Long-term Persistence of Pesticides and TPs in Archived Agricultural Soil Samples and Comparison with Pesticide Application

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Abstract

For polar and more degradable pesticides, not many data on long-term persistence in soil under field conditions and real application practices exist. To assess the persistence of pesticides in soil, a multiple-compound screening method (log K\_ow 1.7-5.5) was developed based on pressurized liquid extraction, QuEChERS and LC-HRMS. The method was applied to study 80 polar pesticides and >90 transformation products (TPs) in archived topsoil samples from the Swiss Soil Monitoring Network (NABO) from 1995 to 2008 with known pesticide application patterns. The results reveal large variations between crop type and field sites. For the majority of the sites 10 to 15 pesticides were identified with a detection rate of 45% at concentration between 1 and 330 µg/kg\_dw in soil. Furthermore, TPs were detected in 47% of the cases where the “parent-compound” was applied. Overall, residues of about 80% of all applied pesticides could be detected with half of these found as TPs with a persistence of more than a decade.

Introduction

Arable soils are exposed to a large number of pesticides by direct application. In Europe several hundreds of pesticides are registered and approximately 300,000 tons (average between 2000
and 2008 for 20 European countries) are applied to agricultural fields per year.\textsuperscript{1} Today, Environmental Quality Standards (EQS) exist to assess the risk of pesticides in freshwater, however, in Europe EQS for soil are absent.\textsuperscript{2,3} Furthermore, the potential impact for freshwater organisms has been shown on the national and international level, but the effects on soil organisms is largely unknown.\textsuperscript{4-6} The long-term behaviour of pesticides in soil depends on a number of factors involving chemical, photochemical and microbial transformation, volatilization, sorption, plant or organism uptake, and leaching to groundwater, with sorption and biodegradation being the most relevant.\textsuperscript{7-9} Systematic studies on the long-term fate and persistence of pesticides including their transformation products (TPs) have been mainly carried out under the regulatory frameworks for pesticide registration in the laboratory and in controlled field dissipation experiments, typically for one or two years for a small number of reference soils.\textsuperscript{9-11} However, these limited studies cannot fully capture the large variety of different environmental scenarios and their interactions (e.g., soil types, pH, organic matter content, temperature) in agricultural practice, which can influence the degradation of pesticides to a great extent.\textsuperscript{9-11} Pesticide fate can vary significantly with the number of applications, spatial and temporal variations, crop rotation and weather conditions. For example, the establishment of microflora capable of rapidly degrade triazines has been observed in soils where triazines have been applied for several years.\textsuperscript{12} Beyond registration studies, there is also a limited number of publications addressing the long-term fate of modern pesticides in soil, mainly from lysimeter experiments and primarily addressing individual compounds or well-studied compound classes such as triazine herbicides.\textsuperscript{13-15} This outlined scarcity of data calls for a more systematic monitoring of pesticides in soils under real agricultural practice, similar to the case for surface and groundwater for decades at national and regional levels.\textsuperscript{16-18} Soil monitoring programs (SMP) aim to observe the soil quality of ‘real-world’ soils in order to detect possible temporal changes in soil quality. In SMP, soils are monitored in a systematic way, so as to determine soil variables and to record their temporal and spatial changes.\textsuperscript{19} Although a wide network of SMP exist in the European Union,\textsuperscript{20} for historical reasons, these networks have focused mainly on the decline of soil organic matter and on soils contaminated with inorganic substances such as trace metals or highly persistent compounds like polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and highly chlorinated first-generation pesticides like DDT or hexachlorocyclohexane (HCH).\textsuperscript{21} In the USA, extensive monitoring is equally scarce with only few studies addressing pesticides in soils such as the National Soil Monitoring Program (NSMP) where organochlorine and
organophosphorus compounds as well as atrazine were studied in agricultural soil and mature
crops from 37 different states.\textsuperscript{22} While some SMP have investigated the fate of a range of
pesticides (e.g. Goncalves and Alpendurada in Portugal) over a short timeframe,\textsuperscript{23} there is little
or no information on the long-term behaviour of “modern” polar and less persistent pesticide
residues at the field scale under real agricultural practices. The lack of information can partly
be attributed to the absence of long-term information on land and pest management of soil
monitoring sites needed for a proper sampling campaign and data interpretation.\textsuperscript{24}

Because of the high number of pesticides registered and their high spatial and temporal
variability, it is difficult to design a proper monitoring campaign and select relevant substances
to assess the exposure of agricultural soils comprehensively. Routine analysis usually focuses
on just 15−40 analytes and analysis is mostly carried out by gas chromatography or liquid
chromatography−mass spectrometry (GC/LC−MS/MS) using single or triple quadrupole
instruments.\textsuperscript{25, 26}

Recent advances in LC−high resolution MS (LC-HRMS) allow efficient screening of a large
number of substances in a single run.\textsuperscript{27, 28} The high mass accuracy and high mass resolution
even allows a suspect screening of compounds without reference standards using exact mass
information, retention time and MS/MS fragmentation. This enables also a screening of TPs for
which standards are often not available or very expensive. This extensive target and suspect
screening was successfully carried out in freshwater\textsuperscript{17, 29, 30} and recently also in sediment\textsuperscript{31, 32},
but not yet in soil.

The aim of this study was to make use out of the available LC-HRMS technology as well as the
comprehensive Swiss archive of soil samples to study the occurrence of a wide variety of
pesticides and their major TPs in soil samples of various agricultural practices. The Swiss Soil
Monitoring Network (NABO) operates about 100 long-term monitoring sites throughout
Switzerland and contains a comprehensive archive featuring soil samples dating back to the
mid-1980s.\textsuperscript{33} Moreover, management data including pesticide application obtained from the
farmers are recorded annually. Thus, the objectives of this study were (i) to select pesticides for
monitoring according to their real application on fields recorded in the monitoring program (ii)
examine the suitability of LC-HRMS analysis for extraction of archived soil samples, and (iii)
to compare the pattern of detected substances and their TPs in soil with the known information
on applied pesticides provided by the farmers on typical agricultural cultures (crop, orchards,
vineyards). As pesticide application data were available from 1995 upwards, long term
persistence of a wide variety of modern pesticides could be studied for the first time.
Methods

Study design

Currently, the NABO comprises 105 observation sites across Switzerland, representing diverse geology, soil types, land use and climate conditions across the country. Monitoring sites were sampled for the first time between 1985 and 1989 and have been continuously re-sampled every five years with the same soil sampling techniques and quality assurance protocols since then.33

Details in the collection and storage of samples are provided in Supporting Information (SI).

In this study, 29 archived soil samples from 14 NABO monitoring sites were selected covering a time period between 1995 and 2008 matching the time span of the pest management data used in this study. The NABO network performed meanwhile six repeated soil surveys (1985 - 2014), but we restricted our study to 1995 – 2008 to limit number of soil samples. The sites were chosen from monitoring sites where crops received pesticides regularly considering land use and the long-term pesticide use pattern recorded as indicated by the farmers. As no constraints or recommendations are given to the farmers managing the soil monitoring sites, the pesticide management at the monitoring sites represents current agricultural practices across Switzerland.

The 14 selected agricultural monitoring sites cover croplands with various crop rotations (12 soil samples from 7 sites), vineyards (8 from 3 sites), orchards (7 from 3 sites), and vegetables (2 from 1 site). Details and physicochemical soil characteristics of each sampling location are reported in Table S1.

Chemical Analysis

Details on the supplier, preparation, and storage of reference standards, reagents, and pesticide-free soil are provided in SI.

Pressurized Liquid Extraction of Soils

Around 6 g of dried soil were homogenized with a mortar and pestle, weighed, and transferred to 11 mL stainless steel extraction cells filled with a glass fiber filter (Whatman GF/F). In addition ~1 g of diatomaceous earth was added to each extraction cell to increase solvent channeling (Hydromatrix, Restek). First, the soils were extracted with a mixture of acetone (Ac) and ethyl acetate (EtAc) at a ratio of 30:70 (% v/v) in two static extraction cycles of 5 minutes at 80°C and 130 bar using an ASE 200 (Dionex). Extraction cells were rinsed with fresh solvent (60% of cell volume) and purged with nitrogen for 120 seconds. Consequently, the soils were extracted with a mixture of Ac and 1% phosphoric acid at 120°C at a ratio of 70:30 (% v/v) in
two static extraction cycles of 5 minutes to release acidic compounds from the soil. The extracts were combined to give a final volume of about 30 mL (pH 4 - 4.5) and spiked with 10 µL of a mixture containing 28 internal standards, resulting in an absolute amount of 100 ng of each labelled compound in the extract. The organic solvents were removed by rotary evaporation at 35°C and the remaining aqueous phase was diluted to 4 mL with HPLC grade water. Information on the overall method development is provided in SI and described in the results.

**Clean-up and Enrichment of Soil Extracts**

Soil matrix was removed from extracts using the “QuEChERS” approach. Briefly, 5 mL of acetonitrile (ACN) were added to 4 mL of aqueous phase and combined with 1.6 g of MgSO₄ and 0.2 g of NH₄Cl. The mixture was shaken for 2 min and centrifuged for 4 min at 1000 x g. After separation, the ACN phase was transferred to a centrifuge tube and combined with 800 mg of MgSO₄, 125 mg of primary-secondary amine (PSA), 125 mg of endcapped C₁₈, and 12.5 mg of graphited carbon black (GCB) (all from Sigma-Aldrich) for d-SPE cleanup. The mixture was shaken for 1 min, centrifuged again for 5 min and the solvent phase was transferred to a graduated centrifuge tube. The d-SPE sorbent was washed with 4 mL of ACN, centrifuged again, and the ACN solvents were combined, evaporated to 50 µL and brought to a volume of 500 µL using methanol. The final extract was filtered into a 2 mL vial using 0.2 µm PTFE syringe filters (BGB analytics, Boeckten).

**Liquid Chromatography Tandem High Resolution Mass Spectrometric Detection**

Analytes were separated on a X-bridge C18 column (2.1×50 mm, 3.5 µm particle size, Waters) equipped with a 2.1×10 mm C18 security guard cartridge at 35 °C at a flow rate of 200 µL/min similar to Kern et al. Compounds were separated by a mobile phase consisting of water (A) and methanol (B), both containing 0.1% of formic acid (v/v). The LC program started at 95% of solvent A for 0.5 min, increasing solvent B up to 95% in 17 min, held for 4 min, returning back to 95% of solvent A in 1 min and re-equilibration for 5 min. As the extracts were in 100% methanol, 5 µL were injected, while the flow rate was raised to 320 µL/min for the first 0.5 min.

Detection of analytes was performed after electrospray ionization (ESI) with tandem linear ion trap-Orbitrap mass spectrometer (LTQ-Orbitrap-XL, Thermo) in positive and negative mode. Full scan accurate mass spectra were acquired from 110 to 600 Da with a nominal resolving power of 60,000 referenced to m/z 400 and a mass accuracy of ±5 ppm. Data-dependent high-resolution product ion spectra (dd-HRMS/MS) were recorded for the five most intense ions.
from a precursor ion list (calculated for the prioritized compounds) at a resolving power of 7,500 using higher energy collisional dissociation at a collision energy of 35%. Additionally, collision-induced dissociation (CID) was performed with a normalized collision energy of 35% and measured at unit resolution in the LTQ as described elsewhere.\textsuperscript{32} Matrix-matched calibration standards (n = 9) were made with concentrations ranging from 2 ng to 500 ng/mL in vial of standard mix solution using a pristine soil extract. Full scan precursor ions were used for detection and quantification, and product ions were used for confirmation.

Quality control accounted for more than 20% of the samples, which included standards, blanks and spiked samples.

Validation of the final method

The method was validated for 64 pesticides and 29 TPs. Relative recoveries were determined by using pesticide-free soil. After homogenization, this soil was distributed into different extraction cells (n=4) and spiked at the top of each cell with 50 µL of 10 mg/L standard mix solution (50 ng absolute) as illustrated in Table S2 in SI. All extracts were spiked with an internal standard mix prior to injection into the LC to calculate extraction and relative recoveries. The method limit of quantification (LOQ) was defined as the lowest point of the matrix-matched calibration curve with a S/N ratio ≥10, or with the lowest visible calibration point in case no background noise was visible due to the noise cut-off algorithm used for data size reduction.

Target and suspect screening of pesticides and TPs

The selection of 80 pesticides for screening was carried out based on a list of pesticides applied to the NABO soils between 1995 and 2008 (Table S3 in SI). Inorganic substances and compounds not ionisable by ESI, based on the absence of corresponding functional groups, were excluded from this study and accounted for ~35% of the entire list of applied pesticides. Furthermore, additional information on known TPs was retrieved using literature data on the applied pesticide and from assessment reports, registration dossiers (US EPA, EU, UK, CA and AUS), the European Food Safety Authority (EFSA), and peer-reviewed publications. Overall, reference standards for 64 pesticides and 29 TPs were used for target screening. Suspect screening was performed for 16 pesticides and 62 TPs by collecting information on the molecular formula and structure. The exact mass of the expected molecular ion, [M+H]\textsuperscript{+} or [M−H]\textsuperscript{−} according to the assumed ionization behaviour, was extracted with a mass window of ±5 ppm from the HR-full scan chromatogram acquired in the positive or negative mode as
described by Kern et al.\textsuperscript{34} Positive detections were further substantiated by a match of observed vs. theoretical isotope patterns and the interpretation of MS/MS spectra. Beyond this analytical approach, circumstantial evidence was also used to validate positive findings, such as the simultaneous presence of parent compounds and TPs at meaningful relative retention times, the presence of compounds detected in samples after a short period of pesticide applications and their absence in samples without known application. Unequivocal confirmation was only possible if a reference standard was available. Without reference standard pesticides and TPs were tentatively identified with a level 3 of confidence as described by Schymanski et al.\textsuperscript{35}

Physicochemical properties of pesticides ($K_{\text{ow}}$ and $pK_a$ in soil) were obtained from literature data, the Pesticide Properties Database (PPDB) or estimated using the Calculator Plugins of Marvin (Chemaxon).\textsuperscript{36, 37}

\textbf{Results and Discussion}

\textit{Optimizing extraction and clean-up for pesticides and TPs by target and suspect screening}

For method development, 39 target screening compounds were selected covering different compound classes and a wide range of physical-chemical properties ($\log K_{\text{ow}}$ 1.7 - 5.5, $pK_a$ 0.1 - 10) as well as known to be persistent in soils, either due to a strong sorption, long half-life, or applied in large amount to the NABO soils as illustrated in Table S2 and S3 in the SI. In a first step, the extraction from diatomaceous earth was tested using different solvent mixtures (Ac/EtAc/H$_2$O 45:40:15, Ac/EtAc 30:70, Ac/H$_2$O 70:30) to cover different polarities. In addition, the influence of temperature (80, 100, 120 and 140°C) during PLE was investigated. All extraction setups were performed on soil samples spiked with a mixture of the mentioned pesticides.

The results show that a mixture of Ac/EtAc yielded higher recoveries over water-containing solvents for most compounds, as illustrated in Figure S1-S3 in the SI, with average extraction recoveries of 70%. The decrease of recoveries for phenmedipham and to a lesser extent for chloridazon, diuron, isoproturon, and bentazone with increasing temperatures suggest thermal degradation of these compounds. Recoveries of acidic substances like 2,4-D, mecoprop, sulcotrione, and the metolachlor TPs were below 40%. The results indicate that Ac/EtAc is a suitable solvent mixture for extracting uncharged and medium polar substances, but not for the most hydrophilic or charged compounds, consistent with previous studies.\textsuperscript{32} Water-containing extraction mixtures resulted in higher recoveries for acidic compounds such as 2,4-D and mecoprop, with extraction recoveries close to 80% and 60% for sulcotrione vs. $\leq 20\%$ when extracted with Ac/EtAc. However, Ac/H$_2$O alone was not suitable for extracting the most
hydrophobic compounds (e.g. pendimethain, chlorpyrifos). Furthermore, thermal degradation of phenylurea herbicides was higher at higher temperatures in aqueous solvents as compared to Ac/EtAc. Thus, a two-step extraction procedure was suggested, utilizing Ac/EtAc at 80°C in the first step and Ac:H₂O at 120°C in the second step, as thermolabile compounds are largely extracted with the first step.

For further optimization, a mixture of acetone with 1% phosphoric acid (70:30, v/v) was tested as second extraction step to improve the recoveries of negatively charged compounds. The results show an increased recovery of the most hydrophilic anionic compounds metolachlor OA and metolachlor ESA when using an acidified aqueous phase (Figure S4). The combination of Ac/EtAc (30:70 v/v) at 80°C, followed by Ac/1% phosphoric acid (70:30 v/v) at 120°C resulted in the best recoveries for the largest number of compounds also from spiked soil (on average 84%) and spiked soil aged for 30 days at 4°C (on average 76%, Figure S4). Low extraction recoveries of 8% were observed for the pyrethroid cypermethrin in aged soils which is consistent with the reduced bioavailability of cypermethrin due to the formation of bound residues and degradation.38 Probably due to the same reason the recoveries for propachlor, dinoseb and fluazinam were ≥30% lower in aged soils than in freshly spiked soils. Due to its short half-life and fast hydrolysis, captan could not be recovered entirely from soil.39

The involved liquid-liquid extraction (QuEChERS) as a clean-up step for soil extract might be followed by an additional purification step using d-SPE, for which some studies have reported a decrease or not significant increase of recoveries.32, 40, 41 Therefore, recoveries of pesticides were determined for the QuEChERS extraction with and without d-SPE (combination of C18/PSA/GCB). The results show similar recoveries for both approaches with relative recoveries of 70 to 110% for most compounds as shown in Figure S5 in SI. However, for some compounds showing recoveries above 150% (likely due to different ion suppression of the analyte and the internal standards used for calibration) such as fenpropidin, isoproturon, metalaxyl and fluazinam, the recoveries moved closer to 100% after d-SPE. As no significant compound losses due to d-SPE were observed, this step was included into the method workflow also to ensure a reduction of the matrix load across samples with different organic matter content.

The final method was validated for all 93 target screening compounds (Table S2). The recoveries for soil samples spiked with 50 ng of each analyte were for 61 of the compounds between 80 and 120%, for 8 above 150%, and for 7 below 40% (mainly hydrophilic acids and hydroxy-triazines). Thus, the developed method worked well for the analysis of a wide range of compounds (~log Kow ~2 to 6), but it is not suitable for the analysis of highly hydrophilic due
to the liquid-liquid extraction used in QuEChERS and the LC conditions employing injection of extracts in 100% of methanol, which results in deterioration of peak shapes for early eluting compounds. Moreover, other compounds are challenging due to their low ionization efficiency in ESI (e.g., chlorothalonil, aclonifen, and most pyrethroids). Therefore, highly hydrophilic pesticides and TPs and those with low ionization efficiencies are out of the range of the analytical method. The LOQ values ranged from 0.7 ng/g<sub>dw</sub> to 25 ng/g<sub>dw</sub> with LOQs ≤ 1.5 ng/g<sub>dw</sub> for more than 80% of the pesticides (Table S2). The method performance is comparable to other studies.32, 40, 41 and the LOQs similar to those acquired with triple-quadrupole based methods.40

The full scan HRMS measurement enabled to screen for suspected pesticides and TPs without reference standards by using exact mass information. This approach allowed to tentatively assign further 15 pesticides and 61 TPs, using evidence from isotopologue patterns, MS/MS spectra, and circumstantial information. Confirmation of four compounds was done retrospectively with the purchase of reference compounds whereas the finding of the others can only be reported tentatively.

This approach is illustrated in Figure 1 for the target compound fenpropidin and four suspected TPs (two hydroxylated, one N-oxide and one carboxylated TP). Their extracted ion chromatograms showed one peak of high intensity for the parent compound at 13.3 min, one peak at 9.7 min for the fenpropiden-carboxylate and five larger peaks for the hydroxy-/N-oxide TP at 9.5, 11.8, 12.5, 12.8 and 13.9 min in the soil sample of the site Vi.2 (Figure S6a). A comparison was performed between a sample from the same soil prior to fenpropidin application, soils without known fenpropidin application and a method blank. The evaluation showed that the peak at 9.5 min is related to a background contamination, while the other TP peaks were not present, substantiating that the observed peaks originate from fenpropidin. The plausibility of retention times was checked in relation to the parent compound; a carboxylation might well result in a negative retention time shift of 3.6 min, a hydroxylation in a negative retention time shift of 1-3 min. The N-oxidation could well explain the positive retention time shift of the TP at 13.9 min as compared to the parent compound as has been described in other studies.42, 43 Finally, MS/MS spectra of the TPs were evaluated in comparison to those of the parent compound (Figure S6b). For the peak of m/z 304.2271, no meaningful MS/MS spectrum could be obtained. For the peaks of m/z 290.2478, all MS/MS spectra showed the presence of an ion at m/z 98.0957±0.0002 (C<sub>6</sub>H<sub>12</sub>N), which could correspond to the piperidine moiety and one additional carbon atom, while for the parent compound the piperidine fragment was present (C<sub>5</sub>H<sub>12</sub>N). The peak at 13.9 min shows additionally the preservation of the tert-butyl-phenyl-
methylene moiety (m/z 147.1167), substantiating the tentative assignment of the N oxide. For
the other three ions of the same mass (at 11.8, 12.5 and 12.8 min), no further MS/MS
information was available, but the ions could possibly be assigned to three different
hydroxylation products instead of the two suspected ones.

**Influence of land use on Pesticide Application Pattern and Frequency of Detection**

Records of applied pesticides in Swiss agriculture between 1995 and 2008 revealed large
variations between crop type and field sites. The intensity of pest control was generally higher
for special crops such as vineyards (Vi) and orchards (Or) with 10 to 20 compounds applied per
year. Moreover, amounts of pesticide applied on vineyards were recorded to be between 12 and
17 kg ha\(^{-1}\) yr\(^{-1}\) and between 4 and 33 kg ha\(^{-1}\) yr\(^{-1}\) for orchards (Figure 2). The number of
pesticides applied yearly to cropland (Cl) and vegetables (Ve) ranged from 1 to 20, but generally
were below 10 pesticides per year. Typical application rates on cropland sites were below 3 kg
ha\(^{-1}\) yr\(^{-1}\), except for potatoes which received between 6 to 15 kg ha\(^{-1}\) yr\(^{-1}\) of various pesticides.
The numbers of pesticides applied were compared with the numbers of pesticides found in the
selected 29 soil samples as illustrated in Figure 2a. For the majority of the sites, 10 to 15
pesticides were detected in the top soil. The detection frequency was in good agreement with
the soil application data obtained from the farmers for special crops, but was generally higher
in cropland soils. The most possible explanation is that for croplands some compounds detected
in soil were applied before 1995 and thus, not reported in our farmer’s records dataset. For all
orchard sites the frequency of application and detection is in good agreement. The less intensive
management of site Or.3, which is a traditional high-stem orchard with only 3 to 10 compounds
applied per year and the high number of pesticides (average > 15) applied to the low-stem
orchard sites (site Or.1 and Or.2) are well mirrored in the detection frequency. The total number
of pesticides applied per site and overall years correlates with the total number of pesticides
detected at each site. While the applied numbers of pesticides per site ranged between 10 and
32 (on average 17), we found between 5 and 30 (on average 12) compounds in soil per site.
Notably, for 75% of the parent compounds measured in soil TPs ranged between 1 and 15 TPs
per site as illustrated in Figure 2b.

**Comparison of Pesticide Residues in Soil with Application Data**

Application data reported by the farmers was compared with the soil chemical analysis in a
qualitative manner defining four categories: (1) true-positive: pesticides detected in soil and
reported as applied by farmers, (2) true-negative: compounds not detected in soil and not
applied, (3) false-positive: compounds detected in soil but not applied, and (4) false-negative:
compounds not detected in soil but reported as applied. The analysis of 80 pesticides for each
of the 29 soil samples at the 14 sites resulted in 2320 cases (80 x 29) within the four categories
as illustrated in Figure 3a with different colors. Pesticides were reportedly applied in 421 cases.
Except for the Vi and Or sites, pest management on the sites depended on the crop type
cultivated and varied largely from year to year. Typically, farmers did not apply the same
pesticides at these sites each year, while for Vi and Or usually the same 5 to 15 compounds
were applied annually. The soil chemical analysis confirmed the presence of pesticides in 309
cases (45%), while for 112 cases (16%) the applied pesticides were not detected. The finding
of 45% of the pesticides applied are rather surprising due to the relative short dissipation half-
lives of these compounds (<1 year) and the relatively long periods of time between pesticide
application and soil sampling, i.e., often longer than one year. Therefore, the analysis of soil
samples demonstrates that although at low concentration levels, applied pesticides may be
present in soils up to decades after their application. Moreover, the results revealed 260 (38%)
false-positive cases in which the pesticide was not reportedly applied but were measured in soil,
indicating that pesticide residues measured may result from applications prior to 1995, as has
been reported in arable soils in Argentina, where some pesticides have been detected before the
application period started.44 The remarkable high numbers of false-positives (38%) was
observed in particular for simazine accounting for 17 false-positive vs. 11 true-positive cases,
followed by atrazine (15 vs. 10) and terbuthylazine (13 vs. 5). The results are consistent with
the large amounts of triazine herbicides in agriculture in the past and with the long term
persistence of residues of these compounds as have been reported for atrazine (14C ring-
labeled), where 50% of 14C residue was found under field condition even after nine years of
application.13, 36, 45 Hence, the detected residues, most likely, might have originated from
applications dating prior to the recorded period or impurities of other pesticides. In addition,
false-positives for the fungicide carbendazim was found in 12 cases. The findings can be
explained by the occurrence of carbendazim as a TP of other pesticides such as thiophanate,
thiophanate-methyl, and benomyl. Furthermore, the high number of false-positives might be
attributed also partly to pesticides applied at neighbouring fields (overspray) or incomplete
records provided by the farmers. For example, weeds might be removed using herbicides prior
to a new crop (preemergence herbicides), which might be not considered as pest control (e.g.,
pendimethalin and oryzalin) by the farmer and therefore, not documented as a pesticide used
during the growing season. Likewise, some farmers may not have reported acaricides or might
have used seeds treated with pesticides (seed coating46) and the information was not considered
in the application data records. Such cases have been reported for imidacloprid particularly in
the treatment of rapeseed.\textsuperscript{46} Imidacloprid leaching from sugar beet seeds to subsurface tile
drains was recently observed on an experimental site.\textsuperscript{46,47} False-positives for imidacloprid were
in fact observed at seven cropland sites and in three orchards sites in our study.
Three herbicides of the triazine class rank in the top five most frequently found pesticides with
simazine, atrazine and terbutylazine detected in 97\%, 86\%, and 62\% of the 29 soil samples,
respectively (Table 1). The use of simazine and atrazine is not approved anymore in Switzerland
(since 2012) and most EU countries since 2003, except Spain for simazine.\textsuperscript{36} Furthermore,
tebutam and carbendazim were detected in 79\% and 72\% of the samples.
Pesticide concentrations in soil samples ranged between 1 and 330 \(\mu g/kg_{dw}\) as illustrated in
Table 1 for the top 20 frequently detected pesticides. A full list of the measured pesticide
concentrations is provided in Table S4 in the SI. The pesticide concentrations are in good
agreement with other measurements of pesticides in agricultural soils. In a monitoring program
of 60 soils from intensive horticulture areas in the North of Portugal, Goncalves and
Alpendurata detected a large array of pesticide residues in 10\% to 30\% of the samples with
concentrations from the low \(\mu g/kg_{dw}\) up to higher than 500 \(\mu g/kg_{dw}\).\textsuperscript{23} In two Finnish arable
soils, ethofumesate was found with concentrations ranging from 10 to 110 \(\mu g/kg_{dw}\).\textsuperscript{48}
Additionally, in 30 soils along the Turia river in Spain, chlorpyrifos was frequently detected
with concentrations up to 65 \(\mu g/kg_{dw}\), while other substances were detected < 10 \(\mu g/kg_{dw}\).\textsuperscript{3}
Low residual amounts of terbuthylazine, metalaxyl and tebuconazole were also detected by
Sanchez-Gonzales et al. in 42 soil samples across the Águeda river basin at the Spanish–
Portuguese border.\textsuperscript{49} However, different to our study, crop and pest application data were not
available for most studies or only a few compounds were investigated which hampers
interpretation and comparison with our results.

**Transformation Products in Soils**

Chemical analysis of 93 TPs resulted in the detection of TPs in 47\% of the cases where the
parent pesticide was reported to be applied as illustrated in Figure 3b. It should be noted that not
for all pesticides given in Figure 3a (n = 569) TP’s were analysed, hence the number of parent
compounds in Figure 3b represent a subset (n = 472). False-positives were found in 11\% of the
cases where TPs were detected in soil but the parent compound was not applied within the
recorded time period. In those cases the parent pesticide might be applied before the registered
time period and degraded completely but residues of its TPs are still present in soil, proving
evidence of the environmental exposure of these pesticides. Most of the TPs detected are
designated “major metabolites“ from registration dossiers with concentrations in soil ranging between 1 and 680 µg/kg_{dw} as illustrated in Table 2 for the top frequently detected 20 TPs. TPs for simazine, diuron and atrazine were found at higher concentrations in soil when compared with other TPs. The isobaric TPs simazine-2-hydroxy and terbuthylazine-desethyl-2-hydroxy had the highest concentration. Furthermore, atrazine-2-hydroxy was detected in all 29 soil samples with concentrations between 2 and 220 µg/kg_{dw}. The findings are consistent with its high persistence as detected in soils after 9 and 22 years of atrazine application.\textsuperscript{13, 45} The complete list of TPs found in soils is provided in Table S5 in the SI.

**Insights and Future Research**

In this work, we have demonstrated the strength of a two-tiered approach to monitor long-term fate of pesticide residues in soil by combining consistent and reliable pest management records and a broad target and suspect screening of archived soil samples of the applied spectra of pesticides and their TPs. The analysis of soil samples demonstrates that although at low concentration levels, applied pesticides and their TPs may be present in soils for decades after their application, resulting in mixtures of typically 10-15 and up to 29 compounds. The reason for this might be a limited bioaccessibility or bioavailability of these low concentrations for further microbial degradation. Bioavailability of these low-level compound mixtures is also critical for their ecotoxicological relevance, but as we used a rather exhaustive extraction procedure, we cannot make any statements on the potential risks for soil organisms.

In several cases, pesticides were detected in soil samples although according to the farmers’ records they were never applied. Generally, these ‘false positive’ findings were related to applications apparently not recorded (e.g., seed coatings) or were applied prior to the recorded period. The outcomes demonstrate that a long-term comprehensive recording is important for results interpretation. However, this goal is hard to achieve since monitoring programs have to work with many sampling sites and reporting pesticide use by farmers is not mandatory. Based on the results of this work, we advocate further investigation on the persistence of pesticides in soil and on the ecotoxicological effects of the pesticide mixtures found in this study with up to 29 pesticides in one soil sample. Moreover, the presented work advocates complementing the required pre-registration fate studies on pesticides with soil monitoring under real agricultural practice.
Acknowledgments

Chemaxon (Budapest, Hungary) is acknowledged for a free academic license of Marvin, the Calculator Plugins and JChem for Excel. Funding by the Federal Agency of Environment (FOEN) in Berne is gratefully acknowledged.
Figure 1. Extracted ion chromatogram of the target compound fenpropidin and of four of its suspected transformation products in the extract from soil sample of the site Vi.2 (NL: signal intensity at 100% of relative abundance).

Figure 2. Applied and measured pesticides for the 14 investigated NABO soil sites (cropland sites (Cl); orchards (Or), vegetable growing (Ve), and viticulture (Vi)) between 1995 and 2008: a) number of pesticides applied per year between 1995 and 2008 (boxplot) with outliers shown.
as circles outside the range of the whiskers and pesticides found in the soil samples (triangles),
b) average number of parent compounds and transformation products (TPs) measured in soil samples.

Figure 3. Qualitative analysis of applied pesticides by farmers (A) and results of the soil chemical analysis for transformation products (TPs) (B) showing (i) true-positives shown in orange (detected and applied), (ii) true-negatives shown in green (not detected and not applied), (iii) false-positives shown in red (detected but not applied); and (iv) false-negatives shown in grey (not detected but applied). Right figure is presented in percentage of applied and detected cases.
Table 1. Concentrations of the 20 most frequently detected pesticides in 29 soil samples (0-20 cm) from 14 NABO monitoring sites (full list is provided in Table S4).

<table>
<thead>
<tr>
<th>Rank</th>
<th>Name</th>
<th>CAS No.</th>
<th>Type</th>
<th>No. of Samples Detected</th>
<th>% of Detected Samples</th>
<th>Concentration Range (µg/kg)</th>
<th>Median Concentration by Land use (µg/kg dw)</th>
<th>Cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Fenpropidin</td>
<td>67306-00-7</td>
<td>Herbicide</td>
<td>10</td>
<td>34</td>
<td>1-14</td>
<td>Cl: 1, Or: ND, Ve: 10</td>
<td>Applied-Detected: 21, Applied-not Detected: 0, Not Applied-Detected: 14, Not Applied-not Detected: 66</td>
</tr>
</tbody>
</table>

Land use: Cropland sites (Cl), orchards (Or), vegetable growing (Ve), and viticulture (Vi)

1Transformation product of other pesticides such as thiophanate, thiophanate-methyl, and benomyl

ND= Not Detectable
Table 2. Concentrations of the most frequently detected 20 transformation products (TPs) in 29 soil samples (0-20 cm) from 14 NABO monitoring sites. Note that a few TPs are formed from two parent pesticides.

<table>
<thead>
<tr>
<th>Rank</th>
<th>Parent Compound</th>
<th>Type</th>
<th>Transformation Product (TP)</th>
<th>TP Type</th>
<th>No. Of Samples Detected</th>
<th>Concentration Range (µg/kg dw)</th>
<th>Median Concentration by Land use (µg/kg dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Atrazine</td>
<td>Herbicide</td>
<td>Atrazine-2-hydroxy</td>
<td>Major</td>
<td>29</td>
<td>2 - 220*</td>
<td>23 2 10 4</td>
</tr>
<tr>
<td>2</td>
<td>Simazine</td>
<td>Herbicide</td>
<td>Simazine-2-hydroxy + Terbuthylazine-desethyl-2-hydroxy</td>
<td>Major</td>
<td>27</td>
<td>2 - 680*</td>
<td>6 208 &lt; LOQ 20</td>
</tr>
<tr>
<td>3</td>
<td>Terbuthylazine</td>
<td>Herbicide</td>
<td>Terbuthylazine-desethyl</td>
<td>Major</td>
<td>26</td>
<td>1 - 2*</td>
<td>1 1 &lt; LOQ 1</td>
</tr>
<tr>
<td>4</td>
<td>Atrazine</td>
<td>Herbicide</td>
<td>Atrazine-desisopropyl</td>
<td>Major</td>
<td>25</td>
<td>1 - 9*</td>
<td>3 5 5 2</td>
</tr>
<tr>
<td>5</td>
<td>Diuron</td>
<td>Herbicide</td>
<td>Diuron-desmonomethyl</td>
<td>Major</td>
<td>22</td>
<td>2 - 130*</td>
<td>3 6 &lt; LOQ 12</td>
</tr>
<tr>
<td>6</td>
<td>Chlorothalonil</td>
<td>Fungicide</td>
<td>Chlorothalonil-hydroxy</td>
<td>Major</td>
<td>19</td>
<td>D</td>
<td>D D D D</td>
</tr>
<tr>
<td>7</td>
<td>Terbuthylazine</td>
<td>Herbicide</td>
<td>Terbuthylazine-hydroxy</td>
<td>Major</td>
<td>16</td>
<td>D</td>
<td>D D D D</td>
</tr>
<tr>
<td>8</td>
<td>Chlorpyrifos</td>
<td>Insecticide</td>
<td>3,5,6-Trichloro-2-pyridinol</td>
<td>Major</td>
<td>15</td>
<td>6 - 70*</td>
<td>&lt; LOQ 27 &lt; LOQ 6</td>
</tr>
<tr>
<td>9</td>
<td>Diuron</td>
<td>Herbicide</td>
<td>Diuron-desdimethyl</td>
<td>Major</td>
<td>15</td>
<td>2 - 30*</td>
<td>&lt; LOQ 3 5 17</td>
</tr>
<tr>
<td>10</td>
<td>Chlorothalonil</td>
<td>Fungicide</td>
<td>3-cyano-6-hydroxy-2,4,5-trichlorobenzamide / 3-cyano-4-hydroxy-2,5,6-trichlorobenzamide</td>
<td>Major</td>
<td>14</td>
<td>D</td>
<td>D D D D</td>
</tr>
<tr>
<td>11</td>
<td>Terbuthylazine</td>
<td>Herbicide</td>
<td>Terbuthylazine-desethyl-hydroxy</td>
<td>Minor</td>
<td>14</td>
<td>D</td>
<td>D D D D</td>
</tr>
<tr>
<td>12</td>
<td>Atrazine</td>
<td>Herbicide</td>
<td>Atrazine-desethyl</td>
<td>Major</td>
<td>12</td>
<td>3 - 9*</td>
<td>5 &lt; LOQ 6 &lt; LOQ</td>
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<tr>
<td>13</td>
<td>Carbendazim</td>
<td>Fungicide &amp; TP</td>
<td>2-Aminobenzimidazole</td>
<td>Major</td>
<td>12</td>
<td>2 - 3*</td>
<td>&lt; LOQ &lt; LOQ 2</td>
</tr>
<tr>
<td>14</td>
<td>Pendimethalin</td>
<td>Herbicide</td>
<td>Pendimethalin-benzimidazole</td>
<td>Major</td>
<td>12</td>
<td>D</td>
<td>D D D D</td>
</tr>
<tr>
<td>15</td>
<td>Metamitron</td>
<td>Herbicide</td>
<td>Metamitron-desaminino</td>
<td>Major</td>
<td>11</td>
<td>2 - 20*</td>
<td>5 &lt; LOQ &lt; LOQ &lt; LOQ</td>
</tr>
<tr>
<td>16</td>
<td>Isoproturon</td>
<td>Herbicide</td>
<td>Isoproturon-monodemethyl</td>
<td>Major</td>
<td>10</td>
<td>1 - 5*</td>
<td>2 &lt; LOQ &lt; LOQ &lt; LOQ</td>
</tr>
<tr>
<td>17</td>
<td>Difenconazole</td>
<td>Fungicide</td>
<td>1-(2-chloro-4-(4-chlorophenoxy)phenyl)-2-(1H-1,2,4-triazol-3-yl)ethanol</td>
<td>Major</td>
<td>9</td>
<td>D</td>
<td>D D D D</td>
</tr>
<tr>
<td>18</td>
<td>Azoxystrobin</td>
<td>Fungicide</td>
<td>Azoxystrobin acid</td>
<td>Major</td>
<td>8</td>
<td>D</td>
<td>D D D D</td>
</tr>
<tr>
<td>19</td>
<td>Dinoseb</td>
<td>Acaricide</td>
<td>Acetyl-dinoseb-6-amino</td>
<td>Unknown</td>
<td>8</td>
<td>D</td>
<td>D D D D</td>
</tr>
<tr>
<td>20</td>
<td>Dinoseb</td>
<td>Acaricide</td>
<td>Dinoseb-6-amino</td>
<td>Unknown</td>
<td>8</td>
<td>D</td>
<td>D D D D</td>
</tr>
</tbody>
</table>

Land use: Cropland sites (Cl), orchards (Or), vegetable growing (Ve), and viticulture (Vi)

*Confirmed by reference standards

D Tentatively detected, but not quantified

< LOQ lower than the limit of quantification
References


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