-term sink for atrazine and/or its metabolites (*15*). Capriel et al. (*25*) demonstrated that most of the herbicide residues were associated with soil organic matter. It can be assumed that >20 years after the last application of ^{14}C-labeled atrazine the soil-associated ^{14}C-labeled atrazine residues have reached a distribution equilibrium. Therefore,

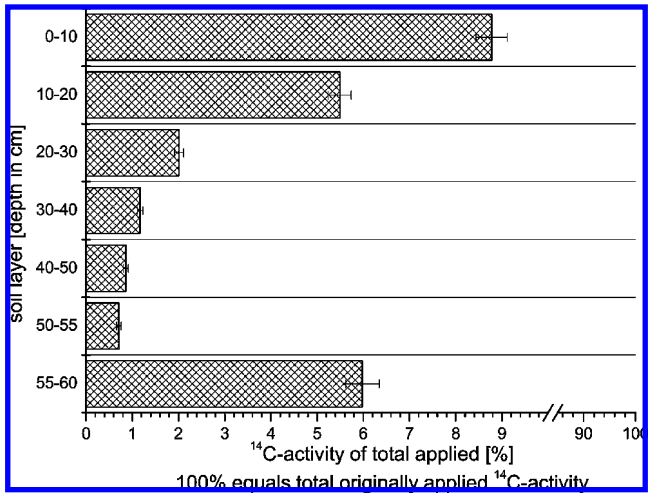

Figure 2. Detected residual atrazine-^{14}C activity in the different soil layers (oxidizer, LSC). Error bars indicate mean standard deviation of $n = 3$. Presented data have previously been published in ref *23*.

these results identify the long-term environmental behavior of the herbicide atrazine and/or its metabolites in an agricultural soil, indicating that most residues are still located in the upper soil layer. As reported by Pignatello et al. (*27*), environmental wet−dry cycles may cause pulse inputs to the subsurface from the resistant herbicide pool. Less organic matter in the subsurface may subsequently cause higher mobility of the residues, and microbial degradation is lower due to limited microbial populations. Since the lowest soil layer functioning as a drainage layer showed similar residual ^{14}C activity to the first 10 cm, it is likely that the major fraction of previously applied atrazine disappeared by leaching. The accumulation of the residual atrazine-^{14}C activity in this soil layer was mainly associated with organic plant detritus and an accumulation of clay-sized aggregates.

Chemicophysical and ^{14}C Activity Analysis: Liquid Fraction. The liquid soil−water fraction or DOM fraction resulted from a previously applied gentle physical soil aggregate fractionation (*23*). The total soil−water contact time was 24 h, including 6 h of rigorous shaking of the mixture. The ^{14}C activity of desorbed ^{14}C residues in the liquid was determined via LSC. The results of desorbed ^{14}C activity are given in **Figure 3**. Results were calculated for 1 L. The pattern of desorbed ^{14}C activity corresponds to the residual activity in the soil matrix, shown in **Figure 2**. The amount of detected ^{14}C-labeled residues decreases from 0.8 to 0.2 μg L^{-1} of sample liquid. This corresponds to 2 and 6% of the ^{14}C activity present in the soil sample, respectively (**Figure 4**). With increasing soil depth the desorbable fraction of ^{14}C residues increased clearly. These results may provide evidence that ^{14}C-labeled atrazine residues in deeper soil layers are potentially less tightly bound and hence more mobile, reaching deeper soil layers by continuous leaching. Herewith naturally occurring desorption processes of atrazine residues from soil and mobilization processes can be assessed. The increase of the desorption capability of ^{14}C-labeled atrazine residues with increasing soil depth is positively correlated with the soil organic carbon content (p value = 0.95) as well as with the TOC content in the DOM fraction (p value = 0.97). This shows the overall relevance of organic carbon as a binding reactant in solid and liquid phases for atrazine and/or its metabolites.

LC-MS/MS analysis. Since the results given in **Figures 3** and **4** represent the quantity of desorbed ^{14}C activity detected via LSC, LC-MS/MS analysis was applied in order to characterize the nature of the desorbed ^{14}C-labeled atrazine residues. LC-

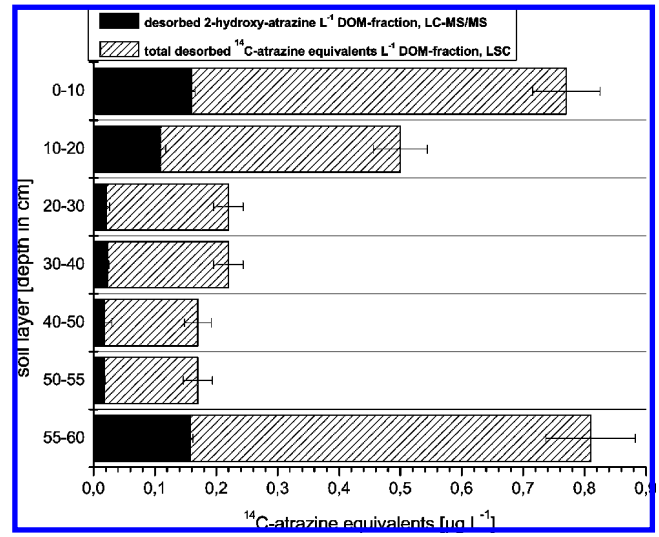

Figure 3. Amounts of total desorbed ^{14}C-atrazine equivalents in μg L^{-1} DOM fraction, measured via LSC detection and desorbed 2-hydroxyatrazine in μg L^{-1} DOM fraction, detected via LC-MS/MS. Error bars indicate mean standard deviation of $n = 9$.

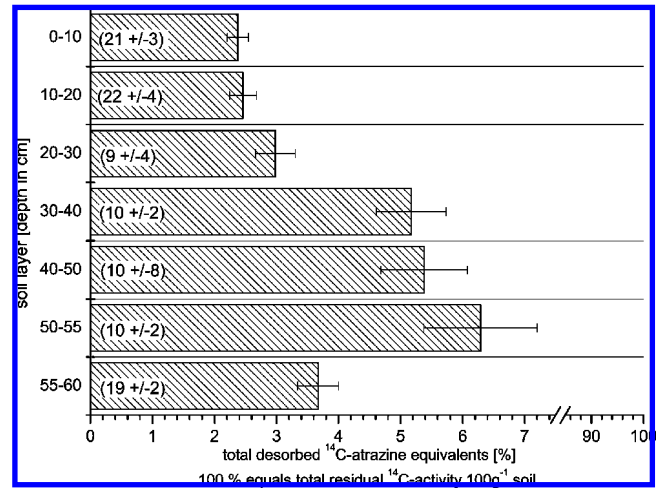

Figure 4. Total desorbed ^{14}C-atrazine equivalents in % of total ^{14}C activity in the sample. 100% equals total residual ^{14}C activity 100 g^{-1} soil of the individual soil layer. Numbers in parentheses indicate the percentage of 2-hydroxyatrazine of total desorbed. Error bars indicate mean standard deviation of $n = 9$.

MS/MS had been successfully applied in previous studies to detect and characterize atrazine and its metabolites (*11, 28, 29*). Liquid samples were analyzed for all MRM transitions.

LC-MS/MS chromatograms for atrazine, 2-hydroxyatrazine and other atrazine metabolites are shown in **Figure 5**, with retention times of 20.99 and 16.67 min for atrazine and 2-hydroxyatrazine, respectively. Atrazine and atrazine-metabolite standard solution were added to noncontaminated soil prior to aqueous shaking extraction. As a control, this figure demonstrates the clear determination of added atrazine and its metabolites via LC-MS/MS analysis applied as a standard mixture in the aqueous fraction.

The environmental behavior of hydroxyatrazine is a matter of controversy. As reported by Brouwer et al. (*30*) and Sorenson et al. (*16*), hydroxyatrazine shows high adsorption and low desorption in soil, suggesting that at depths greater than 20 cm hydroxyatrazine is due to in situ degradation of atrazine previously translocated to those depths. However, the amount of desorbed 2-hydroxyatrazine from subsamples of each soil

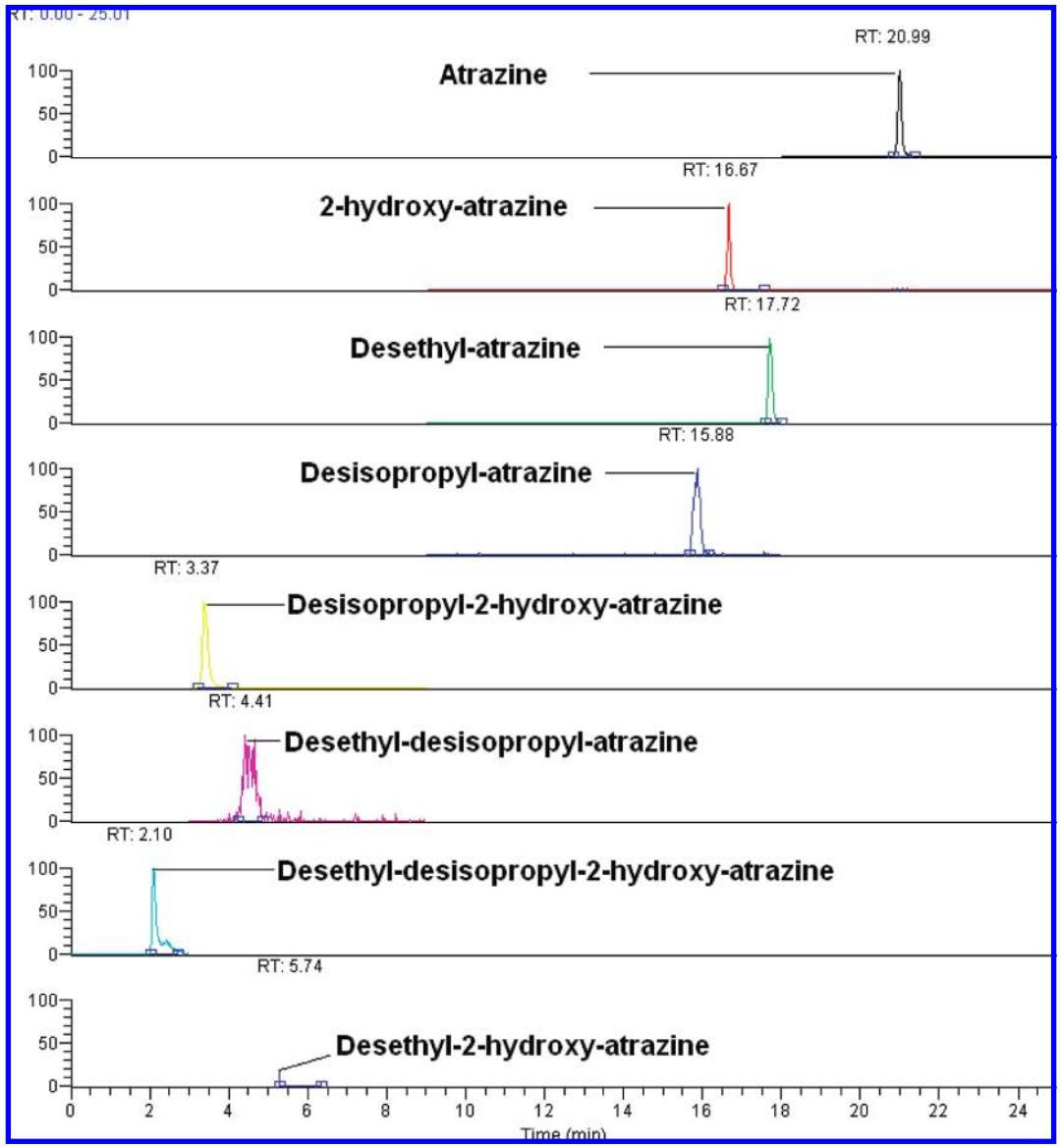

Figure 5. LC-MS/MS chromatograms showing the mass scale of all metabolite fragments.

layer given in **Figure 3** ranges from 0.16 in the upper layer to 0.017 μg L^{-1} DOM-fraction in the 50−55 cm soil layer. From the lowest coarse layer at 55−60 cm the amount desorbed was 0.16 μg L^{-1} DOM fraction and was therefore similar to soil from the top layer. As given in **Figure 4**, the amounts desorbed represent 21% of the total desorbed ^{14}C activity in the two upper soil layers and 10% in the 50−55 cm soil layer, respectively. The amount of 2-hydroxyatrazine with respect to the total desorbed ^{14}C activity from samples of the intermediate soil layers (20−55 cm depth) was around 10%. These results are in accordance with those obtained by Mahía et al. (*31*) and Lerch et al. (*32*) showing that hydroxyatrazine was the main long-term metabolite found in soil. It is unclear whether these results are due to the previously mentioned in situ transformation of atrazine in deeper soil layers or due to direct leaching of hydroxyatrazine from the surface layer. But the accumulation in the 55−60 cm layer underlines the leaching character of 2-hydroxyatrazine. The major fraction of noncharacterized ^{14}C activity in the liquid is due to nondetectable quantities of other atrazine metabolites and/or represents non-substance-specific ^{14}C. These ^{14}C compounds resulting from the degradation of the ^{14}C-labeled atrazine are likely associated with the dissolved organic carbon pool, since the amount of ^{14}C activity and DOM concentration is very well correlated (p value = 0.97).

2-Hydroxyatrazine is considered to be the main atrazine metabolite occurring in a first degradation step (*11*), due to biotic and/or abiotic factors. Kruger and Coats (*33*) showed that hydroxyatrazine was equally persistent in surface and subsurface soils. Under conditions of groundwater contamination, the order of leaching potential determined by Schiavon (*34*), desethylatrazine > atrazine > desethyldesisopropylatrazine > deisopropylatrazine > hydroxyatrazine, might be questionable since in the study presented here only water was used to desorb the aged atrazine residues; these residues resulted from the originally applied atrazine as the only parent compound. Since 2-hydroxyatrazine was found to be the only water-desorbable metabolite, with a high concentration detected in the lowest layer (**Figures 3** and **4**), this indicates a considerable leaching potential in soil. This assumption contradicts the results presented by Sorenson et al. (*16*) and Clay and Koskinen (*35*) where hydroxyatrazine is tightly adsorbed to the soil and could not be easily leached.

Nondesorbable ^{14}C activity originating from ^{14}C-labeled atrazine residues may be due to the parent compound or

metabolites, corresponding to the assumption by Clay and Koskinen (*36*) after a desorption study of atrazine from soil. Since Capriel et al. (*25*) demonstrated the long-term persistence of atrazine in soil, it could be assumed that these compounds might represent a long-term source of soil pollutants and might be leached to deeper soil layers by DOM- or soil-particle-associated transport processes (*17*). However, the fate and leaching of atrazine and/or its metabolites is mainly due to soil conditions and carbon contents less than the water solubility of these pesticide compounds (*37*−*40*). In conclusion, atrazine and/or hydroxyatrazine as its main metabolite are persistent in soil, even in trials being managed similar to agricultural practice under outdoor conditions. These data derived from such a long-term experiment will be very valuable for future hazard assessment strategies.

ABBREVIATIONS USED

(1) LC-MS/MS, liquid chromatography tandem mass spectrometry; (2) ICP-OES, inductively coupled plasma optical emission spectroscopy; (3) LSC, liquid scintillation counter; (4) DOM, dissolved organic matter; (5) TOC, total organic carbon; (6) NPOC, nonpurgeable organic carbon; (7) HPLC, high performance liquid chromatography; (8) MRM, multiple reaction monitoring; (9) D_5 STD, deuterated standard (abbreviation used within the paper as deuterated standard solution of 2-hydroxyatrazine).

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