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Multi-elemental compound-specific isotope analysis of pesticides for source identification and monitoring of degradation in soil: a review

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ABSTRACT

The transfer of pesticides from agricultural soils to food and drinking water is a huge environmental problem in many countries. Robust approaches to identify, characterize and quantify the dissipation of pesticides in soils at the field scale are scarce. A relatively mature tool to study industrial pollutants in groundwater is multi-elemental compound-specific isotope analysis (ME-CSIA) which may help to follow-up pesticide residues in soils. Direct adaptation to soils is challenging because pesticide concentrations are generally low, and soil constituents may hamper chromatographic separation and accurate analysis of pesticide residues.

We reviewed recent literature on ME-CSIA as an approach to improve the evaluation of sources and transformations of synthetic pesticides in agricultural soils. First, we discuss current extraction and purification techniques for pesticides in soils. Then, the analytical techniques for reliable measurements of the stable isotope composition of the pesticides are examined. Five hundred and fortyseven isotopic compositions for seventy-one active molecules and issued from different pesticide manufacturers were retrieved and listed in a database, for the following isotopes: ^{13}C (61%), ^{15}N (14%), ^{37}Cl (14%), and ^{2}H (11%). This unique database reflects the overall isotopic variability and trackability of pesticide residues in the environment. Since the environmental fate of pesticides is governed by multiple transformation processes, including bio- and photodegradation, and other abiotic degradations, isotope fractionation values were retrieved from the peer-reviewed literature. This results in a catalogue of two hundred seventy fractionation values for thirty-three compounds to guide the interpretation of isotopic fractionation in the environment, including legacy pesticides such as atrazine, lindane, dichlorodiphenyltrichloroethane, chlordecone and organophosphorus compounds. Specific transformation processes may be identified and quantified using the Rayleigh concept for isotope fractionation during reaction. The review article summarizes existing modelling approaches and future trends to predict the reactive transport of pesticides in soils and associated hydrological systems.

1. Introduction

Soil contamination by organic compounds such as pesticides, hydrocarbons or chlorinated solvents is a widespread problem occurring on agricultural soils, urban and industrial sites. In particular, an estimated 1 to 2.5 million tons of active pesticide ingredients were used annually in

2012, mainly in agriculture (Fenner et al. 2013). In principle, pesticides are only registered for use if they are demonstrated not to persist too long in the environment beyond their intended period of action. However, many pesticides persist for long periods in soil, mainly in the form of non-extractable residues. On the one hand, pesticides leaching from the soil into surface and groundwater may cause severe deterioration of aquatic ecosystems (Brock et al. 2009). On the other hand, uptake of recalcitrant pesticides into crops can lead to human health risks and economic losses for farmers and distributors. Additionally, pesticide residues can impact soil living organisms with consequences on soil ecosystemic services. Over the last decades, efforts to reduce the use of pesticides have mostly failed, while global warming is expected to expand the prevalence of pests in the future and increase the societal dependence on chemical crop protection (Stokstad, 2018). The continuous and partially increasing use of synthetic pesticides in agriculture jeopardizes the ecological and chemical status of soil and aquatic ecosystems, threatening human health (Mateo-Sagasta et al. 2017). In the future, agroecology and organic farming may globally increase and clean and green chemistry in industry may give hope for change in the conception of modern pesticides. But the issue of the persistence in soils of historical and current pesticides and other pollutants still needs to be solved.

The recurrent detection and persistence of pesticides in aquatic ecosystems also emphasizes difficulties to extrapolate pesticide testing in the laboratory or in lysimeter to field conditions (Fenner et al. 2013). Previous research postulated several reasons for the slow degradation of pesticides in soils, including: i) the overall low bioavailability, due to sorption and sequestration of pesticides into soil organic matter (Allen-King et al. 2002), and diffusion-limited desorption from soil aggregates or from minerals (Grathwohl and Reinhard 1993), ii) the poor ability of microorganisms to degrade at low concentrations (Roch and Alexander 1997), iii) the absence of adapted microorganisms with genes encoding for degrading enzymes (Vogel 1996), iv) the inability of microorganisms to degrade due to insufficient available nutrients (N, C source) for co-metabolism, and v) combinations of either reasons. For example, the soil fumigant 1,2-dibromoethane was found in agricultural soils up to 19 years after its last reported application (Steinberg et al. 1987), while half-life at 20°C in laboratory is 70 days (Lewis et al. 2016). In contrast to freshly added 1,2-dibromoethane, residual 1,2-dibromoethane remains recalcitrant to both mobilization (desorption into air and water) and microbial degradation, while diffusion-limited desorption was the main process driving 1,2-dibromoethane removal (Steinberg et al. 1987).

Back in 1962, the publication of the book “Silent spring” by Rachel Carson (1962) revealed for the first time how dangerous persistent organic pesticides in soils can be for man and nature. Since

88 then, huge advancements in analytical chemistry have been made to identify, follow-up and predict
89 pesticides in soils and to evaluate their degradation.

90 Some bioreporter bacteria, such as e.g., luminescent PAH degraders, have been developed in the
91 past to identify less bioavailable pollutants such as hydrocarbons. However, such approaches are
92 restricted to a narrow range of pollutants and proved to be generally inefficient in the case of
93 strongly adsorbing soils. Often, conventional methods cannot resolve whether pollutants are truly
94 recalcitrant, or degradation is just too slow for being monitored. The occurrence of biodegradation
95 of persistent pollutants in the soil is of fundamental importance to improve their monitoring, to
96 evaluate their potential ecotoxicological effects, and to predict ensuing risks following their
97 exposure.

98 One of the latest important analytical developments was the coupling of gas/liquid-chromatography
99 to continuous-flow isotope ratio mass spectrometry to study various stable isotopes ratios specific
100 to each pesticide molecule. When at least two different isotope ratios are analyzed, one speaks of
101 multi-elemental compound-specific isotope analysis (ME-CSIA; Elsner and Imfeld 2016; Vogt et al.
102 2016). Starting from the early 2000s, ME-CSIA has successfully been applied to some common
103 pollutants in many different environments (Lichtfouse, 2000), but especially in groundwater. The
104 Environmental Protection Agency of the United States of America (US-EPA) published in 2008 a
105 guideline for the application of CSIA to groundwater contamination (Hunkeler et al. 2008). More
106 recently, current research on ME-CSIA was reviewed in two short review articles on hydrocarbons
107 (Vogt et al. 2016) and on pesticides (Elsner and Imfeld 2016).

108 In pesticide-contaminated soils, ME-CSIA is much more difficult to apply compared to surface or
109 groundwater applications mainly due to three reasons. First, soil is a complex matrix and contains
110 compounds, such as humic and fulvic acids, which may hamper the extraction (Kalathoor et al.
111 2015), the chromatographic separation and the identification of pollutants due to the so-called
112 matrix effect (Bakkour et al. 2018). This is generally less of a problem in groundwater which is
113 often a well-filtered matrix. Second, pesticides in soil water are usually in the microgram-per-liter
114 range or below it, while industrial pollutants in groundwater often exceed the mg/L range. In
115 addition, pesticides in soil cannot be as easily concentrated as aqueous samples. Third, pesticides
116 in agricultural soils differ from fuel compounds and solvents by their polarity and by a generally
117 larger molecular mass, limiting measurement of stable isotope fractionation using traditional
118 techniques.

Nevertheless, in the last years many studies revealed that ME-CSIA can not only be applied to several pesticides such as *s*-triazine herbicides (Meyer et al. 2008; Reinnicke et al. 2012), phenoxyacid herbicides (Milosevic et al. 2013), substituted phenylureas such as isoproturon (Penning and Elsner 2007), glyphosate (Kujawinski et al. 2013), bentazone (Reinnicke et al. 2010), organophosphorus insecticides (Wu et al. 2014), hexachlorocyclohexanes (Badea et al. 2011), DDT (Holmstrand et al. 2004) and chlordecone (Chevallier et al. 2018) but also to transformation products amino methyl phosphonic acid (AMPA, Kujawinski et al. 2013) and 2,6-Dichlorobenzamide (Reinnicke et al. 2012). Analytical developments were made toward novel sample clean-up techniques, using for instance preparative HPLC (Schreglmann et al. 2013) or molecularly imprinted polymers (Bakkour et al. 2018), and novel injection systems including pre-concentration, like solid-phase microextraction (SPME) or large-volume injections. While all these studies brought a proof of concept for soil ME-CSIA to the scientific literature, they were sometimes restricted to artificially contaminated laboratory systems (e.g., soil microcosms or lysimeters) and higher concentrations, while field studies remain scarce (Alvarez-Zaldivar et al. 2018).

The purpose of this review article is thus to describe recent developments of ME-CSIA for pesticides in soils and to provide an overview on what ME-CSIA offers to better understand sources and the fate of pesticides in soils. The underlying hypothesis of this review is that ME-CSIA bears the potential to identify, characterize, quantify and predict, when combined with reactive transport models, *in situ* transformation of pesticides in soils, even if no transformation products are found. A list of isotopic compositions of active molecules from available pesticide formulations, analytical standards and major degradation products is provided to gain insight in the overall variability of molecules from different sources. Furthermore, stable isotope fractionation processes were compiled in another list to help interpret the fate of pesticides in soils. The knowledge gathered in this article will help not only to take actions to assist the self-restoration of soils (e.g., by nutrient addition or plant introduction) and evaluate its resilience, but also to provide an expected timeframe to manage pesticide ecotoxicity when remediation is not feasible, as e.g. for Chlordecone in the French West-Indies (Chevallier et al, 2018).

2. Analysis of soils

2.1 Extraction and clean-up for ME-CSIA of pesticide residues in soils: analytical challenges and prospects

Challenges. The extension of ME-CSIA of pesticide residues in soils faces three main analytical challenges limiting the evaluation of pesticide degradation in soils: (i) the need of a sufficient mass of analyte for ME-CSIA combined with low pesticide concentrations (sub-ug/g_{soil}) following degradation, ageing or leaching, (ii) the wide range of pesticide physico-chemical characteristics (polar to apolar, non-volatile to volatile), and (iii) the co-enrichment of non-volatile soil components leading to the so-called 'matrix effect' during chromatographic separation. As for water samples, the development of appropriate sample preparation procedures, including optimized robust extraction and cleanup, is a fundamental key to maximize the analytical performance for precise and sensitive ME-CSIA without altering the isotope ratio of the target compounds (Torrento et al. 2019).

The low concentrations of pesticides typically found in soils, the chemical properties of pesticides and the complexity of soil matrices, impose a significant pre-concentration step (from 1000 to 100,000-fold to allow >10-15 ng C or Cl, >30 ng N or H on column) depending on the elemental contribution to the molar mass of the target analyte(s), as well as its solubility, polarity and volatility. In addition, the selectivity and efficiency of pesticide extraction largely varies according to the soil moisture, pH, texture and the amount of organic matter content controlling the amount of available pesticide in soils. For instance, pesticides typically sorb to soils with high clay and organic matter content and low water content (e.g. (Correia-Sa et al. 2012). Pesticide sequestration and extraction also depends on pesticide ageing, i.e., the pesticide physical and chemical distribution and diffusion over time in soil pore water, minerals and organic matter.

Extraction. Pesticides from different families were already extracted from diverse soils for CSIA in several recent analytical (Ivdra et al. 2014; Wu et al. 2019a), laboratory (Masbou et al. 2018; Liu et al. 2020; Liu et al. 2021; Perez-Rodriguez et al. 2021; Torrento et al. 2021) and field studies (Alvarez-Zaldivar et al. 2018; Wu et al. 2019a). Both classical and modern methods of pesticide extraction, described in several leading reviews (e.g. (Tadeo et al. 2012; Bruzzoniti et al. 2014), were adapted for the target analytes. Extraction methods include quick, easy, cheap, effective, rugged and safe procedure (QuEChERS), accelerated solvent extraction, ultrasonic assisted extraction (USE) and focused ultrasonic extraction (FUSE). An accelerated solvent extraction method was validated for determining carbon isotope ratios of Hexachlorocyclohexanes, DDT, and their chlorinated metabolites (soil concentrations from 0.3 to 0.8 mg/kg) in two different types of soil matrices (Ivdra et al. 2014). However, accelerated solvent extraction using three static cycles to increase the recovery rate (>85%) was privileged in Wu et al. 2019a and further related studies. accelerated solvent extraction enabled reproducible extraction of hexachlorocyclohexanes from

soil for C, H and Cl isotope analysis within the analytical precision of ±0.5‰, ±5‰ and ±0.3‰, respectively. QuEChERS approaches (Anastassiades et al. 2003) involve an initial extraction with acetonitrile followed by salt addition to induce phase separation. QuEChERS with acetonitrile (Torrento et al. 2021) or modified for the use of ethylacetate (Alvarez-Zaldivar et al. 2018) or pentane:dichloromethane (3:1, v/v;Perèz et al. 2021) as extraction solvent, in pH buffered or unbuffered media, were used to extract atrazine, metolachlor, alachlor, acetochlor or metalaxyl from soil samples without causing significant isotope fractionation for carbon ($\Delta\delta^{13}C \leq 1‰$) and nitrogen ($\Delta\delta^{15}N \leq 0.5‰$), despite variable extraction efficiencies. However, extraction from two agricultural soils induced a systematic but reproducible isotope fractionation for Cl ($\Delta\delta^{37}Cl$ between +2.5 and +3.5‰) using a QuEChERS extraction kit with acetonitrile (Torrento et al. 2021).

Overall, these results emphasize the versatility of QuEChERS approaches as a standard preparation method for pesticide CSIA from soil samples and possible adaptations for specific matrix-analyte combinations to reach more selective extraction. Acidic pesticides are usually extracted from soil with solvents of medium polarity (ethyl acetate or methanol) in a solvent-water mixture in buffered extraction (pH = 5), by citrate or acetate buffering, to enhance recoveries of pesticides showing pH dependence (Tadeo et al. 2012). The effect of pH on extraction from soil and matrix interferences should also be consirered. Indeed, humic acids at low pH values are protonated and carry fewer negative charges (Melsbach et al.,2020). For less-polar pesticides, such as chloroacetamides, triazines and their metabolites and acetamides, organic solvents like methanol and acetonitrile are commonly used, alone or with mixtures with water (Perez-Rodriguez et al., 2021). The solvent selection should be balanced with the soil matrix co-elution and reduction of the matrix effect before CSIA. In this respect, acetonitrile has the advantages to extract less lipophilic compounds from the soil, to facilitate the removal of residual water with drying agents, and to form distinct partitioning phases with nonpolar solvents to facilitate further cleanup (Anastassiades et al., 2003). Other methods, such as ultrasonic-assisted extraction, are faster since several extractions can be performed simultaneously at relatively low-cost without specialized laboratory equipment (Anastassiades et al., 2003). However, classical shaking or QuEChERS approaches remain adaptative, low-cost and robust in routine extraction for pesticide CSIA. Analyte extraction efficiency using QuEChERS usually increases with an additional step of ultrasound radiation, in a water bath, probes or sonoreactors, enhancing solvent penetration into soil and mass transfer (e.g., Santalad et al. 2010). More selective extraction procedures may simplify sample purification prior to a chromatographic analysis.

Cleanup. The pre-concentration step for pesticide CSIA and coextraction of matrix components requires cleanup strategies to purify soil extracts (e.g., Tadeo et al. 2012) and reduce co-enriched soil organic components of unknown isotopic composition. Among liquid–solid extraction techniques, the most common procedure is solid phase extraction (SPE) with cartridges or columns using sorbents, such as Florisil, silica, alumina, C18 or graphitized carbon black, depending on the selected pesticides (Buszewski et al., 2012). A current trend is to develop analytical methods where the extraction and purification are carried out simultaneously. Most soil QuEChERS approaches developed for pesticide CSIA rely on dispersive solid-phase extraction, in which a bulk amount of solid phase extraction sorbent is mixed with the extract rather than loading the extract in a column or cartridge packed with the sorbent. Primary Secondary Amine (PSA) sorbent was added to improve the cleanup of hexachlorocyclohexanes (Liu et al. 2020; Liu et al. 2021) chloroacetanilide (Perez-Rodriguez et al. 2021; Torrento et al. 2021; Perez-Rodriguez et al. 2021) and metalaxyl extracts (Masbou et al. 2018) with anhydrous magnesium or sodium sulfate as a cleanup and drying agent to remove soil matrix, ensure pesticide recovery and reduce GC-IRMS background. In the future, stir bar sorptive extraction (SBSE) coated with polydimethylsiloxane (PDMS) or newer materials may be used for additional clean up and enrichment following QuEChERS extraction of soil matrices for more selective pesticide extraction (Abdulra'uf and Tan 2014). For instance, molecularly imprinted polymers are synthetic polymeric materials with specific cavities designed for a template molecule that can retain the target pesticide (Farooq et al., 2018). Molecularly imprinted polymers could drastically reduce the matrix effect due to dissolved organic carbon for accurate C and N-CSIA measurements of 1*H*-benzotriazol, a typical corrosion inhibitor (Bakkour et al. 2018). In comparison with other methods, molecularly imprinted polymers offer high thermo-chemical stability and reusability and can be used for solid phase extraction as a highly selective sorbent for different types of pesticides in trace concentrations in soils (Farooq et al. 2018).

2.2 Isotope analysis of pesticides

Isotope ratios in pesticides are measured as the ratio of the relative abundance of heavy and light isotopes in a sample (with R_{sample} = abundance of heavy isotope/abundance of light isotope) and equation 1:

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \qquad \text{eq. 1}$$

using the international $R_{standards}$ given as e.g. in Aelion et al. (2010). The abundances of the heavy and light isotopes are generally quantified by an isotope ratio mass spectrometer (IRMS). Pesticides are often commercialized as formulations where the active molecule is mixed with additives in a solid or liquid matrix to facilitate their use. In order to trace its fate, only the abundances of isotopes from the active substance must be measured. Hence, it becomes obvious that purification and chromatographic separation of the pesticide is essential before measurement by IRMS. A large majority of isotope data on pesticides in this study (Supplementary Material Annex 1) were measured on gas chromatography coupled to an IRMS (GC-IRMS). For carbon isotope analysis of the polar herbicide glyphosate, the IRMS was coupled to liquid chromatography (LC-IRMS). And finally in an intermediate number of cases where pesticides were available as pure substances, the measurement was made on an elemental analyzer coupled to an IRMS (EA-IRMS). The latter is widely used in many labs since it permits measuring international standard materials as references and calibrate the IRMS by multiple point calibration (Schimmelmann et al. 2016). The three analytical techniques are illustrated in Figure 1 and are presented in the following in more detail.

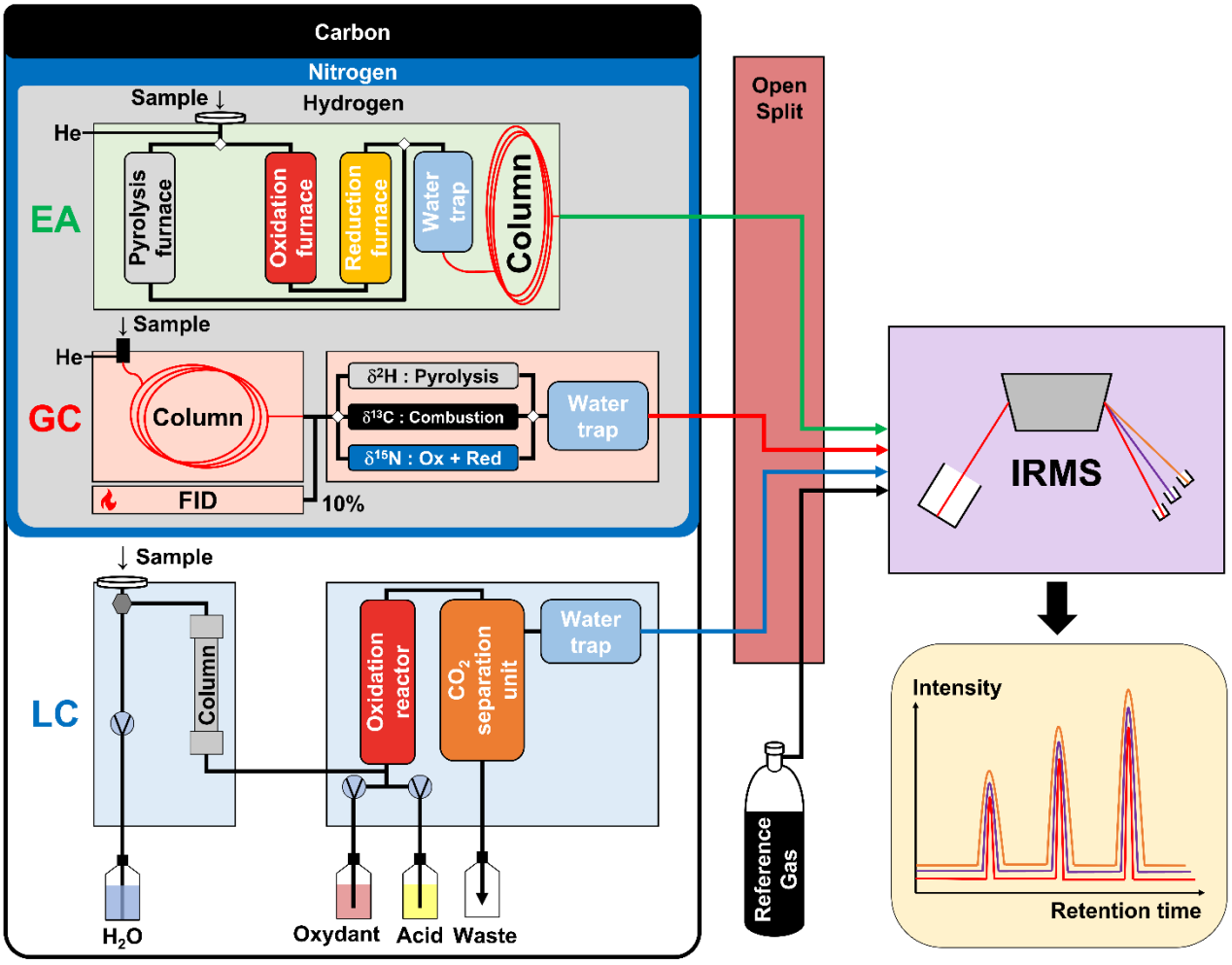


Fig. 1: Common analytical techniques for isotope ratios measurement in pesticide formulations and bulk samples, for the three most common elements carbon, nitrogen and hydrogen. EA: Elemental analysis. GC Gas chromatography. LC Liquid chromatography. IRMS Isotope Ratio Mass Spectrometry. He: Helium gas. Ox: Oxidation. Red: Reduction. FID: Flame ionization detector

EA-IRMS: The five elements H, O, C, N and S can be analyzed in elemental analyzers. However, sulfur isotopes were analyzed in pesticides in only one article (Schürner et al. 2015), and also only one single publication included oxygen isotopes in glyphosate (Sandy et al. 2013). Therefore, we present here mainly H, C and N analysis. For hydrogen isotope analysis, the sample is pyrolyzed at 1400°C in a stream of helium gas and the produced hydrogen gas is separated from other gasses, including CO and H_2O, by a chromatographic column and transferred via an open split system to the IRMS for the measurement of the abundance ratio $^2H/^1H$. The open split system was developed when continuous-flow IRMS became available and permits an accurate dilution of the sample. For C and N elemental analysis, the sample is combusted at 1000°C in an oxidation

furnace with a pulse of oxygen. Then, the nitrous oxides are reduced to N_2, and the latter is separated from CO_2 and other gases by the chromatographic column in the EA. The IRMS is first set to masses 28, 29, 30 to quantify the abundances of $^{14}N_2$, $^{14}N^{15}N$ and $^{15}N_2$ for the calculation of $\delta^{15}N$. The magnet of the IRMS is then switched by a peak jump to the masses 44-46 to quantify the CO_2 isotopologues with different masses. Correction for ^{18}O is made with the method of (Santrock et al. 1985). The mass of sample needed for accurate EA-IRMS measurement is mostly between 0.2 to 0.5 milligrams of pure pesticide, which is weighted in tin or silver capsules.

GC-IRMS. This is the most widely applied method for pesticide isotope analysis, especially for real field samples, capable of measuring isotope ratios of H, C or N (Fig. 1). The main differences compared to EA-IRMS is that chromatographic separation of the compounds is made before pyrolysis or combustion, and that the injected mass of compound is much lower. The mass of carbon to reach reliable IRMS measurements of carbon stable isotope ratio is typically around 10-15 nanograms (Aelion et al. 2010). Knowing this value, the minimum injection amount into the GC can be back calculated according to dilution factors in the injector. For the hydrogen analysis, again a pyrolysis is operated at 1400°C to produce H_2. For N and C analysis, a combustion furnace is used, also with reduction of NOx compounds to N_2. The analysis of ^{15}N in pesticides requires, however, a separation of N_2 from CO_2, which is done by freezing out the CO_2 in a cooled trap.

LC-IRMS. This technique has so far only been applied to C isotope analysis in the polar pesticide glyphosate (Fig. 1). The eluent was a 2.5 mM NaH_2PO_4 solution adjusted to pH 1.9. The reagents to convert glyphosate to CO_2 were phosphoric acid and peroxodisulfate in a reactor kept at 99.9°C. Then the CO_2 was flushed with He to the IRMS. Details are provided in (Kujawinski et al. 2013) and Mogusu et al. (2015). This technique may also improve knowledge on the persistence of the transformation product amino methyl phosphonic acid, or other mostly polar products, following degradation processes of pesticides in soils. Apart from the three most-used techniques shown in Figure 1, other techniques were used. They were:

GC-qMS. This technique couples a gas chromatograph to a quadrupole mass spectrometer and has been first described in 2007 (Sakaguchi-Soder et al. 2007) and first applied to the insecticide DDT in 2010 (Aeppli et al. 2010). It is only available to chlorine isotopes due to the large abundance of the ^{37}Cl isotope which contributes to about 24% of all chlorine atoms in the world. GC-qMS results have been compared in an interlaboratory study to continuous-flow IRMS (Bernstein et al. 2011) and proved to yield sufficient accuracy in isotope ratios when a correct bracketing was made using two standard compounds of different isotope ratios which lie slightly

outside of the measurement range of the samples. The technique was recently applied to chlorine isotope analyses in acetochlor, metolachlor and atrazine (Ponsin et al. 2019; Torrento et al. 2021).

GC-MC-ICPMS. This technique consists of coupling a GC to a Multi-Collector Inductively Coupled Plasma Mass Spectrometer, first described by (Van Acker et al. 2006). For pesticides it was only used for chlorine isotope analysis. In the plasma, the masses of ^{35}Cl and ^{37}Cl are generated for the calculation of the isotope ratio, but care must be taken not to count also the mass of ^{36}ArH when water vapor is reacting with argon gas (Horst et al. 2017). It has been applied in two studies looking at hexachlorocyclohexanes (Wu et al. 2019b), acetochlor and S-metolachlor (Lihl et al. 2019). GC-MC-ICPMS measurements of pesticides may open the way to analyze the stable isotope ratios of heteroatoms, such as bromine or sulfur, in pesticide molecules.

Offline MS. Other studies converted pesticide samples by off-line methods to gaseous molecules containing chlorine or bromine and injected them to Dual-Inlet IRMS for the analysis of ratios of $^{37}Cl/^{35}Cl$ (Drenzek et al. 2002; Drenzek et al. 2004; Reddy et al. 2002) or $^{81}Br/^{79}Br$ (Kuntze et al. 2016).

2.3 Isotopic compositions of pesticides from literature

Isotopic compositions of pesticides and of key degradation products were compiled from the peer-reviewed literature (Figure 2 and Supplementary material Annex 1). The main criteria for selection of isotopic compositions of pesticides were the existence of a reliable stable isotope composition of the active molecule. Publications in which the isotope analysis by EA-IRMS targeted commercial formulations, meaning the active molecule was not pure, were not included (e.g. (Annable et al. 2007). All hexachlorocyclohexane isomers were included although only the gamma isomer is a pesticide. The method of analysis is provided along with the isotopic composition. Isotopic compositions were found for 71 different compounds, in publications which appeared between 2002 and 2021. A total of 547 isotopic compositions were listed, with 337 values for ^{13}C, 75 values for ^{37}Cl, 78 for ^{15}N and 57 for ^{2}H. One publication was found for ^{18}O in glyphosate (Sandy et al. 2013), and one for ^{34}S in amethryn (Schürner et al 2015).

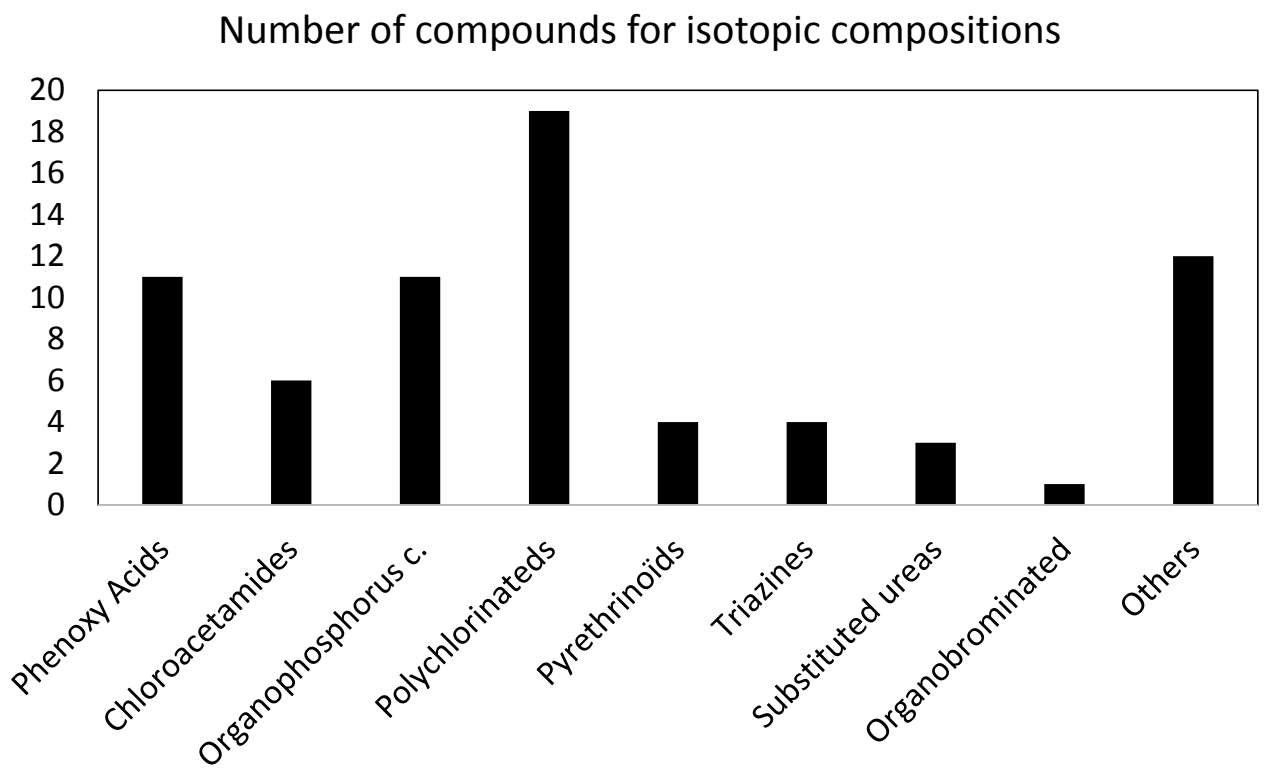


Figure 2: Pesticide classes for which isotopic compositions are reported in literature (see Supplementary material, Annex 1).

2.4 Isotope fractionation values of pesticides

Isotope fractionation values (enrichment factors) of pesticides and key degradation products were also compiled from the peer-reviewed literature (Supp. Mat. Annex 2). The criteria for selection included (i) the presentation of a well-described degradation pathway, (ii) a clear description of reaction conditions, and (iii) the quantification of isotope fractionation values from isotope data using the Rayleigh approach.

Isotope fractionation values were found for 32 compounds (Supp. Mat., Annex 2). A total of 263 isotope enrichment factors were retrieved, with 174 values for ^{13}C, 19 values for ^{37}Cl, 50 for ^{15}N, 13 for ^{2}H, and 7 for ^{81}Br, 2 for ^{34}S and 1 for ^{18}O. The brominated compounds all concerned reactions acting on the fumigant ethylene dibromide. For ^{13}C, all enrichments were normal (i.e., negative isotope fractionation values) except two for photooxidation of atrazine where inverse effects were observed. The most pronounced normal isotope effect (the most negative enrichment factor for ^{13}C, -30.9 ‰) was observed for the abiotic reduction of ethylene dibromide, a small compound with

only two carbon atoms, both positioned at reactive positions of the molecule. This resulted in a strong enrichment in ^{13}C, as observed for the reductive dechlorination of chloroethenes (see e.g. (Hunkeler et al. 2002)).

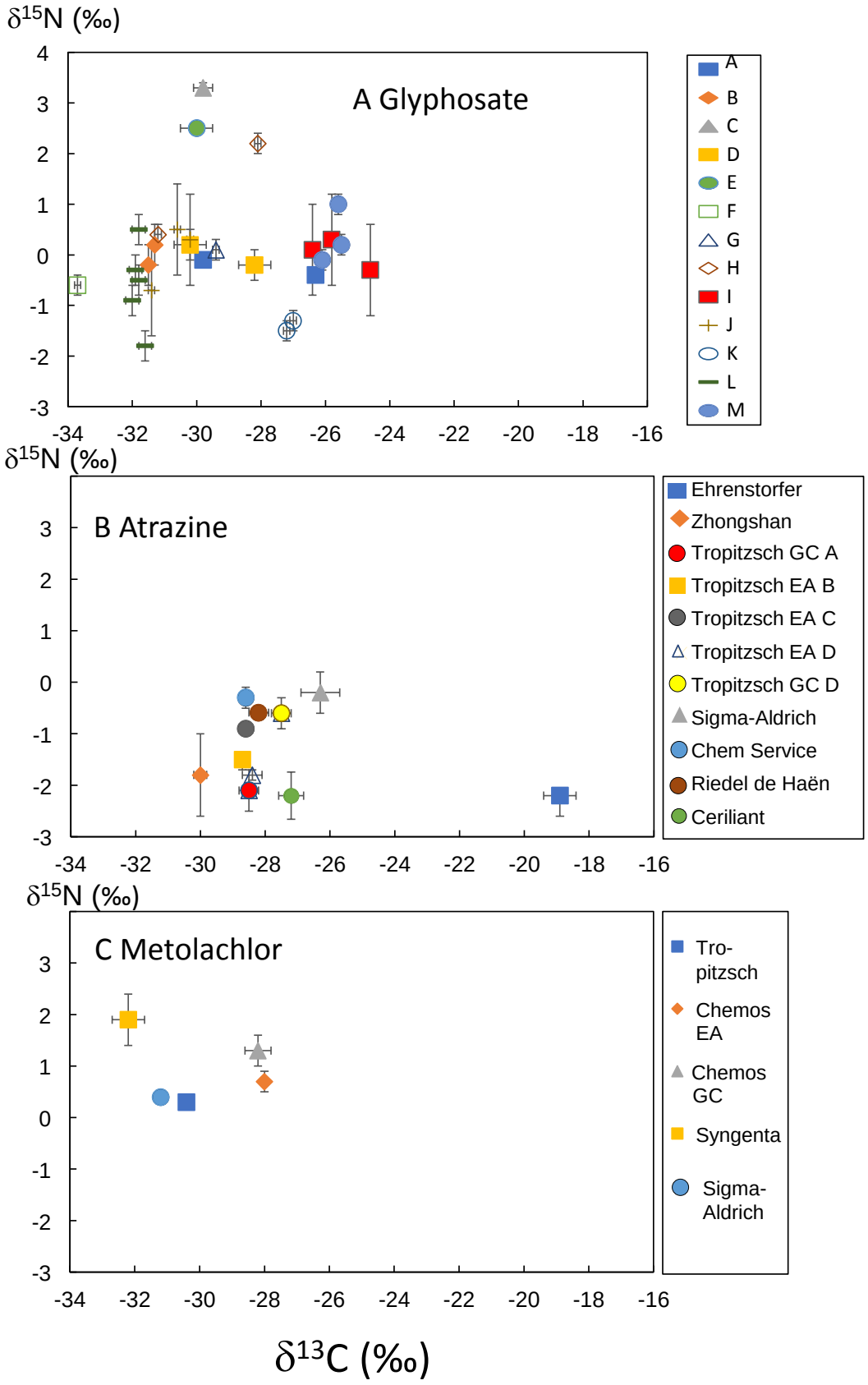
For ^{15}N, both inverse and normal isotope effects were observed for biotic transformations. Inverse effects with positive isotope fractionation values were observed for the biotic degradation of triazine herbicides (Schürner et al. 2015; Chen et al. 2019), the aerobic degradation of bromoxynil (Knossow et al. 2020) and also for the acid hydrolysis of atrazine (Meyer et al. 2009; Masbou et al. 2018).

The most pronounced normal isotope effect for ^{15}N was the alkaline hydrolysis of isoproturon, a substituted urea (Penning et al, 2008). In this compound family isotope effects were generally normal for ^{15}N (SI, Annex 2). An inverse effect was also observed for ^{15}N in atrazine photooxidation, like for carbon (Hartenbach et al. 2008).

3. Applications of pesticide ME-CSIA

3.1 Source identification, Forensics

Contaminations of surface waters, groundwaters or springs by pesticides are relatively frequent and call usually for actions to identify the polluters. The isotopic compositions of pesticides might help here for a better identification of the pollution source, especially when pesticides from different manufacturers have distinct isotopic signatures. For instance, a C and N CSIA of glyphosate (Mogusu et al. 2015) revealed that glyphosate is a molecule with a highly variable isotopic composition, depending on the commercial formulation (Fig. 3).


δ15N (‰)
A Glyphosate
A
B
C
D
E
F
G
H
I
J
K
L
M
δ15N (‰)
B Atrazine
Ehrenstorfer
Zhongshan
Tropitzsch GC A
Tropitzsch EA B
Tropitzsch EA C
Tropitzsch EA D
Tropitzsch GC D
Sigma-Aldrich
Chem Service
Riedel de Haën
Ceriliant
δ15N (‰)
C Metolachlor
Tro-pitzsch
Chemos EA
Chemos GC
Syngenta
Sigma-Aldrich
δ13C (‰)

368

Figure 3: Isotopic compositions of A) glyphosate from 13 manufacturers. Data from Mogusu et al. 2015, including also data from Kujawinski et al. 2013. For list of manufacturers see Supp. Inf. Annex A. B) atrazine from 7 manufacturers. Data from Chen et al (2017), Masbou et al. (2018), Meyer et al. (2008, 2013), Ponsin et al. 2019, Torrento et al. 2019+2021. Schreglmann et al. 2013. C) metolachlor from 5 manufacturers. Data from Alvarez-Zaldivar et al. (2018), Masbou et al. (2018) and Torrento et al (2021).

The $\delta^{13}C$ ratios of glyphosate from different sources span from -33.7 to -24.6 ‰, while those for $\delta^{15}N$ range from -1.8 to +3.3 ‰. Figure 3 shows that $\delta^{13}C$ alone bears already a good potential for discrimination between two manufacturers, and that the dual element isotope assessment may improve further distinction among distinct glyphosate sources. While the authors did not discuss why the manufacturing process leads to such a high variability, they concluded that this variability would allow forensic investigations in cases of pollutions of glyphosate or its key metabolite, AMPA.

Another famous legacy pesticide, atrazine, also features highly variable isotopic composition (Fig. 3B). However, the $\delta^{13}C$ ratios of atrazine span only from -26.3 to -30 ‰ except for the atrazine of Ehrenstorfer which is at - 19 ‰, while $\delta^{15}N$ ratios range from -2.2 to -0.2 ‰. This highlights that the power for discrimination of distinct atrazine sources is far below that of glyphosate. However, stable chlorine isotope analysis has been developed for atrazine (Ponsin et al. 2019). Further data on the stable chlorine isotope composition may help in the future to establish a more powerful basis for forensic investigations of atrazine pollution.

In contrast to glyphosate and atrazine, *S*-metolachlor analyzed from five manufacturers shows relatively little isotopic variation for carbon and nitrogen (Figure 3C), suggesting that the chlorine isotope analysis must be included for forensic studies relying on *S*-metolachlor (Torrento et al. 2021). If isotopic signatures of *S*-metolachor in commercial formulations do not vary significantly, transformation processes involving bond breaking may be followed up in the environment, independent of the used commercial formulation. This may allow catchment-scale evaluation of metolachlor transformation.

The largest forensic investigation of a pesticide so far found in literature concerned 26 different pure samples of the insecticide lindane (γ-Hexachlorocyclohexane), including three analyses of stable isotopes of carbon, chlorine and hydrogen (Ivdra et al. 2017). In addition, other samples from commercial formulations, soils or wastes were also analyzed, as well as other

hexachlorocyclohexane isomers. Lindane is a banned persistent organic compound which is still found in many soils worldwide. Stable isotope ratios of hexachlorocyclohexanes isomers and lindane covered the ranges from −233‰ to +1‰ for $\delta^{2}H$, from −35.9‰ to −22.7‰ for $\delta^{13}C$, and from −6.69‰ to +0.54‰ for $\delta^{37}Cl$.

To conclude this section, an example is presented where the stable isotope composition of a pesticide was used to elucidate a criminal food intoxication case, presented by Kawashima (2015). In December 2007 and January 2008, people suffered from food intoxication in two provinces of Japan after eating frozen dumplings (gyosa) imported from China. These dumplings contained very high concentrations (1490-19,290 ppm) of methamidophos, an organophosphorus pesticide. The $\delta^{13}C$ of methamidophos from seven Japanese formulations and a Chinese one was analyzed by GC-C-IRMS. The isotope signatures of methamidophos ranged from -31.9 to -49.2 ‰, potentially sufficient to distinguish most products without the addition of a second element. As this study permitted to clearly point the Chinese source for the food contamination, a worker in the Chinese food factory was arrested and later confessed the injection of stolen methamidophos into the food. The study highlights the interest to have a good trackability of pesticide sources as it is relatively easy for criminals to obtain pesticides and use them for poisoning.

3.2 Process identification by ME-CSIA

A common feature of diffuse agricultural pollution is that soil microorganisms cannot or only partly degrade organic pollutants after a long period of ageing in soils. This has been observed for instance for several banned or restricted agrochemicals, such as the *s*-triazine herbicides atrazine and simazine (Stucki et al. 1995), racemic metolachlor, the insecticide chlordecone (Martin-Laurent et al. 2014) or the soil fumigant 1,2-dibromoethane (Steinberg et al. 1987). All listed compounds are known to be degraded to different extents by microorganisms in pure cultures and soil microcosms under laboratory conditions. In contrast, in agricultural soils, degradation extent and pathways of degradation could often not be evidenced based on conventional monitoring methods, the molecules persist over decades, and they are still detected in the underlying groundwater or nearby surface waters. This phenomenon has been called dormant pollution and resurrection (Charles et al. 2021), and is also observable for some industrial pollutants, such as chlorinated hydrocarbons (Zimmermann et al. 2020).

The collected information on isotope fractionation values can be used to predict changes of isotopic ratios during the advancement of pesticide dissipation in soils. This is best illustrated in

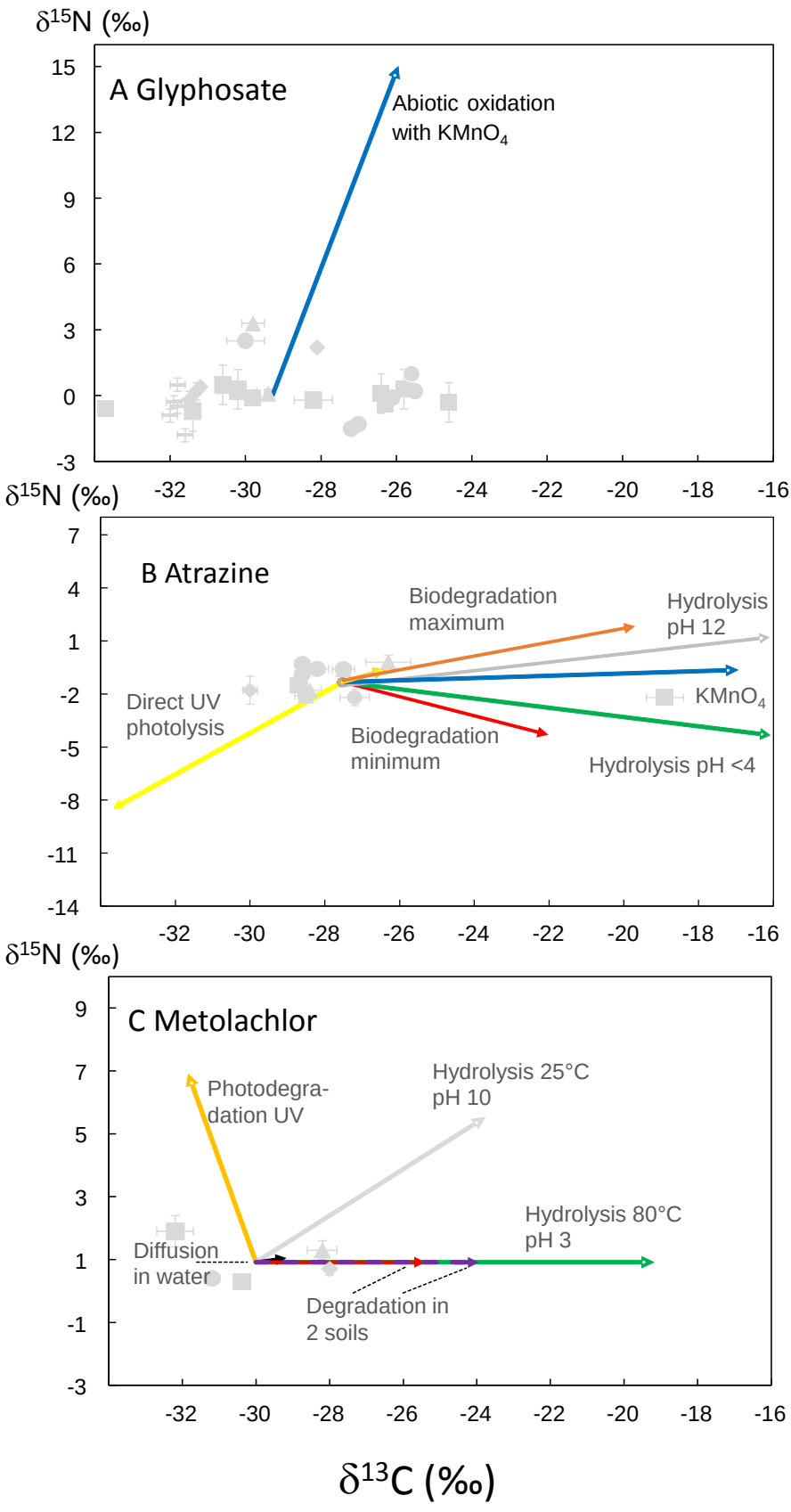
multi-elemental plots, like in section 3.1, for glyphosate, atrazine and metolachlor, for both carbon and nitrogen isotopes. Changes in the isotopic ratios for each process were calculated using the approximate Rayleigh equation (eq. 2), assuming a removal of 90% of the initial mass

$$\Delta\delta^{13}C = \varepsilon_C \ln(f) \qquad \text{eq. 2}$$

Where f is the remaining mass fraction in soil (0.1 in our calculations). Δ is the isotope shift with respect to the initial isotope composition. For the three compounds, we calculated an average isotope composition for all manufacturers, and drew calculated isotope shifts with arrows away from this average composition (Figure 4). It is worth noting that the slope of these arrows in Figure 4 corresponds to Lambda, which is generally used in the literature on ME-CSIA as (eq. 3, here for nitrogen and carbon isotopes):

$$\Lambda_{N/C} = \frac{\epsilon_N}{\epsilon_C} \qquad \text{eq.3}$$

Lambda (Λ) expresses the relative magnitude of isotope enrichment of two elements, but does not express absolute magnitude of enrichment, i.e., whether the final isotope ratios expected in samples which underwent a removal process are far away from the initial composition, or not. The Λ value may also be partly elusive since a process involving two inverse isotope effects for both elements may have the same Λ as a process with two normal isotope effects. Hence, tables of Λ were not printed in this review, but all Λ values can easily be calculated from the Excel sheet in annex 2.


δ15N (‰)
A Glyphosate
Abiotic oxidation with KMnO4
δ15N (‰)
B Atrazine
Biodegradation maximum
Hydrolysis pH 12
KMnO4
Direct UV photolysis
Biodegradation minimum
Hydrolysis pH <4
δ15N (‰)
C Metolachlor
Photodegra-dation UV
Hydrolysis 25°C pH 10
Hydrolysis 80°C pH 3
Diffusion in water
Degradation in 2 soils
δ13C (‰)

449

Figure 4: *Change of stable isotope ratios (^{15}N vs. ^{13}C) for selected processes acting on average glyphosate (A), atrazine (B) and metolachlor (C). Grey shaded points represent variable compositions from different manufacturers, already shown in Figure 3. Note that photolysis at natural sunlight does not create an isotope shift for atrazine (Drouin et al, 2021). $KMnO_4$: Potassium permanganate*

For glyphosate, only the abiotic oxidation with $KMnO_4$ was characterized for fractionation of both stable carbon and nitrogen isotopes (Fig. 4A redrawn from (Mogusu et al. 2015)). This oxidation leads to a strong N enrichment, with a much higher shift than the range of variability of different $\delta^{15}N$. The nitrogen isotope analysis may confirm the abiotic oxidation in soil even if the initial composition of the glyphosate was not known. In contrast, the carbon enrichment is small for abiotic oxidation, and carbon isotope analysis would not be able to confirm this removal process in soil, especially when the initial $\delta^{13}C$ of the glyphosate was not known.

The C and N isotope enrichment of atrazine was studied for various processes (Torrento et al. 2021; Drouin et al. 2021; Meyer et al. 2014; Meyer and Elsner 2013; Hartenbach et al. 2008), and for a range of pure bacterial strains known to degrade atrazine (Schürner et al. 2015; Chen et al. 2017; Chen et al. 2019, Fig. 4B). Generally large carbon isotope shifts are expected after 90% atrazine degradation, whereas nitrogen isotope shifts remain modest. However, nitrogen shifts are either positive or negative, which can help for process identification. But the slopes of the isotope shifts between various abiotic and biotic degradations of atrazine are not very distinctive, and for a correct process identification one should add also stable chlorine isotope analysis (Ponsin et al. 2019).

For metolachlor removal processes, large C and N isotope shifts are illustrated in Fig. 4C, with data from (Drouin et al. 2021; Masbou et al. 2018; Sun et al. 2021; Torrento et al. 2021). Photolysis under UV lamp and alkaline hydrolysis result in well-distinct isotope fractionation patterns in Figure 4C, while acid hydrolysis, soil degradation and diffusion in water do not differ in their slope Λ. They differ, however, in the magnitude of carbon isotope shifts: diffusion does almost not fractionate, and soil biodegradation leads to lower carbon isotope shifts than acid hydrolysis. To tease apart those two processes, additional analyses are needed. It is also worth noting that enantiomers of chiral pesticides, such as metolachlor, have identical physico-chemical properties, and transport processes or abiotic reactions appear not to change enantiomer proportions (Bidleman et al. 2013). In contrast, degradation of chiral pesticides may be mainly microbiologically mediated and enantioselective (Liu et al. 2005). Because degradation rates often differ among enantiomers and

result in deviation from racemic ratios, changes in the enantiomeric fraction (EF) of metolachlor may be used to follow degradation (Hegeman and Laane, 2002; Milosevic et al, 2013). In particular, combining CSIA and enantioselective analysis techniques, enantioselective stable isotope analysis (ESIA) may enhance the evaluation of sources and transformation processes of chiral micropollutants in the environment (Badea and Danet, 2015).

3.3 Identification and modeling of pesticide degradation in soil and across the soil-hillslope-water continuum

Isotope data derived from ME-CSIA require adapted modeling approaches to interpret and predict the persistence and the transport of pesticides in soil and water. Reactive transport models can support this deployment as they explore numerically and quantitatively the interactions of physical, chemical and biological processes in surface and subsurface systems (Li et al. 2017, Prommer et al. 2019). Historically, the first reactive transport models integrating isotopic signatures aimed at interpreting monitored natural attenuation of legacy industrial pollutants in aquifers, e.g. (Hunkeler et al. 2008; Thouement et al. 2019; Antelmi et al. 2021) or in wetlands e.g. (Alvarez-Zaldivara et al. 2016). Some reactive transport models incorporating isotope fractionation also derived the physico-chemical conditions, including redox conditions, dissolved oxygen, temperature, or pH, to predict pollutant degradation and associated isotopic fractionation across the aquifer domain (D'Affonseca et al. 2011).

While soil reactive transport models evaluating pesticide persistence and transport exist since decades (Gassmann 2021), models including pesticides CSIA or ME-CSIA data remain scarce. Indeed, ME-CSIA of pesticides was limited and no isotopic dataset existed in the early 2010. Nevertheless, the virtual 2D hillslope reactive transport model HydroGeoSphere, including pesticide CSIA data ($\delta^{13}C$ and $\delta^{2}H$) across the soil-hillslope-waters continuum (Lutz et al. 2013), highlighted the potential to examine pesticide degradation on the catchment scale. Worthy of note, pesticide transport was simplified in HydroGeoSphere by neglecting volatilization and sorption processes, assuming soluble, non-volatile and non-sorptive pesticides. A main limitation of lumped approaches, i.e., considering the catchment as an undivided entity (Surdyk et al. 2021), is to neglect spatial information, including soil heterogeneity, water content or temperature, hampering the capacity to identify degradation hot spot across the catchment (Grundmann et al. 2007). Combining carbon CSIA data ($\delta^{13}C$) of the herbicide S-metolachlor in a river and a parsimonious lumped agro-hydrological model may, however, improve interpretation of pesticide transport and degradation at the catchment scale (Lutz et al. 2017). Complementarily to a soil-hillslope-waters

514 scale approach, modeling of water-sediment phase transfer or mass transfer limited transport
515 supported lab-scale biodegradation of pesticides with CSIA for complex systems, including
516 groundwater (Sun et al. 2021) or river-sediment interface (Droz et al. 2021).

517 There are several reasons explaining the limited development and use of soil reactive transport
518 models incorporating pesticide ME-CSIA data, which are also opportunities (Table 1). First, an
519 isotopic signature database of pesticides in commercial formulations is necessary for ME-CSIA in
520 soil reactive transport models to identify sources and processes (see sections 2.3; Table 1). If 546
521 isotopic compositions of pesticides were found in the literature, only 13% of them were associated
522 with commercial formulations. These isotopic compositions of applied pesticides can differ from
523 analytical standards often used for reference laboratory experiments (see section 2.3). Whenever
524 the range of $\delta^{13}C$ of the applied formulations (Figure 3) is larger than $\Delta\delta^{13}C$ during pesticide
525 degradation, it becomes difficult to evaluate the contribution of degradation in soil or water. At the
526 field scale, a unique commercial formulation may be used, allowing an evaluation of degradation
527 (e.g., Alvarez-Zaldivar et al, 2018). At larger scale, e.g., from a few hectares to hundred km^2, a
528 survey of the main commercial formulations is required to constrain $\delta^{13}C$ and $\delta^{15}N$ initial values of
529 target pesticides and and to reduce the uncertainty to identify pesticide sources and dissipation.

530 In addition, estimating isotope fractionation values (e.g., ε_c) from reference laboratory experiments
531 under controlled conditions (e.g., a degradative process occurring under a specific redox condition)
532 is a preliminary requirement to evaluate in situ degradation (Centler et al. 2013; Fischer et al.
533 2016). However, the reference isotope fractionation values should be carefully selected prior to
534 interpreting and predicting *in situ* biodegradative processes in the field. It is thus essential that such
535 isotope fractionation values correspond to the established hydrochemical conditions *in situ* and the
536 corresponding degradation pathways. Bacterial isolates associated with micropollutant degradation
537 are less known than bacteria associated with degradation of industrial solvents (Elsner and Imfeld
538 2016). This severely limits the field interpretation of degradative processes and definition of
539 remediation strategies. It has been suggested that degradation of micropollutants may rather occur
540 via co-metabolism, where enzymes may no longer be harbored by a single microorganism only but
541 may be distributed in a range of microbial populations constituting the microbial community
542 (Fenner et al. 2021). Therefore, microcosm experiments with the targeted soil and pesticide remain
543 an essential step identify the degradation mechanisms and to derive isotope fractionation values
544 associated with complex degrading microbial communities for *in situ* application of ME-CSIA
545 (Alvarez-Zaldivar et al. 2018). Additional characterization of transformation products of the targeted

546 pesticides may also help identify ongoing degradation pathways both in the reference microcosm
547 experiments and at the field scale.

548 In addition, degradation is generally represented with a lumped soil half-life parameter, hampering
549 the incorporation of ME-CSIA data in soil reactive transport model codes (Table 1).

550 ***Table 1****: Challenges, bottlenecks and opportunities to identify and predict pesticides degradation in*
551 *soil with CSIA and ME-CSIA in soil and across soil-hillslope-water continuum.*

Challenge	**Bottleneck**	**Opportunities**
Source tracking - Isotopic signature ($\delta^{13}C$, $\delta^{15}N$, $\delta^{37}Cl$, …) of targeted pesticides?	Limited knowledge of isotopic signatures of pesticides in commercial products	i) Isotopic signature database for commercial formulations of applied and legacy pesticides, ii) Characterization strategy from field to catchment scales
Processe identifications - Validity of reference stable isotope fractionation factors (ε_C, $\varepsilon_{N,}$ $\varepsilon_{Cl, ...}$)?	Transposing reference stable isotope fractionation factors to interpret pesticide degradation in soil in relation with soil characteristics	Evaluating the impact of soil physico-chemical and microbial conditions on stable isotope fractionation for field estimation of pesticide degradation
Reactivity - *Reactivity* - Open the degradation black-box in soil reactive transport models by considering impact of soil bio-physico-chemical properties and by differentiating degradation pathways?	Degradation in soil reactive transport models as a lumped process with one soil half-life parameter	i) Introducing in soil reactive transport models the effect of soil temperature and moisture and the soil biological activity on degradation intensity, ii) considering different degradation pathways in soil reactive transport models

Transport - Integrate preferential flows in soil reactive transport models for better prediction of pesticides transit time?	Mischaracterization of preferential flow in soil reactive transport models and their impacts on pesticide leaching	i) *in situ* measurements with geophysical techniques and tracer experiments for soil profiles, ii) geostatistical methods, pedotransfer functions or meta-models to parametrize preferential flow in soil reactive transport models

Therefore, the use of a single reference isotope fractionation factor (see sections 3.1 and 3.2) may bias the interpretation of pesticide degradation by neglecting multiple and possibly simultaneous degradative processes in soil. Here, the effect of soil temperature and moisture and the soil biological activity on degradation intensity may be introduced in soil reactive transport models to strengthen and expand the description of degradation within the catchment and across the hydrological or growing season (Gassmann 2021), even when degradation is considered as a lumped process.

Finally, a major interest of introducing CSIA data in soil reactive transport models is to prove, characterize and quantify pesticide transformation. This, however, depends on the ability to interpret the transport of water and pesticides by preferential and matrix flows (Jarvis et al. 2012; Köhne et al. 2009a+b; Beven and Germann 2013; Gassmann et al. 2015; Weiler 2017). Including preferential flow framework in soil reactive transport models may help to improve prediction of transit times in soil. Here, CSIA may further help tease apart fast flows, mobilizing “fresh” pools of pesticides with isotopic signatures mirroring that of the applied products, from the “aged” pools of pesticides in soil, reflecting degradation in the soil matrix. *In situ* measurements of macropores, e.g., earthworm channels, soil pipes and larger soil cracks, in the soil profile may help identifying preferential flow and providing parameter values for soil reactive transport models using geophysical techniques and tracer experiments (Weiler 2017). At the hillslope and catchment scales, geostatistical methods, pedotransfer functions or meta-models, i.e., simplified statistical models built with 1D soil reactive transport models such as MACRO (Lindahl et al. 2008), may provide spatially distributed parameters. This, in turn, allows predicting preferential and soil matrix flows in Horizontal 2D soil reactive transport models with associated pesticide loads (Jarvis et al. 2012).

4. Conclusions and perspectives

ME-CSIA was originally developed for industrial pollutants, which are frequently encountered in groundwater at concentrations often in the mg/L range. These organic pollutants have often relatively small molar masses and are therefore prone to undergo relatively large isotope fractionations. ME-CSIA applied to pesticides was originally thought to be hardly possible, especially since they occur mostly in the µg/L range as diffuse sources and are rather large molecules which might exhibit little isotope fractionation. However, this review shows that large current and future improvements of extraction techniques of pesticides from soils, combined with lower limits of detection of analytical techniques, and new analytical methods make it possible to apply ME-CSIA to study, follow and predict pesticide degradation in soil. It is shown also that this opens ways to model the fate of pesticides on the catchment scale, which may help to design new management practices.

While a lot of recent literature summarized in this work have significantly advanced science to use ME-CSIA for a better understanding of the fate of pesticides at the field scale, prominent research gaps still remain that must be tackled in the future.

First, our database of isotopic compositions of pesticides is strongly lacking analyses of pesticides from commercial formulations available on the market worldwide. More work is thus needed to systematically analyze commercial formulations from different manufacturers and to study their variability from past to present. In the framework of the French project who is funding this research, over 120 commercial formulations available on the French market of pesticides have been collected and are currently analysed for better insight to be provided in a follow-up publication.

In addition, the database of isotope fractionation values of pesticides during degradation processes has mainly been built on laboratory experiments performed at relatively high concentrations of active molecules. Processes in real soils, in contrast, occur mostly at low concentrations. More work is thus needed to investigate whether at low concentrations the same fractionation patterns occur as at high concentrations. Depending on whether substrate uptake into the microbial cell, or slow enzyme kinetics is rate-determining, the extent of stable isotope fractionation may also be smaller than expected (Elsner and Imfeld, 2016). More reference biodegradation studies involving bioavailability limitations and aging of residues in soil should be performed.

Third, the analytical techniques so far developed are not equally good for all elements in pesticide molecules. As shown in the supplementary material, isotope analysis of ^{13}C is well developed and practiced in many labs. Fewer but still abundant analyses were made for ^{15}N and ^{37}Cl. For these three isotopes, accuracy is generally better than ±0.5 ‰. For ^{2}H, less measurements were made so far. The robustness of ^{2}H analysis is hampered by the low natural abundance of deuterium and the presence of water vapor in mass spectrometers and yields at best an accuracy of ±5 ‰.

Furthermore, the presence of chlorine atoms in a pesticide can cause problems during the pyrolysis of the molecule before mass spectrometry of the produced H_2. Only very few studies of isotope analysis of other elements in pesticides, such as ^{81}Br (1 study, Kuntze et al. 2016), ^{18}O (1 study, Sandy et al. 2013), or ^{34}S (1 study, Schürner et al. 2015) were found in literature. Future research may foster the development of stable isotope analysis for these elements to strengthen the ME-CSIA approach and enhance the distinction between transformation pathways in both laboratory and field settings.

More fundamentally, the mechanistic understanding of isotope fractionation and related processes should still be improved. Fractionation by diffusion has been studied in soil air (e.g. Bouchard et al. 2008), water (Jeannottat and Hunkeler, 2012; Sun et al. 2021) or clay (Wanner and Hunkeler, 2015). More knowledge should be gained on isotope fractionation of pesticides during diffusion in narrow soil pores, since this may be affected by Knudsen diffusion, where molecules are subject to collisions with pore walls, in addition to collisions with other gas or water molecules. Also, the fundamental understanding of isotope fractionation by sorption/desorption during multi-step partitioning and volatilization should be improved. For instance, a recent study by Zamane et al (2020) showed quite large isotope effects during volatilization of toluene and propan-2-ol from sandy soil.

Finally, while stable isotopes are good markers of different sources of pollutants, and markers of sinks, it remains difficult to tease apart both multiple sources and fractionating sinks. Combined source apportionment and degradation quantification of organic pollutants with ME-CSIA has been shown to be possible in groundwater (Lutz van Breukelen, 2014) but its adaptation to soils has not yet been developed.

Supplementary Material:

Annex 1: Table of isotopic compositions of pesticides from Literature. Annex 2: Table of enrichment factors from Literature. Both tables are integrated in the EXCEL sheet given in the supplementary material on the journal website.

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