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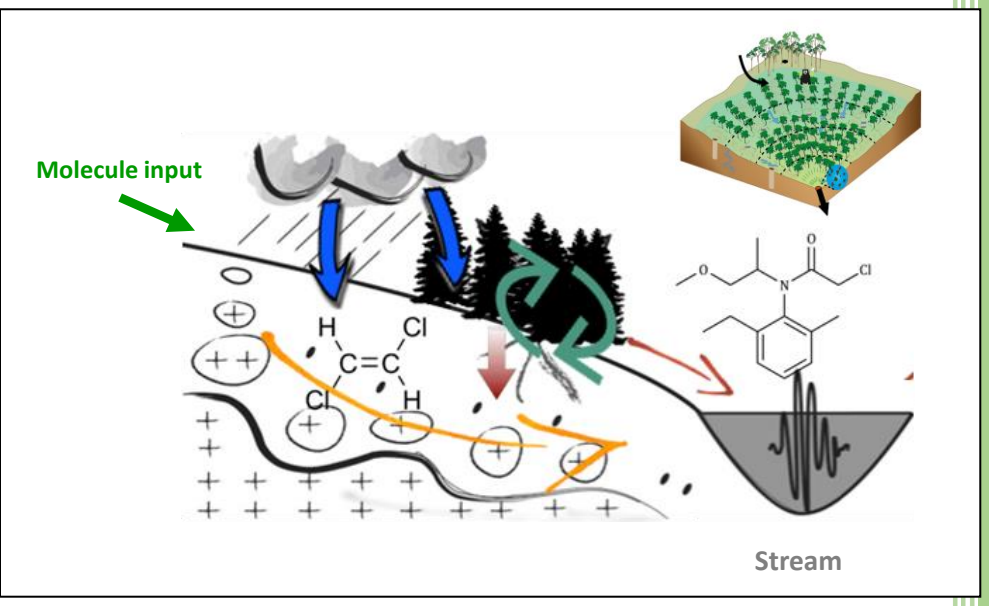
Guideline for assessing degradation and/or source identification of pesticides in soil by compound-specific isotope analysis (CSIA)



HOHENER Patrick et al.

Report: Final product of the project DECISIVE sponsored by the French National Science foundation ANR

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Notice

The French National Science Foundation ANR funded the research project DECiSIvE - Tracking degradation of soil pollutants with multi-elemental compound-specific isotope analysis through grant ANR-18-CE04-0004-01. The project lasted from 2019 to September 2023. This guideline is the final deliverable of the project Decisive and is distributed free of charge to any end user.

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Foreword

Despite the pioneering work conducted in the early 2000's within the framework of the EU soil thematic strategy (Van-Camp et al., 2004), the European Commission has still not launched the soil protection directive. This has become a crucial issue as we all know that soils are non-renewable resources at human-life span. They host a huge diversity of living organisms and together support many ecosystem services crucial for agricultural production, for climate change mitigation and adaptation as well as for the preservation the 'One Health'. The EU commission recently wrote a report indicating that 95% of European foods comes from soils and that 70% of European soils are degraded and unhealthy causing annual loss of 50 billion €. Soil degradation is mainly due to human activities and is an important threat for the key objectives of the European Green Deal. For this reason, the European Commission recently launched a soil strategy for 2030 which aims to set a vision to have healthy soils by 2050 by setting up a range of measures to protect, restore, use soils in sustainable way. To achieve this strategy, EU will establish a new Soil Health Law to provide a comprehensive legal framework for soil protection.

Within this context soil monitoring in EU is more and more an important issue as it is necessary to monitor changes in indicators of soil quality. EU Soil Observatory, Lucas soil program (Orgiazzi et al., 2018) and national soil survey programs such as RMQS (Réseau de la Mesure et de la Qualité des Sols, (Arrouays et al., 2003)) are conducted to establish references to monitor soil quality. Among the pressures exerted on soil resources, agrochemicals (fertilizers and pesticides) used for conventional agriculture to ensure the quality of crop production, are of great concern. Indeed, (Silva et al., 2019) showed that 80 % of 317 agricultural top-soils collected in 2015 in 11 EU member states and 6 main cropping systems were contaminated with pesticide residues. This study reveals that 'the presence of mixtures of pesticide residues in soils are the rule rather than the exception'. Later on, (Riedo et al., 2021) showed that pesticide residues can be found in all the 100 fields sampled in Switzerland conducted under organic and conventional management. Up to 16 different pesticide residues were detected in organic soils even 20 years after their conversion. More recently (Froger et al., 2023) showed that 98 % of the 47 soils sampled across France under a range of land uses were contaminated with pesticide residues including areas supposed not to be exposed to pesticides (organic fields, forests and grasslands). In these three studies, glyphosate and AMPA, its main metabolite, were the main contributor to the contamination of soils, highlighting persistence longer than that expecting regarding half-life reported in the homologation dossier questioning the real persistence of pesticides in soils.

Until now detection of pesticide residues in soils relies classically on their extraction and further analyzes using high-performance liquid chromatography coupled to a quadrupole mass spectrometer (HPLC-MS/MS) and/or gas chromatography coupled to a triple quadrupole mass spectrometer (GC-MS/MS). These two analytical methods allow the detection and quantification of the pesticides and of their known metabolites found in soil samples. The recent implementation of suspect screening using QTOF mass spectrometry allows the unknown metabolites of the active

compound contained in soil samples (Storck et al., 2016). This gives insight in the transformation of pesticide ongoing in soils but not allows the discrimination of the relative importance of abiotic and biotic processes involved in its transformation. Compound stable isotope analysis (CSIA) has the potential to overcome this limitation by not only giving information on the processes involved in the transformation of the pesticide in soils but also by tracking the origin of the observed pesticide contamination (Höhener et al., 2022).

However, CSIA remains a confidential method mastered by a handful of expert laboratories and cannot readily be implemented in European or national soil survey program. Therefore, within this context, this guideline is aiming to give the basics to assess degradation and/or source identification of pesticides in soil by compound-specific isotope analysis (CSIA).

June 16, 2023, Fabrice Martin-Laurent

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Acronyms

AKIE	Apparent Kinetic Isotope Effect
CSIA	Compound-specific isotope analysis
EA	Elemental analysis
GC	Gas Chromatography
GC-C-IRMS	Gas Chromatography coupled to Isotope Ratio Mass Spectrometry via a combustion interface
GC-qMS	Gas Chromatography coupled to Quadrupol Mass Spectrometry
IRMS	Isotope Ratio Mass Spectrometry
IUPAC	International Union of Pure and Applied Chemistry
LC	Liquid Chromatography
KIE	Kinetic Isotope Effect
ME-CSIA	Multi Elemental Compound-specific isotope analysis
PSIA	Position-specific isotope analysis
NMR	Nuclear Magnetic Resonance
VCDT	Vienna Cañon Diablo Troilïte
VPDB	Vienna Pee Dee Belemnite
VSMOC	Vienna Standard Mean Ocean Chloride
VSMOW	Vienna Standard Mean Ocean Water

Symbols

Symbol	Parameter	Unit	Reference/Comment
f	Fraction of compound which has not reacted (Remaining fraction)	No unit	
Index $_h$	Index designating the heavy isotope		
Index $_l$	Index designating the light isotope		
Index $_p$	Index designating the product in a transformation reaction		
Index $_s$	Index designating the substrate		
ln	Natural logarithm		
log	Logarithm of 10		
R	Isotope ratio	No unit	
$R_{standard}$	Isotope ratio of a standard	No unit	
R_0	Isotope ratio of initial compound (before transformation)	No unit	
α	Fractionation factor	No unit	
α_{bulk}	Average fractionation factor over the whole compound	No unit	
$\alpha_{reactive\_position}$	Fractionation factor observed at a reactive position in compound	No unit	
δ	"delta" notation	‰	
$\Delta\delta$	Change of δ with respect to its initial value δ	‰	
ε	Enrichment factor	‰	
Λ	Slope of a regression in a graph of multi-elemental isotope data	No unit	

For further definitions see Annex III

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Executive Summary

Monitoring and management of polluted environmental systems such as groundwater aquifers, soils or sediments requires generally insights into the changes of concentrations of pollutants and their fate. Every risk analysis considers the pathways of pollutant losses, such as volatilization, transformation (biotic or abiotic), sorption and sequestration, runoff, and dilution. While transformation may convert the pollutants to innocuous products and therefore might be regarded as a positive process, other processes, including runoff, dilution of volatilization do not result in a net decrease of contaminant mass and environmental risks in the environment, and are thus considered as inacceptable.

The traditional approach of monitoring a reduction in the concentrations of contaminants in polluted systems is often not able to demonstrate that the contaminants are actually being transformed to harmless products. When data on concentrations are the only data available, it is difficult or impossible to prove transformation in presence of co-occurring processes such as dilution or runoff. However, when organic contaminants are transformed in the environment, the ratio of stable isotopes will generally change, while the extent of transformation can be recognized and predicted from the change in the ratio of stable isotopes. Recent advances in analytical chemistry makes it possible to perform Compound-Specific Isotope Analysis (CSIA) on various organic compounds, targeting mostly the carbon isotopes $^{13}C/^{12}C$, but also other stable isotopes such as hydrogen, nitrogen, or chlorine.

Compound-specific isotope analysis (CSIA) of organic pollutants was developed at the end of the 1990s and first applications emerged thereafter for two classes of industrial pollutants, namely hydrocarbons and chlorinated solvents. In 2008, CSIA was approved by the US Environmental Protection Agency as a complementary assessment method for monitoring the remediation of contaminated sites, and a guideline was published with the goal to promote and harmonize the use of the new tool CSIA (Hunkeler et al., 2008). Two years later, a complete book on CSIA for bioremediation of contaminated sites was published (Aelion et al., 2009). At that time, applications of CSIA were basically all for dissolved industrial pollutants at relatively high concentrations in aqueous solutions, mainly groundwater. The isotope analysis of such high concentrations of mostly apolar pollutants in groundwater with low matrix effect was relatively easy.

To open the application of CSIA to other pollutants in various contexts, many new developments had to be made. For instance, for pesticides in soils, one is confronted with very low concentrations of mostly polar compounds on one hand, and high matrix effects in soil water and soil runoff due to the presence of natural organic matter. Furthermore, abiotic processes such as photochemistry, sorption, or chemical reduction have to be considered. To distinguish between these processes, two or more isotope systems are increasingly applied to do multi-elementary CSIA. Novel isotope analysis techniques have been developed in the last years to measure isotopes such as $^{37}Cl/^{35}Cl$, $^{81}Br/^{79}Br$, $^{18}O/^{16}O$, or $^{34}S/^{32}S$ in organic pollutants. With the appearance of many new research studies on all these aspects after 2010, the authors of this work

resented that a publication of a new guideline devoted to soil contaminants, especially pesticides, would be a welcome addition to literature. The French National Research Foundation kindly funded the project DECISIVE to fill some knowledge gaps on soils pollutants by experimental work, retrieve further published studies from literature and elaborate such a new guideline.

The guideline starts with a chapter on sampling strategy and sample preparation, focusing in detail on the extraction of pesticides from soil, and sample clean-up before isotope analysis. It is essential to take care that during all these procedures the isotope ratios stay constant, or to quantify isotope shifts if they occur in a reproducible manner. The next chapter thus provides the basics for the most-applied isotope analysis techniques and highlights the importance of using standards for referring to the international isotope scales, and to monitor the stability and the linearity of the instruments. The following chapter presents basic concepts and practices to follow-up the fate of pesticide in field experiments using CSIA and eventually modelling. A further chapter is devoted to track the sources of pesticides by using CSIA and presents a database of measured isotopic compositions of pesticides in either pesticide analytical standards, or pesticides from manufacturers in commercial formulations. Finally, a collection of equations is given that permits the evaluation of isotope data obtained by CSIA, notably to relate the change in isotope ratios to the progress of pollutant transformation. These relationships require the use of fractionation factors (or enrichment factors) which must be characterized in laboratory studies in reference experiment under controlled conditions. A large collection of these factors is given in the second-last chapter of this guideline, which ends thereafter with all references to the cited studies.

The annex of the guideline provides a list of definitions of terms used in this work, tables of data on isotopic compositions of pesticides and fractionation factors, and finally a list of addresses of laboratories which might offer compound-specific isotope analysis of organic soil pollutants.

1. Introduction

Contamination of soils by organic compounds such as pesticides, polycyclic aromatic hydrocarbons (PAHs) or chlorinated solvents is a widespread problem in agricultural soils as well as urban and industrial sites. On one hand, leaching of organic contaminants from the soil into surface and groundwater may cause severe deterioration of the soil and water ecosystems, affect their sustainable uses. On the other hand, uptake of recalcitrant pesticides into crops can lead to human health risk and economic loss for farmers and distributors. Although the intended 50% reduction of pesticide use in agriculture (e.g., Plan Ecophyto in France, https://agriculture.gouv.fr/le-plan-ecophyto-quest-ce-que-cest) as well as clean and green chemistry in industry may give hope for a future change of this situation, the problem of persistent historical soil pollutions still needs to be solved. A common issue of both diffuse agricultural pollution and industrial point-source pollution is that soil microorganisms cannot or only partly degrade organic pollutants after an extended period of ageing in soils. This has been observed for instance for several banned or restricted agrochemicals, such as *s*-triazine herbicides atrazine (Stucki et al., 1995) and simazine, or racemic metolachlor, the insecticide chlordecone (Chevallier et al., 2018) or the soil fumigant 1,2-dibromoethane (Steinberg et al., 1987). All listed compounds are known to be degraded by microorganisms in pure culture and soil microcosms under laboratory conditions. In contrast, in field soils, degradation extent and pathways could often not be evidenced based on conventional monitoring methods, the substances persist over decades, and they are still detected in the underlying groundwater or nearby rivers. The same phenomenon is observable for industrial pollutants like PAHs and chlorinated hydrocarbons.

At contaminated urban sites, there is a strong need for risk characterization, management, and definition of the remediation strategy. A variety of tools were developed that help to carry out these tasks. Following the year 2000, compound-specific isotope analysis (CSIA) was proposed to characterize the extent of degradation of industrial pollutants (Phillips et al., 2022). This then new analytical tool was based on the quantification of heavy and light stable isotopes in the remaining pollutants and since bacteria generally transform pollutants with light isotopes faster than pollutants with heavy isotopes. Several studies have demonstrated that the quantification of degradation with CSIA is possible for typical industrial pollutants like hydrocarbons or chlorinated solvents. The US EPA approved CSIA as a tool for site characterization and published a guideline on CSIA in 2008 (Hunkeler et al., 2008). Ongoing research thereafter has extended CSIA to multi-elementary compound-specific isotope analysis (ME-CSIA) by including isotope analysis of different elements in one organic pollutant. See the Text Box 1 for an explanation how ME-CSIA works.

An advantage of multi-elementary compound-specific isotope analysis (ME-CSIA) in the context of persistent organic pollutants in soils is to enable assessment of transformation rates and mechanisms without detection of transformation products that might evade targeted chemical analysis. The underlying hypothesis is that the occurrence, reaction pathways and extent of degradation of persistent organic pollutant in soils is not well understood, thereby leading to sub-optimal use and management of industrial and agricultural soils. This results in runoff to surface waters, contamination of fish and

potential biomagnification in the food chain and leaching to groundwater and contamination of drinking water resources and aquatic ecosystems, as well as risk and economic loss when adequate remediation strategies cannot be selected.

This guideline aims at presenting and applying the innovative approach of ME-CSIA for understanding and evaluating the nature of slow transformation of organic pollutants in soil, with a special emphasis on diffuse pollutions at small concentrations. For point-source contaminations and high concentrations, the reader is referred to the EPA guideline (Hunkeler et al., 2008).

ME-CSIA investigates isotopic enrichments in the remaining non-degraded pollutants to draw conclusions on which process has acted on them and to which extent degradation is achieved. The way how ME-CSIA works ideally is shown in the Text Box and in Figure 1. Stable isotope fractionation for pesticides and other listed persistent pollutant in soil has very rarely been described so far.

TEXT BOX 1: How does ME-CSIA work?

Isotope ratios of two elements (e.g., carbon and nitrogen in a s-triazine herbicide) are measured in a specific compound in both the pure product from the principal manufacturer and in soil samples. The evolution of the isotope ratios can be followed in laboratory experiments under controlled conditions for different abiotic and biotic destructive or non-destructive dissipation processes, and fractionation factors for each process are determined.

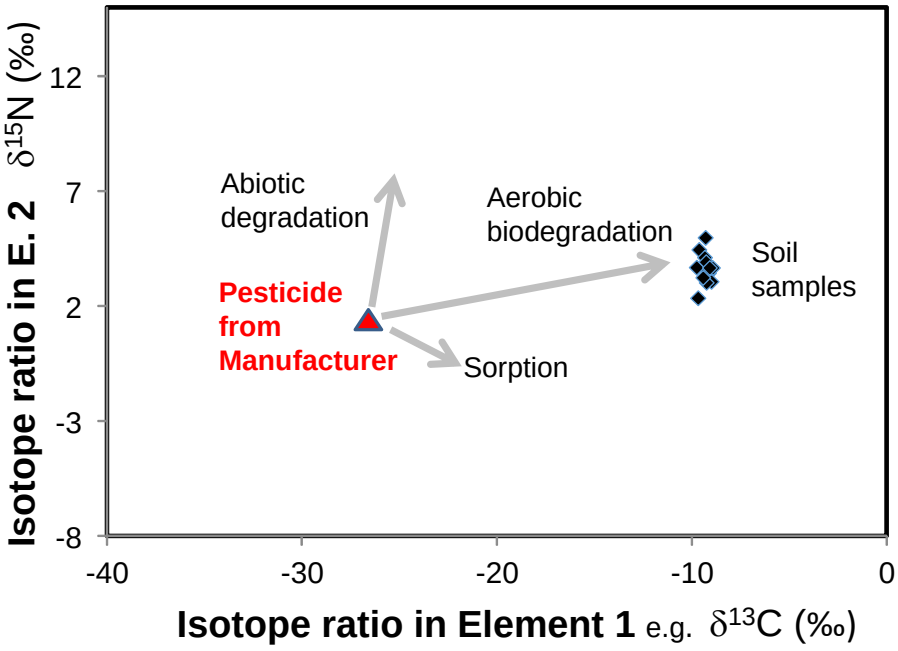


Figure 1: Two-dimensional application of Multi-Element – Compound-Specific Isotope Analysis: Hypothetic example for a s-triazine pesticide with stable carbon and nitrogen isotopes

Text Box 1 continued

In the case of s-triazine, it could be that abiotic degradation results in a strong enrichment of ^{15}N, whereas the enrichment of ^{13}C would be small. This would lead to a characteristic slope of the vector for abiotic degradation in Figure 1. In contrast, if the s-triazine in soil was partly removed by aerobic biodegradation, then the points for soil samples could lie on the vector with a smaller slope (less enrichment for N and more for C). Ideally, all different removal processes result in different vectors. In the illustrated example it would be possible to conclude that the soil samples were affected by aerobic biodegradation. If, under bad circumstances, two processes fall on similar vectors with similar slopes, then one could add a third isotope (e.g., hydrogen or chlorine).

The guideline contains data on initial isotope compositions for several relevant and persistent soil pollutants, and information on the slopes of process vectors. These data will be comprehensively presented, together with tested protocols for the measurement of soil samples, which avoid bias in isotope ratio measurements. Furthermore, the state of the art of isotope analysis is summarized.

This Guide is intended for managers of catchment areas and water bodies who may design sampling plans that will include CSIA and specify data quality objectives for CSIA analyses, for analytical chemists who may carry out the analyses, and for staff of regulatory agencies who may review and approve the sampling plans and data quality objectives, and who must review and interpret the data provided from the analyses. This Guide provides recommendations and suggestions to land managers, chemists and regulators.

2. Sampling strategy and sample preparation

2.1 Challenges of pesticide CSIA.

The occurrence of very low (sub-µg/L) concentrations of pesticides and their polarity are two major analytical challenges to be addressed to extend CSIA approaches to pesticides. First, the enrichment of sufficient analyte (typically a few ng of C or N on column per injection are needed) requires the extraction of large amounts of water, soil or vegetal material. The detection limits for nitrogen CSIA of pesticides are much higher than for carbon, as typically there are fewer atoms of N than C in the pesticide molecule, making it difficult to apply ME-CSIA *in situ* (Elsner and Imfeld 2016). Any scaling up of the extraction method should be monitored for potential isotope effects. As shown by (Melsbach et al., 2021), increasing volume of water during SPE above 10 L may alter the $\delta^{13}C$ of atrazine.

For accurate and precise CSIA, complete chromatographic separation of all compounds is required. Therefore, any chromatographic interferences can strongly limit the applicability of the CSIA. As most pesticides are non-volatile or semi-volatile on-column liquid injection is typically used as a sample introduction technique when pesticides are analyzed with GC-IRMS. This technique, in contrast to static or dynamic headspace sampling used for volatile organic compounds, can introduce many matrix interferences

from compounds with similar physicochemical properties from soil or water co-enriched during extraction procedures. For example, during large volume SPE, non-volatile matrix components from environmental samples are thus enriched together with the target compounds, which is defined here as the matrix effect. Extract clean-up procedures are thus often necessary prior to the application of CSIA to environmental samples (see section 2.4).

The second challenge lies in the polarity of the more polar pesticides and their transformation products. This generally requires the use of a derivatization step prior to GC separation or the use of LC-IRMS, which is currently limited to $\delta^{13}C$ analyses. LC-IRMS is further constricted due to high detection limits (at least one order of magnitude higher than GC-IRMS). LC-IRMS has also limited application due the incompatibility of the method with organic solvents containing carbon atoms, such as methanol, typically used for chromatographic separation (Gilevska et al., 2014; Perini and Bontempo, 2021). Therefore, the most commonly derivatization step is chosen to circumvent the polarity of the parent compound or daughter products. The choice of the derivatization method strongly depends on the chemical structure of the pesticide. Methylation of hydroxyl and amino groups is typically achieved with trimethylsilyldiazomethane (TMSD) and trimethylsulfonium hydroxide (TMSH) (Melsbach et al., 2019; Mogusu et al., 2015; Reinnicke et al., 2010; Torrentó et al., 2019). Any additional step, such as derivatization, requires strict screening for any isotope fractionation during the sample preparation. Additionally, the stable carbon isotope composition should be corrected if additional carbon atoms are introduced during derivatization.

2.2 Soil and water sample preparation for CSIA

Sampling strategy: The following sampling strategy is suggested to quantify pesticide degradation extent under field conditions in small agricultural catchments (i.e., 10-100 hectares). Both sampling and interpretation should be adapted for larger scales to include multiple sources and events over an agricultural season.

First, sufficient knowledge regarding hydrological and hydroclimatic conditions and functioning is mandatory for the application of pesticide CSIA at the catchment scale (Table 1). Hydroclimatic data should provide sufficient resolution to evaluate (i) mean daily rainfall, (ii) mean rainfall intensity, (iii) total rainfall, (iv) mean daily reference evapotranspiration, (v) mean daily temperature, (vi) mean daily discharge normalized by the total catchment area, (vii) time of concentration and (viii) percent of days in a month when rainfall occurred (% Wet Days). Subsurface travel time should be defined precisely, possibly with preliminary hydrological studies using the stable hydrogen and oxygen isotope composition of water ($\delta^{2}H$ and $\delta^{18}O$), refer to Chapter 6.3.1.

The soil sampling frequency should be adapted to the pesticide application and the expected degradation kinetics. Transects should be selected to account for soil type, heterogeneity and the variability of moisture conditions, drainage characteristics and to maximize the number of plots where the pesticide is applied. In addition, a Digital Elevation Model (DEM), may be used to determine local slopes and to estimate the

topographical wetness index (TWI) [-]. TWI mainly quantifies the impact of topography on soil moisture. Soil crust development should be characterized across the catchment after a precipitation event as a function of mm of cumulative rainfall. This should allow us to evaluate the reduction in the soil infiltration capacity due to crusting, and to interpret the temporal water evolution along with rainfall-runoff data.

During sampling, the water discharge at the catchment outlet should be continuously monitored to evaluate hydrological functioning and establish water and pesticide mass balances. After sampling, the sub-samples of water may be pooled into composite samples according to hydrograph characteristics and pesticide concentrations, yielding one or more samples weekly with the chosen water volume. This volume should be selected to ensure that the concentrations of each pesticide are sufficient for CSIA analysis. An example of the sample size and the potential for pesticide and isotope analysis is illustrated in Fig. 2.

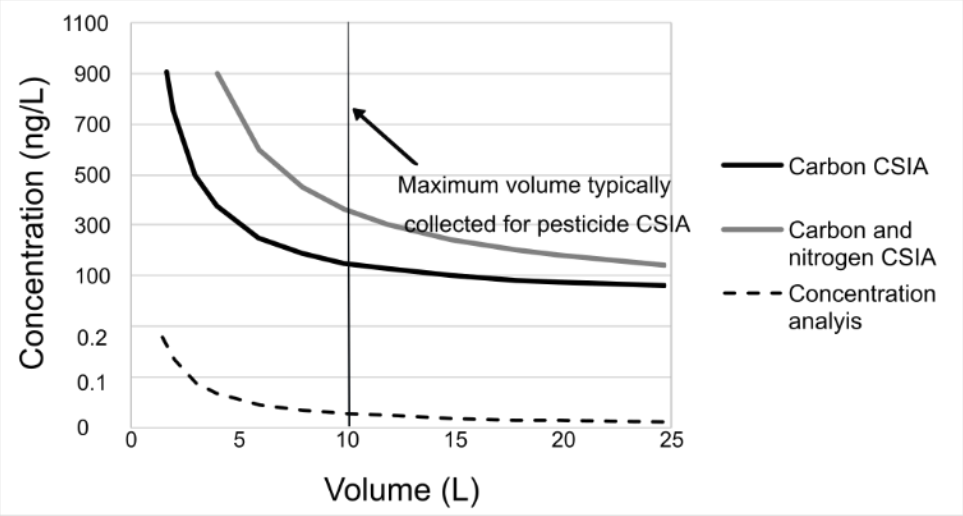


Figure 2: Relationship between the necessary S-metolachlor concentrations in water and collected water volume for reliable carbon or nitrogen CSIA and concentration analysis (Alvarez-Zaldivar et al., 2018; Torrentó et al., 2019). The solid black line represents feasibility of carbon CSIA, solid grey line represents feasibility of carbon and nitrogen CSIA, dashed line represents feasibility of concentration analysis. Note a different scale for the concentration analysis. Note that this relationship is site specific.

2.3 Water and soil sample processing and conservation

Water samples should be collected using a refrigerated autosampler in the field, stored in the dark at 4 °C during collection (to avoid photolysis and limit further biodegradation) and placed on ice during transportation to the laboratory for immediate filtering (on 0.7 µm glass fiber). Water samples should be kept at 4 °C from the collection up to the extraction and should be preferably further filtered and analyzed (using SPE, see section 2.3) within 24 h. Preliminary tests on the effect of water sample sampling, transport and storage should be done prior to the study. Water samples may also be frozen for longer-

term conservation, but preliminary tests of the effect of freezing on both extraction yield and stable isotope fraction are required.

After collection, soil samples should be kept in an ice box during transport to the laboratory and kept frozen at -20 °C until analysis. Soils should be homogenized, quartered before sieving (e.g., according to NF X 31100 standard) and sieved at 2 mm. Water content, pH, organic content, cation exchange capacity, and other parameters can be measured to characterize soil samples and help in data interpretation.

Table 1: Sampling scheme for water and soil samples for implementing pesticide CSIA on the catchment scale.

	Prior to sampling	**Measurements and analyses**	**Sampling**
Water	*Hydrological and climatic data* - Daily precipitation and temperature data - Contribution of surface, sub-surface, and base-flows - Concentration time of the catchment	- Discharge measurements - Multiparameter probes *in situ* - Hydrochemical analysis	Flow proportional sampling with refrigerated automatic sampler with glass vials Applied commercial pesticide formulations
Soil	*Pedological and surface soil data* - Pedological characterization - Digital Elevation Model - Agricultural plots (owners, limits, etc.) - Typical agricultural scheme	- Farmers survey for the period of pesticide application - Plant growth and coverage - Soil moisture characteristics - Soil physico-chemical characterization - Erosion budget	Top soil (1 cm) and/or discrete sub-surface soil (in different layers from 0 to 20 cm or deeper) across several transects. Applied commercial pesticide formulations

2.4. Extraction methods for CSIA from water and soil

To expand the use of pesticide CSIA to agricultural catchments, it is crucial to use an appropriate extraction method. Extraction methods for pesticide residues from environment matrices for ME-CSIA should: (i) provide sufficient analyte mass for reliable isotope analysis, (ii) cause no isotope fractionation, (iii) be applicable to a wide range of pesticides and matrices, and (iv) limit matrix co-enrichment to avoid co-elution during chromatographic separation.

Extraction methods from water. To date, solid phase extraction has been the most common method used to extract water samples for pesticide CSIA, although liquid-liquid extraction has been used for small amounts of water (from 0.5 mL) in laboratory experiments (Chevallier et al., 2018; Knossow et al., 2020). The extraction methods should be tested within the expected concentration range and target environmental matrix to determine the feasibility of pesticide CSIA from water samples. SPE has been tested previously in combination with CSIA of atrazine, acetochlor, S-metolachlor, metalaxyl, butachlor, alachlor, terbutryn, chlordizon, bentazone, dichlorvos, dimethoate, omethoate and several of their metabolites (Drouin, 2021; Droz, 2021; Elsayed et al., 2014; Masbou et al., 2018a; Pérez-Rodríguez et al., 2021; Schreglmann et al., 2013; Schürner et al., 2016; Torrentó et al., 2019; Wu et al., 2014). To ensure maximum recovery, the type and amount of sorbents and extraction eluents should be preliminary adjusted to the physical properties of the analytes and matrix and thoroughly tested. As the physicochemical properties of parent compounds and their transformation products (TPs) may differ significantly, different sorbents or sorbent combinations may be used to preconcentrate the parent compounds and the TPs (Torrentó et al., 2019).

The majority of pesticide CSIA studied did not show isotope fractionation using SPE (Droz, 2021; Elsayed et al., 2014; Masbou et al., 2018a; Pérez-Rodríguez et al., 2021; Schreglmann et al., 2013; Torrentó et al., 2019; Wu et al., 2014). A sample size of up to 10 L is more frequently used for SPE and pesticide CSIA, as increasing the volume >10 L could shift $\delta(^{13}C)$ of pesticides (Melsbach et al., 2021). However, this shift may not be correlated with the SPE procedure but rather with the matrix effect on the measurement, decreasing oxidation capacity, and increasing background levels and instrument maintenance issues. Therefore, prior to the pesticide measurements, clean-up strategies (discussed below) are typically applied to minimize the matrix effect. Nevertheless, current SPE methods allowed carbon and nitrogen pesticide CSIA in the ng/L to µg/L of pesticide concentration range (Alvarez-Zaldivar et al., 2018; Schreglmann et al., 2013; Torrentó et al., 2019). This underscores the feasibility of carbon and nitrogen CSIA from water samples in agricultural settings throughout the agricultural season.

Extraction methods from soil. Physicochemical characteristics of pesticides, such as their hydrophobicity and acid dissociation constant, and soil characteristics, including pH, organic matter and water contents, should be considered when selecting the extraction method and extraction solvent. For optimal results, extraction tests must be carried out

with the target soil or sediment to ensure high recovery and non-significant isotope fractionation and evaluate the matrix effect due to the co-extraction of soil organic matter. Modifications to the existing method, such as increasing the sample volume from 5 to 20 g or sequential use of extraction solvents, should also be evaluated.

Pesticide extraction methods from soil and sediment for reliable pesticide CSIA have been already used in both laboratory and field studies (Alvarez-Zaldivar et al., 2018; Masbou et al., 2018b; Pérez-Rodríguez et al., 2021; Wu et al., 2019)(Ivdra et al., 2014)) proposed a modified ultrasonic-assisted extraction (MUSE) without carbon isotope fractionation associated with extraction ($\Delta^{13}C \leq 0.4$) for hexachlorocyclohexanes (HCHs). A modified MUSE method (Ivdra et al., 2014) was also tested with ethylacetate (Alvarez-Zaldivar et al., 2018; Masbou et al., 2018b) or dichloromethane:pentane (Droz et al., 2021; Pérez-Rodríguez et al., 2021) as the extraction solvent. Another study used accelerated solvent extraction (ASE) for the extraction of HCHs from soil and plants, which enabled stable C, H, and Cl isotope analysis (Liu et al., 2021, 2020; Wu et al., 2019). The application of QuEChERS for the extraction of metolachlor from two agricultural soils led to an insignificant (<1 ‰) isotope fractionation for carbon CSIA (Torrentó et al., 2021).

Current methods allow for carbon and nitrogen CSIA in soil samples in the range of ng/g to µg/g range for carbon and several µg/g for nitrogen CSIA (Alvarez-Zaldivar et al., 2018; Droz, 2021; Masbou et al., 2018b). This currently restricts the application of carbon and nitrogen CSIA of pesticides to source areas and laboratory studies, respectively. To decrease the matrix effect and thus reach lower pesticides concentrations for ME-CSIA on a broad range of soil samples, fractionation-free clean-up methods applicable to a broad range of environmental soils and pesticides are necessary.

Clean-up procedures. A number of clean-up procedures can be applied to address the issue of co-enrichment to maximize the analytical performance of pesticide CSIA extraction from environmental matrices without altering the isotope ratio of the target compounds. These include: i) the addition of a sorbent, such as PSA or graphitized carbon black (GCB), to remove pigments, such as chlorophyll (Anastassiades et al., 2003; Wilkowska and Biziuk, 2011), ii) chromatography HPLC separation or column chromatography (Mogusu, 2016; Schreglmann et al., 2013), and/or iii) the use of molecularly imprinted polymers (MIP) (Bakkour et al., 2018). MIP is likely the most effective clean-up method for CSIA. However, MIP is not commercially available for all classes of compounds, and therefore must be specifically synthesized prior to clean-up. Furthermore, samples should only be processed in glass when using organic solvents, especially dichloromethane, to reduce matrix interferences from the extraction procedure.

Carbon and nitrogen CSIA in complex matrices can also benefit from two-dimensional gas chromatography (GC×GC)-IRMS. This approach has been applied to polychlorinated biphenyls, chloronaphthalenes, and chlorofluorocarbons (Horii et al., 2005; Horst et al., 2015). In GC×GC-IRMS, the system needs to be equipped with a column-switching device, such as moving capillary stream switching (MCSS), or with a 6-port valve. By

column switching parts of the effluent from the first column are cut and transferred to a second column, where separation of compounds of interest can be enhanced. In addition, the use of (GC×GC)-IRMS can improve sensitivity by 8-fold (Horst et al. 2015) by enhancing chromatographic separation. Therefore, this approach has potential for pesticide CSIA application.

Whenever possible, one or several clean-up strategies, depending on the complexity of the matrix, should be applied during pesticide CSIA. Along with improving the background of pesticide CSIA, it will reduce the need for increased maintenance of the GC-IRMS instrument, including oven and column replacement as well as blockage of capillaries. Complementarily with clean-up procedures, it may be possible to adjust the chromatographic parameters, e.g., the. extend the temperature program to improve the level of background and the chromatographic separation when the background compromises the GC-IRMS measurements.

It is worth mentioning that pesticide CSIA must be systematically preceded by a quantitative evaluation of the pesticides in the extracts to optimize injections of the target analytes. Prior to CSIA, rigorous quality assurance practices and referencing strategies must be established to ensure that isotope measurements are accurate and reproducible.

3. Isotope analysis

3.1 Instruments and methods

Isotope ratios in pesticides are measured as the ratio of the relative abundance of heavy and light isotopes in a sample which are compared to the international $R_{standards}$ given explained in detail in section 6, equation 6.1. 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 field conditions. To trace the fate of pesticides, 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 literature (Höhener et al., 2022) 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 laboratories since it permits measuring solid 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.

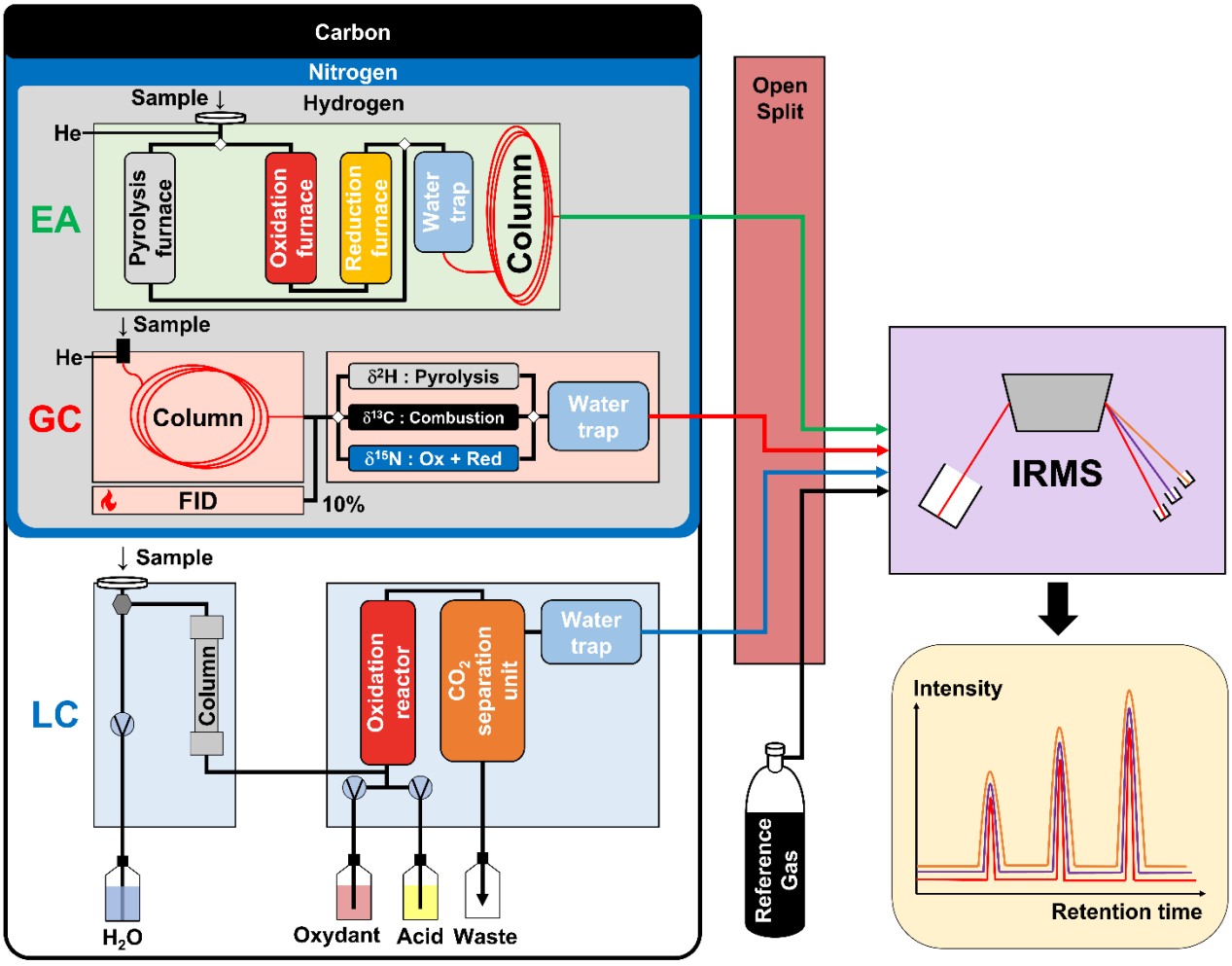


Figure 3: Analytical techniques for isotope ratios measurement in pesticide formulations and bulk samples, for the three most common elements carbon, nitrogen and hydrogen. EA: Elemental analysis. GC Gas chromatography. LC Liquid chromatography. IRMS Isotope Ratio Mass Spectrometry. He: Helium gas. Ox: 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 analyses. 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 $^{15}N_2$, respectively 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-Söder et al., 2007) for analysis of $^{37}Cl/^{35}Cl$ isotope ratio in chlorinated solvents. It was first applied to the insecticide DDT in 2010 (Aeppli et al., 2010b). 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; Torrentó 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., 2015). It has been applied in two studies looking at hexachlorocyclohexanes (Wu et al., 2019), 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.

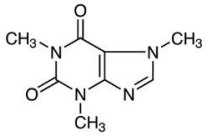
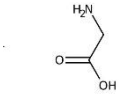
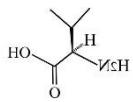
Offline MS. Other studies converted pesticide samples by off-line methods to gaseous molecules containing chlorine or bromine and injected them to Dual-Inlet IRMS for the analysis of ratios of $^{37}Cl/^{35}Cl$ (Drenzek et al., 2004, 2002; Reddy et al., 2002) or $^{81}Br/^{79}Br$ (Kuntze et al., 2016).

3.2 Standards (Reference materials)

Reporting isotope data requires that the deviations of these from international standards are accurately measured. That means that during the experimental analysis of pesticide samples, there must be also experimental analysis of a certified standard material. This allows to thoroughly monitor instrumental drift and apply – if needed – a correction for this.

Classical reference materials are historically destined to elemental analysis and were mostly solids distributed as fine powders. A few liquids were also widely used (water for O and H isotopes, NBS oil for C and H). However, all these standards are not suited for injection into a GC system. Only in 2016, new reference materials became available which can be used in GC analysis (Schimmelmann et al., 2016). These include caffeine, amino acids, hexadecane and a few other compounds (Table 2), although pesticide standards are mostly lacking.

Table 2: Examples of standard materials from USGS and IAEA, prepared as described in (Schimmelmann et al., 2016).

Reference ID	Chemical name	Structure/ Composition	Reference values with standard uncertainties		
			$\delta^{2}H_{VSMOW}$	$\delta^{13}C_{VPDB}$	$\delta^{15}N_{Air}$
USGS61	caffeine		+96.9 ± 0.9	-35.05 ± 0.04	-2.87 ± 0.04
USGS62	caffeine		-156.1 ± 2.1	-14.79 ± 0.04	+20.17 ± 0.06
USGS63	caffeine		+174.5 ± 0.9	-1.17 ± 0.04	+37.83 ± 0.06
IAEA-600	caffeine		-156.1 ± 1.3	-27.73 ± 0.04	+1.02 ± 0.05
USGS64	glycine		*	-40.81 ± 0.04	+1.76 ± 0.06
USGS65	glycine		*	-20.29 ± 0.04	+20.68 ± 0.06
USGS66	glycine		*	-0.67 ± 0.05	+40.83 ± 0.06
USGS67	n-hexadecane	$C_{16}H_{34}$	-166.2 ± 1.0	-34.50 ± 0.05	na
USGS68	n-hexadecane	$C_{16}H_{34}$	-10.2 ± 0.9	-10.55 ± 0.04	na
USGS69	n-hexadecane	$C_{16}H_{34}$	+381.4± 3.5	-0.57± 0.04	na
USGS70	C_{20} FAME	$C_{20}H_{39}OOCH_3$	-183.9 ± 1.4	-30.53 ± 0.03	na
USGS71	C_{20} FAME	$C_{20}H_{39}OOCH_3$	-4.9 ± 1.0	-10.5 ± 0.03	na
USGS72	C_{20} FAME	$C_{20}H_{39}OOCH_3$	+348.3 ± 1.5	-1.54 ± 0.03	na
USGS73	L-valine		*	-24.03 ± 0.04	-5.21 ± 0.05
USGS74	L-valine		*	-9.30 ± 0.04	+30.19 ± 0.07
USGS75	L-valine		*	+0.49 ± 0.07	+61.53 ± 0.14
USGS76	C_{17} FAME	$C_{17}H_{33}OOCH_3$	-210.8 ± 1.2	-31.36 ± 0.04	na

*Material is very hydroscopic na not applicable.

With the help of these materials, two-point calibrations can be made for many isotope measurement systems, using a light and a heavy reference of the same material. The availability of these materials thus has enhanced the robustness of measurements (especially GC-IRMS, but also LC-IRMS for carbon).

3.3 Data quality management

An important component of quality assurance is the use of analytical standards of pesticides calibrated on international isotope scales. The referencing strategy of the 'identical treatment principle' (Werner and Brand, 2001) should be implemented: (i) to measure external standards before and after the sample to correct for an offset, and (ii) to use a reference material that is identical to the target substance. For obtaining in-house isotopic standards, a mass spectrometer equipped with an elemental analyzer and isotope ratio mass spectrometer (e.g., FlashEA IsoLinkTM CN IRMS, Thermo Fisher Scientific, Bremen, Germany) or offline conversion followed by dual-inlet-IRMS are typically used. The $\delta^{13}C$ and $\delta^{15}N$ values should be normalized to VPDB and Air scales, respectively, using a minimum of two international reference materials with the range of isotope delta values, that would encompass all the measured samples, which are chosen as an anchor point for the regression line, e.g. IAEA600 [$\delta(^{13}C)$ =–27.77 ‰] and USGS41 [$\delta^{13}C$ =+37.63 ‰] (Coplen et al., 2006). Then, the third standard, which has the value between the chosen anchors is treated as an unknown sample for quality control (QC) and is used to evaluate combined analytical uncertainty. As a part of inter-laboratory comparison, it is recommended to measure the same standards at other lab facilities using another EA-IRMS.

Unless otherwise specified, the uncertainties for pesticide CSIA are reported as standard deviation (1σ) calculated from replicate measurements. Combined analytical uncertainty of $\delta^{13}C$ and $\delta^{15}N$ values obtained with GC- or LC-IRMS should not differ (≤0.5 ‰) from values obtained by EA-IRMS.

The standard injection frequency depends on the pesticides, the element measured, the matrix, and the number of pesticides analyses. For relatively simple environmental matrices (e.g., groundwater), it is recommended to measure an in-house pesticide mix standard with known isotope composition at least every six samples to control the retention time of the target compounds and assess the instrument performance (e.g., conversion efficiency). The standard mixtures are injected every three samples, however, for simultaneous $\delta^{15}N$ analysis of several pesticides for complex environmental matrix (e.g., soil). The amplitudes of peaks of injected standards should closely match those of analyzed samples (Sherwood Lollar et al., 2007). To conserve the combustion capacity of the oven, the measurement window (effluent mode) should be kept to a minimum: opened before the targeted peak elution and closed shortly thereafter.

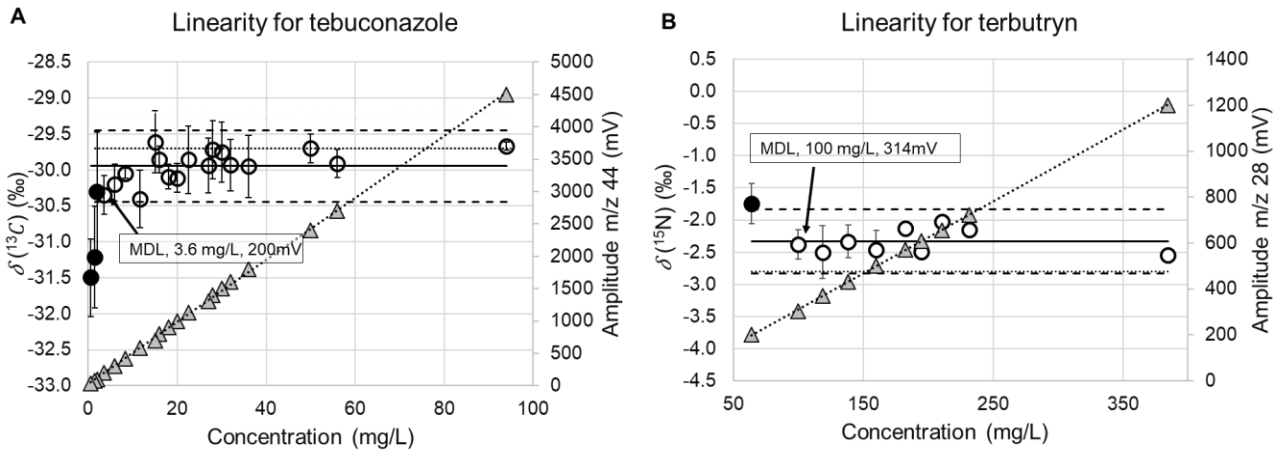


Figure 4: *Concentration and A – $\delta^{13}C$ measurements and amplitude of the mass 44 for tebuconazole B - $\delta^{15}N$ measurements and amplitude of the mass 28 for terbutryn. Circles represent stable carbon and nitrogen isotope compositions. Triangles indicate the amplitude of mass 44 and 28 peaks. The solid line represents the calculated mean $\delta(^{13}C)$ value (–29.9 ± 0.2 ‰, n=51) and $\delta(^{15}N)$ value (–2.3 ± 0.2 ‰, n=30); dashed lines indicate the ±0.5 ‰ interval. Measurements were performed in triplicate; the standard deviation of each point is indicated by error bars. The dotted line represents the $\delta(^{13}C)$ value of tebuconazole (–29.7 ± 0.1 ‰, n=3) and $\delta(^{15}N)$ value of terbutryn (–2.8 ± 0.1 ‰, n=3) measured by EA-IRMS. Values outside the linear range – filled circles are excluded from the mean $\delta(^{13}C)$ and $\delta(^{15}N)$ value calculation due to either being outside ±0.5 ‰ interval from the mean value or due to low reproducibility of triplicate measurement (>0.5 ‰). MDL – method detection limit. The major principles illustrated in this figure are described in (Jochmann et al., 2006).*

As part of CSIA method validation, the linearity range should be determined for each pesticide. The linearity range specifies the range of measurements with sufficient precision and accuracy, indicating, within an acceptable range [e.g. ±0.5 ‰ for $\delta^{13}C$], that the stable isotope composition is independent of the amount of compound injected (Jochmann et al., 2006). The method detection limit (MDL) is the point with the lowest (or highest) concentration within a ± 0.5 ‰ linear interval of the mean value for the standard measured with GC-IRMS and with good reproducibility in triplicate measurements (<0.5 ‰). Figure 4A shows the linearity for $\delta^{13}C$ values for the pesticide tebuconazole, with three data points (filled circles) outside ±0.5 ‰ linear interval from the mean value or with low reproducibility of triplicate measurements (>0.5 ‰). Such measurements are not taken into consideration and are outside the linear range. Figure 4B shows the linearity for $\delta^{15}N$ for terbutryn, with MDL corresponding to a significantly higher concentration of the compound in the sample.

4. Field studies using pesticide CSIA

Integrative strategies relying on isotope data have been conducted for more than three decades in the case of nitrate and have significantly contributed to the development of water management policies (Lutz et al., 2013; Moschet et al., 2013; Nestler et al., 2011).

A similar strategy has been developed for legacy pollutants (Hunkeler et al., 2008), and may be now developed for pesticides in agricultural catchments, as recently described (Alvarez-Zaldivar et al., 2018).

Prior to the sampling campaign, which would include pesticide CSIA, information on the type and amount of the applied pesticides, the application periods, the published ε and Λ values for pesticides used, and the isotope signature of the pesticide formulation should be gathered.

4.1 Insights from pesticide CSIA

When $\Delta^{13}C$ or $\Delta^{15}N$ >2 ‰, assuming combined analytical uncertainty of ± 0.5 ‰, of pesticide molecules is observed across space, time, or source, i.e., compared to the isotope signatures of applied pesticides, pesticide degradation is likely occurring *in situ*. Further, the degradation pathways may be identified based on the determined ε and Λ values. For example, in the biotic hydrolysis of atrazine by *Arthrobacter aurescens* TC1, an unusual trend towards more negative $\delta^{15}N$ values is observed. Protonation of nitrogen atoms during the reaction makes ^{14}N react more rapidly while ^{15}N accumulates in the remaining atrazine (inverse isotope effect) (Meyer et al., 2009). In contrast, oxidative dealkylation by *Rhodococcus* sp. strain NI86/21 would result in more positive $\delta^{15}N$ values. As mentioned in Text Box 1. the use of dual element isotope plots is preferred, especially when multiple sources are present.

4.2. Quantitative assessment of in situ degradation.

When the degradation pathway is identified in the field, and an enrichment factor has been quantified for this pathway, then equations 6.7 and 6.8 can be used to calculate the extent of transformation. For pesticides, these calculations typically require a large extent of degradation to fulfil $\Delta^{13}C$ >2 ‰ requirement. For example, for S-metolachlor (using ε_C = -1.5±0.5 ‰ for biodegradation in soil (Alvarez-Zaldivar et al., 2018; Droz et al., 2021)), the extent of degradation of the applied pesticide should be higher than 74 % to apply carbon CSIA to identify and quantify degradation *in situ.* Nevertheless, due to the multiplicity of processes affecting pesticide residues at the agricultural fields, e.g., contamination pulses during run-off events, these estimates of the extent of biodegradation are likely conservative. Therefore, a subsurface-surface reactive transport model incorporating CSIA isotope source ratios, ε values for different elements can help to examine sources and dissipation of pulses of diffuse contaminants (Lutz et al., 2017; Van Breukelen, 2007).

4.3. Case study

To illustrate the applicability of CSIA to evaluate the transformation of S-metolachlor, a widely used herbicide, we discuss here a study case at the agricultural catchment scale (Alvarez-Zaldivar et al., 2018). Typically, pesticide reactive transport in agricultural fields is driven by pesticide pulses that can be traced in event-based studies. In such case, pesticide CSIA may enhance the interpretation of pesticide transformation in heterogeneous reactive compartments during runoff from soil to surface water. The study

case shows the interest of pesticide CSIA to detect the occurrence and to estimate the extent of pesticide transformation in the field. Such information often remains elusive despite the large amount of data on pesticide degradation gathered from regulatory testing and routine pesticide monitoring. Indeed, pesticide concentrations may largely vary in space and time due to confounding degradative and non-degradative dissipation processes and re-mobilization during rainfall or irrigation events. Therefore, concentration data of pesticides and their transformation products (TPs) alone can be misleading because they may not be indicative of the re-mobilization or the transformation of the pesticide. Further, transformation of TPs, whenever known and quantified, may be confounded by their simultaneous formation. In the present case, soil and sub-event CSIA data helped to evaluate the natural attenuation of pesticides at the catchment scale by delineating catchment areas controlling pesticide transformation and estimating transformation and export losses (Elsner and Imfeld 2016).

The degradation of the herbicide S-metolachlor was evaluated in a typical agricultural headwater catchment (47 ha) during a growing season (March to August). Detailed knowledge on hydrological and hydroclimatic conditions, functioning of the catchment and a Digital Elevation Model to determine local slopes and the topographical wetness index were obtained to define the sampling strategy and transects. Topsoil (1 cm) S-metolachlor concentrations and $\delta^{13}C$ were determined by weekly sampling on three transects across the catchment. Water discharge was continuously monitored at the catchment outlet to establish the hydrological and pesticide mass balances. Weekly water sub-samples collected using a refrigerated autosampler were pooled into composite samples according to hydrograph characteristics and pesticide concentrations to ensure enough S-metolachlor for carbon CSIA analysis. Samples were immediately placed on ice and in the dark during transportation to the laboratory for immediate sample preparation (homogenization, filtration, and sieving), and S-metolachlor extraction within 24 h. Extraction from soil and water samples were established beforehand (Gilevska et al., 2022) and did not cause significant isotope fractionation ($\Delta\delta^{13}C$ <1 ‰). The minimum peak amplitudes needed for accurate $\delta^{13}C$ measurements (300 mV) corresponded to 10 ng of carbon injected on column. The GC-C-IRMS system consisted of a TRACE™ Ultra Gas Chromatograph (ThermoFisher Scientific) coupled via a GC IsoLink/Conflow IV interface to an isotope ratio mass spectrometer (DeltaV Plus, ThermoFisher Scientific). The reproducibility of triplicate measurements was <0.2‰ (1σ) for $\delta^{13}C$. The range of $\delta^{13}C$ of S-metolachlor (from –31.7 ± 0.2‰ to –32.6 ± 0.5‰) for four different pesticide formulations was narrow. Therefore, a significant change of the stable isotope composition ($\Delta\delta^{13}C$ >2‰, about 75% of S-metolachlor degradation) may indicate S-metolachlor degradation.

Compared to the commercial formulation of S-metolachlor applied on the field, a significant change in carbon isotope composition ($\delta^{13}C$) of S-metolachlor were observed with time in topsoil and runoff water at the catchment's outlet. Together with the formation of S-metolachlor TPs (i.e., ESA- and OXA-metolachlor), this indicated S-metolachlor degradation in the catchment. To estimate S-metolachlor attenuation by degradative and non-degradative dissipation, carbon isotope fractionation factors (ε_C) were retrieved from reference laboratory degradation experiments with agricultural topsoil from the same catchment. Both the classical mass balance, based on hydrological and concentration data, and the CSIA approach, using changes in $\delta^{13}C$ values with time and the Rayleigh approach (Eq. 6.7), underscored consistent variations of degradation extent (T%, eq. 6.8) between the catchment topsoil and the outlet despite changing hydrological regimes. This demonstrated the monitoring applicability of CSIA methods despite changing hydrological regimes over the growing season. From the CSIA data, it could be inferred that S-metolachlor degradation mainly occurred in topsoil and reached more than 90% of the initially applied amount three months after the first S-metolachlor application. In addition, the CSIA approach enabled delineation of the catchment areas mainly contributing to S-metolachlor degradation and estimated that losses in runoff and leaching accounted for 8% of applied S-metolachlor.

Overall, this case study highlights that high-resolution sampling scheme involving carbon-based CSIA enables quantification of pesticide degradation across multiple events and catchment areas to improve understanding of pesticide fate during periods of high transfer risk. It also shows the potential of CSIA to evaluate pesticide fate at catchment scale and paves the road to upscale application to even more complex situations, e.g., multiple sources, pathways, and reactive zones, and during longer periods. It also encourages to retrieve at the catchment scale multi-element CSIA data at high resolution from both soil and runoff water samples.

When CSIA data are interpreted at the catchment scale, hydrological modeling can also help to characterize dominant pesticide transport pathways and response times that may affect stable isotope ratios. Previous reactive transport models integrating isotopic signatures have been developed to interpret 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-Zaldivar et al. 2016. However, models including pesticides CSIA data remain scarce, mainly due to the lack of valid CSIA datasets, although soil reactive transport models evaluating pesticide persistence and transport exist since decades (Gassmann 2021). For instance, the virtual 2D hillslope reactive transport model Hydro-GeoSphere, 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 at the catchment scale. A major drawback of lumped approaches of is to consider the catchment as an undivided entity. While neglecting spatial information

on soil heterogeneity, water content or temperature, this currently limits the identification of degradation hot spots across the catchment. Nevertheless, combining carbon CSIA data ($\delta^{13}C$) of the herbicide S-metolachlor in the above-mentioned agricultural catchment and a parsimonious lumped agro-hydrological model helped to interpret pesticide transport and degradation at the catchment scale (Lutz et al. 2017).

5. Use of Stable Isotopes for Source Differentiation

5.1. Variability of Isotope Ratios of Different Sources

The initial stable isotope composition of organic chemicals depends on the conditions and the pathways used to synthesize the compound, and thus depends on the manufacturers and the time frame of production. This variation may serve to identify chemical sources or trace the time of contaminant release in the environment. The variability of isotope ratios in various pesticides and some degradation products in the literature has been reviewed including publications that appeared before May 2022 (Höhener et al., 2022). 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. 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 2022. A total of 547 isotopic compositions were listed (Höhener et al., 2022), 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, and one for ^{34}S in amethryn.

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. 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 ‰. While Mogusu and co-workers 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. However, the $\delta^{13}C$ ratios of atrazine span only from -30 to -26.3 ‰ except for the atrazine of Ehrenstorfer which is at -19 ‰, while $\delta^{15}N$ ratios range from -2.2 to -0.2 ‰ (Höhener et al., 2022). 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 (Höhener et al., 2022), suggesting that the chlorine isotope analysis must be included for forensic studies relying on S-metolachlor (Torrentó 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.

5.2. Measurements of commercial formulations during the project DECISIVE

A systematic analysis of the isotopic composition of commonly or formerly used pesticides in formulations produced by different manufacturers worldwide is of interest for pesticide CSIA because the initial stable isotope composition is generally unknown. Such a database of isotope composition for different elements of micropollutant molecules would help to identify sources and to interpret transformation more systematically in field studies and treatment systems.

The pesticide commercial formulations, i.e., 5 mL in clean (burned) glass vials, were collected directly at the farms, following official requests to regional and national pesticide providers and manufacturers, and stored at 4 °C until analysis. The active compounds of the different pesticide formulations can be isolated by either liquid-liquid or solid phase extraction and measured subsequently by GC-C-IRMS.

During the DECISIVE project, 120 commercial formulations, including 102 different active molecules were collected. 59 pesticide formulations from 14 different manufacturers were analyzed for both carbon and nitrogen CSIA, including 40 fungicides, 17 herbicides, 2 insecticides. The carbon isotope of pesticides in formulations ranged from −26 to −36 ‰ (mean = –31.0 ± 2.4 ‰; n=59) but a narrow range of $\delta^{13}C$ values may be found for individual pesticides. For example, a narrow range of $\delta^{13}C$ of S-Metolachlor for four different pesticide formulations (from –31.7 ± 0.2‰ to –32.6 ± 0.5‰) and slightly different composition of an analytical standard (–31.0 ± 0.5 ‰) was found. Based on that range, if the $\delta^{13}C$ of S-Metolachlor measured *in situ* is higher than –29.0 ‰, it may be indicative of degradation. However, it may not be possible to differentiate sources of S-metolachlor in the field (see Section 4.0).

The IsotoPest database (https://ites.unistra.fr/isotopest) was created to fulfill the community's need of a pesticide database of stable isotope signatures to i) archive stable isotope signatures of pesticide in commercial formulations, ii) create inter-comparison exercise opportunities to ensure accuracy and consistency (across different methods and settings) and iii) facilitate application of stable isotope data in future studies on pesticide transformation and sources in the environment.

5.3. Applications of source identification using stable isotope data

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 δ^{2}H, from −35.9‰ to −22.7‰ for δ^{13}C, and from −6.69‰ to +0.54‰ for δ^{37}Cl.

Stable isotope analysis has been proposed as a management tool to characterize pesticides in crops and food (Won et al., 2021). That review article summarizes analytical methods for CSIA in crops and food residues and concludes that CSIA has a high applicability for tracking unintended pesticide pollution in the environment, with possibilities to unravel different sources and their evolution in history. Nevertheless, only few real studies are available so far.

In Japan, 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 δ^{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.

6. Derivation of Equations to Describe Isotope Fractionation

6.1. Expressing Isotope Ratios

Isotope ratios 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 3.1:

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \qquad \text{eq. 6.1}$$

using the international $R_{standards}$ given in Table 2:

Table 3: List of international standards and their abundance ratio $R_{standard}$

Element	Ratio of isotopes	Standard	Abbreviation	$R_{standard}$ Abundance Ratio	References
Hydrogen	$^{2}H/^{1}H$	Standard Ocean Sea Water	VSMOW	$1.5575\ 10^{-4}$	a
Carbon	$^{13}C/^{12}C$	Carbonate of Belemnite from Pee Dee formation	VPDB	0.0112372	a
Nitrogen	$^{15}N/^{14}N$	Atmospheric air	AIR N_2	$3.677\ 10^{-3}$	a
Oxygen	$^{18}O/^{16}O$	Standard Ocean Sea Water	VSMOW	$2.0052\ 10^{-3}$	a
Bromine	$^{81}Br/^{79}Br$	Bromide from standard Ocean Sea Water	SMOB	0.9729	a
Sulfur	$^{34}S/^{32}S$	H_2S in Troïlite from Cañon Diablo	VCDT	$4.5005\ 10^{-2}$	b
Chlorine	$^{37}Cl/^{35}Cl$	Chloride ion from Standard Ocean Sea Water	SMOC	0.319766	c,d

References a) (Clark and Fritz, 1997); b) (Qi and Coplen, 2003) c) (Godon et al., 2004) d) the value of 0.324 is sometimes given but does not correspond to the abundance ratio of chloride in sea water.

6.2. Expressing and Quantifying Isotope Fractionation

Isotope fractionation is usually described by an isotope fractionation factor α characterizing the magnitude of isotopic separation. They are derived from the magnitude of an isotopic shift. For a phase exchange reaction (e.g., fractionation between vapor and liquid), the fractionation factor α is defined as in eq. 6.2:

$$\alpha_{v-l} = \frac{R_v}{R_l} \qquad \text{eq. 6.2}$$

where $\alpha_{v\text{-}l}$ is the fractionation factor between vapor and liquid, R_v is the isotope ratio measured in the vapor, and R_l is the one in the liquid phase.

For a kinetic chemical reaction far away from equilibrium, the fractionation factor is defined as (eq. 6.3)

$$\alpha = \frac{k_h}{k_l} \qquad \text{eq. 6.3}$$

where k_h and k_l are the reaction rates of heavy and light isotopes, respectively. Fractionation factors do not have units and are numbers usually close to one. Usually, heavy isotopes react slower than light isotopes, thus α values are more often smaller than one. One speaks then of normal isotope fractionation effects. However, inverse isotope fractionation effects are also possible, but are rather the exception. They have $\alpha > 1$.

For reporting fractionation effects, it is more convenient to report isotope enrichment factors ε. They are defined as in eq. 6.4:

$$\varepsilon = (\alpha - 1) * 1000 \qquad \text{eq. 6.4}$$

and are expressed in per thousand. Normal isotope effect is characterized by a negative ε, while an inverse effect has a positive ε.

6.3. The Rayleigh Equation

For evaluating field and laboratory data, a mathematical equation is needed that link the change in isotope ratios to the extent of reaction that occurred in the system. For field data, the approximation given by equation 6.4 is very much used. It allows to estimate how the delta value in a degrading pesticide increases as a function of the remaining (unreacted) fraction of pesticide and the enrichment factor (eq. 6.5)

$$\Delta\delta \approx \varepsilon \, ln f \qquad \text{eq. 6.5}$$

$\Delta\delta$ is the increase of the isotope ratio above its initial value, ln is natural logarithm, and f is the remaining fraction, usually expressed as $C/C_{initial}$. This approximation is mostly quite accurate, except when the delta values are far off from zero (Elsner, 2010).

In laboratory experiments, one wants to quantify enrichment factors as accurately as possible and uses therefore the exact Rayleigh equation 6.6:

$$1000 \, ln\left(\frac{1000+\delta}{1000+\delta_0}\right) = \varepsilon \, ln \, f \qquad \text{eq. 6.6}$$

Plotting the delta values in the form of the left-hand side of equation 6.6 versus ln f should yield a straight line with the slope ε.

Equation 6.6 can be rearranged to yield f, eq. 6.7:

$$f = \left(\frac{1000+\delta}{1000+\delta_0}\right)^{\frac{1000}{\varepsilon}} \qquad \text{eq. 6.7}$$

Through this equation, f can be obtained from isotope measurements and a known ε value, and then the percent transformation T(%) observed in the system can be calculated with eq. 6.8:

$$T(\%) = (1 - f) * 100 \qquad \text{eq. 6.8}$$

6.4. Equations for ME-CSIA

When using isotope ratios of two different elements, one usually calculates the slope Λ of the dual isotope plot. It is defined as expressed in equation 6.9:

$$\Lambda = \frac{\varepsilon_1}{\epsilon_2} \qquad \text{eq. 6.9}$$

Where ε_1 and ε_2 are enrichment factors of elements 1 and 2.

To obtain Λ from measured data of delta values, one must apply equation 6.9 (here for carbon and hydrogen isotopes).

$$\Lambda = \frac{ln[(\delta\ ^2H/1000 + 1)/(\delta\ ^2H_0/1000 + 1)]}{ln[(\delta^{13}C/1000 + 1)/(\delta^{13}C_0/1000 + 1)]} \approx \frac{\varepsilon_H}{\varepsilon_C} \qquad \text{eq. 6.10}$$

This equation was introduced by (Wijker et al., 2013) and is discussed in (Höhener and Imfeld, 2021).

7. Stable Isotope Fractionation Factors

During the last two decades, many laboratory experiments were performed to quantify the isotope fractionation of various processes that affect the fate of organic pollutants in environmental systems. These include volatilization, diffusion, sorption, photochemical reactions, abiotic and biotic transformations. A recent review paper (Höhener et al., 2022) has summarized fractionation factors occurring for pesticides, while other reviews have addressed also other organic micropollutants (Blessing and Baran, 2022), or industrial pollutants (Aelion et al., 2009). For pesticides, a total of 263 fractionation factors were published for thirty-three pesticides, including legacy pesticides such as atrazine, lindane, dichlorodiphenyltrichloroethane (DDT), chlordecone and organophosphorus compounds (Höhener et al., 2022). These data represent the state of the art of peer-reviewed literature by May 2022 and are given in an electronic annex (Excel database).

The compilation of enrichment factors includes 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 factors) 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)). The higher the number of carbon atoms is in a pesticide,

the lower the enrichment is expected because dilution with carbon atoms not interfering with the initial bond which is broken is observed.

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

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. An inverse effect was observed for ^{15}N in atrazine photooxidation, like for carbon (Hartenbach et al., 2008).

8. Recommendations for the Application of CSIA

Compound Specific Isotope Analysis (CSIA) provides a new type of information on pollutants in the environment to complement data on concentrations. The isotope data can be used either for understanding the variability of different sources, or for understanding the fate of the pollutant, especially by transformation reactions. Modern instrumentation can provide valid determinations of isotope ratios at low concentrations of contaminants at the field concentrations from different environmental matrices.

Application of CSIA at a contaminated site should start with a clear idea of the information that is sought from stable isotope analysis. Basically, there are two distinct goals: (i) source characterization or differentiation; (ii) qualitative or quantitative proof of biodegradation or abiotic transformation. The use of CSIA for these purposes has been discussed in detail throughout this guideline.

If the specific interest is the identification of an unknown source, the database of isotopic compositions of pesticides should first be consulted to check whether there is a significant variability in isotope composition between different manufactured pesticides. The isotope analysis should target those elements which show the highest variability. Analyses should then be performed on potential sources, and on the pollutant found at a receptor site (a ground water well/a surface water/ in a crop/ etc.).

If the specific interest is a qualitative proof of degradation, then both the database on isotope composition and the database on isotope enriching processes should be consulted. The extent of variability of isotope ratios in your target compound should first be assessed. The variability should be small, so the isotope analysis should include the stable isotope with the smallest variability. Furthermore, one should check the magnitude and direction of isotope enrichment of processes of interest. If for example you are interested to know whether your pesticide undergoes biotic transformation, you must check whether there is a significant isotope enriching process for a certain isotope, and whether this effect is normal or inverse. You may find for example that your pesticide varies usually in $\delta^{13}C$ between -27 ‰ and -25 ‰ in different formulations, and

that biotransformation will have a normal isotope enrichment (i.e., a negative enrichment factor ε). Then, you might define a target value as a proof of degradation, including safety margins for uncertainties of the analytical measurement and the uncertainty of the variability of the pesticides. Here in the present example, this limit might be safely set to -23 ‰, meaning that all samples in which you find higher isotope ratios will be qualified as having undergone some biotic degradation.

If the specific interest is quantification of degradation, again the database and literature on isotope enriching processes must be consulted and an appropriate isotopic fractionation factor must be selected. Using the fractionation factor, the second step is to estimate whether the observed changes in concentration of the contaminant at the field site are sufficient to produce a significant change in the isotope ratio. Best is if you have a precise idea of the initial isotope ratio of your source. If these two prerequisites are met this may be possible to set a conservative boundary on the extent of biodegradation or abiotic transformation at the field site.

Regarding the sampling strategy, it is recommended that first an ample analysis of pesticide concentrations is performed. The cost of concentration analysis is significantly lower than that for isotope analysis, because isotope ratio mass spectrometers are costly machines. Based on concentration data, a subset of samples can then be decided to be sent to isotope analysis, including only samples with concentrations above detection limits of isotope ratio mass spectrometers.

While compound specific isotope analysis can bring new dimensions in insights into processes occurring at field sites, it should not be applied as sole tool. CSIA cannot replace a proper hydrological and geochemical characterization or measurements of contaminant concentrations, and transformation products (if detectable). Multiple lines of evidence are needed to come to a meaningful assessment of the risks associate with the contaminants and the selection of an appropriate remedy. At sites contaminated with chlorinated solvents undergoing transformations to various less-chlorinated products, such multi-line approaches were developed, for example by establishing isotope mass balances with concentration and isotope data of the pollutants and the products, and by interpreting them based on flow and transport models (Aeppli et al., 2010a; Höhener et al., 2015).

It is also worth noting some limits for the application of CSIA in some cases for soil pesticides. As discussed in sections 2 and 3, when extracting a pesticide from a soil, a matrix effect can make it difficult or impossible to analyze the isotope ratio in pesticide properly. Also, isotope enrichment tends to be small for large molecules having a high number of one element, due to a dilution of the isotope effect during analysis when the sample is combusted or pyrolyzed before analysis in the mass spectrometer. Still more research is needed to push back such limits. With the recent appearance of high-resolution mass spectrometers of the type ORBITRAP (Hilkert et al., 2021), progress may be made in the next few years on that.

It is our hope that this Guide will be a useful practical extension of the earlier EPA CSIA guide (Hunkeler et al., 2008) and literature on CSIA for pesticides in soil. We expect that CSIA will have a growing role to investigate sources and transformation of pesticides at sites with diffuse pesticide pollutions. CSIA can guide decisions on selection and implementation of remediation strategies and may be used to monitor the performance of remedial technology in an early stage of implementation. The growth in the application of CSIA is driven by continued improvements in analytical methods, by more widespread availability of the instruments used in CSIA, by an increasing number of publications showing the broad applicability of CSIA to a variety of contaminants, and by an increasing appreciation for the unique information provided by CSIA.

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Annexes

Annex I: Isotopic compositions of pesticides from Literature and measured in this project.

ISOTOPEST: The pesticide isotopic database

https://ites.unistra.fr/isotopest

At ITES, Strasbourg, équipe Biogéochimie ISotopique et Expérimentale

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Annex II Fractionation factors for pesticides

In excel File named Annex II Guideline CSIA Decisive.xlsx, Worksheet contains fractionation factors from literature.

https://lce.univ-amu.fr/fr/projet-anr-decisive

Download

Annex III: Definitions

The purpose of this section of the annex is to clarify certain definitions, expressions and terms used in the context of compound-specific isotopic analysis. We are referring to recent literature in the field of environmental sciences, which has seen the emergence of numerous publications on the application of stable isotopes for the study of environmental compounds, and this from just about the year 2000. It should be noted, however, that there is extensive ancient literature on the applications of stable isotopes in fundamental studies in biochemistry, chemistry-physics, hydrology, geology, and many other fields.

In this "classic" literature, the terminology is sometimes not the same as that used today in recent environmental publications. For example, the isotopic ratios as defined in classical literature were given by dividing the abundance of the light isotope by that of the heavy, while in recent environmental literature the opposite, i.e., heavy/light, is retained. So, we want to clarify this from the beginning here and we ask the reader to fully understand that we are mostly quoting recent literature and we will not refer here to the old definitions and conventions.

Isotope

Form of an element with the same number of protons but a different number of neutrons.

Stable Isotope

Isotope that does not undergo any radioactive decomposition.

Isotopologues

Chemically identical compounds but having a different isotope composition.

Examples: H_2O, HDO, $H_2{}^{18}O$, ….

Isotopomers

Isotopologues having the same isotopic composition but a difference between the positions of each isotope. Two isotopomers have the same molar mass.

Examples: CH_3CHDCH_3 et $CH_2DCH_2CH_3$

Compound-specific isotope analysis

This analysis aims at measuring a specific isotopic ratio for a compound, for example for the active molecule of a pesticide formulation. This molecule must therefore first be separated from the matrix of its formulation, which is typically achieved by gas chromatography or liquid phase chromatography (Aelion et al., 2009). Compound-specific isotopic analysis gives an average ratio on all atoms of the compound, unlike position-specific isotopic analysis.

Multi-elemental compound-specific isotope analysis

Aims at measuring at least two different specific isotopic ratios for a compound. Produces a graph isotopic ratio element 1 vs. isotopic ratio element 2 with a slope called Λ.

« Bulk » isotope analysis

This analysis quantifies an isotopic ratio in a sample (a matrix) without separating the different constituents, for example in a crude pesticide formulation without separating the active compound from the matrix. It therefore does not target the ratio of the active compound. The commonly used technique is mass spectrometry coupled with elemental analysis. The term "bulk" is also used to express that a specific isotopic ratio for a compound is an average of ratios of all positions in the compound (see below).

Position-specific isotope analysis

This analysis measures a specific isotopic ratio for a position of an element in a compound. For the analysis, the compound must be in its pure phase. The techniques that can do this analysis are nuclear magnetic resonance NMR or mass spectrometry.

The latter technique requires fragmentation of the molecule, for example by pyrolysis. Both techniques can document a deviation from the isotopic ratio from the average of all positions in the compound. Thus, a position-specific analysis must be supplemented by a compound-specific analysis, called "bulk" in the sense of "average over the entire molecule." The definition of bulk here is therefore not the same as that given a few lines above.

Kinetic isotope effect KIE

The kinetic isotope effect KIE refers to the change in a chemical reaction rate when one of the atoms of a compound is replaced by an isotope. Formally, it is the ratio of reaction velocity of light (l) to heavy (h) KIE = kl/kh isotopes. KIE is a magnitude defined in the classical physical-chemical literature where the light isotope/heavy isotope l/h convention is used.

Apparent Kinetic Isotope effect AKIE

The observable kinetic isotopic effect is an KIE observed during a chemical reaction transformation in an environmental environment. It refers to fractionation at the reactive position and reflects the expression of the KIE under the constraints of environmental limitations where the reaction takes place (for example in a microbiological cell, with limitations of transfer through the cell walls of microbes). The AKIE cannot necessarily be compared to KIE without precaution, but a comparison is interesting to study the mechanisms of the chemical reaction that acted on the compound (Elsner, 2010).

Isotope fractionation factor

Factor characterizing the magnitude of isotopic separation. They are derived from the magnitude of an isotopic shift. For phase exchange reactions, $\alpha_{1\text{-}2}=R_1/R_2$ where the R_s are the isotopic ratios in the two phases involved. During a kinetic chemical reaction far away from equilibrium,$\alpha=k_h/k_l$ where k are the reaction rates of heavy and light isotopes, respectively.

Bulk enrichment factor

This is the fractionation factor obtained by looking at a transformation of an entire compound, expressed as α_{bulk}. This factor is obtained by compound-specific isotopic analyses. The majority of α in literature are α_{bulk}.

Position-specific enrichment factor

This enrichment factor $\alpha_{reactive\_position}$ is obtained by measuring the fractionation of a specific position in a compound during a transformation. This requires a position-specific isotopic analysis, but very few studies have been carried out so far. This factor can now also be estimated from the α_{bulk} by an approach developed and described in (Elsner et al., 2005). The value of

$\alpha_{reactive\_position}$ is inverse to the value of the AKIE.

Enrichment factor

The enrichment factor e is a more practical form of the fractionation factor α. We note:

$\varepsilon = (\alpha - 1) * 1000$ and we express ε in per thousand. In the ANR project DECiSIvE, measuring enrichment factors for the most used pesticides is the primary objective.

Isotope ratio

The relationship between the abundance of an isotope and the abundance of its associated isotope, for example.

$^{13/12}R=[^{13}C] / [^{12}C]$. If nothing is specified before R, the abundance of the heavy isotope is divided by that of the light.

Isotope ratio of a standard

The relationship between the abundance of an isotope and the abundance of its associated isotope in a standard (reference material), $\mathbf{R_{standard}}$. Table 3 gives the $\mathbf{R_{standard}}$ currently used to define the international scales for stable isotopes in common light elements. The reference materials that are distributed are described on the website of IAEA and also by the Geological Service of the United States USGS (Schimmelmann et al. 2016).

Initial isotope ratio

Relationship between the abundance of an isotope and the abundance of its associated isotope in a compound in its original state before a transformation, R_0.

δ Notation,"delta"

The isotopic **R** ratio is transformed into a change of deviation δ from the isotopic ratio of the standard, $\mathbf{R_{standard}}$ using equation 1. Note that in same publications it is preferred to omit the factor of thousand.

$$\delta = \left(\frac{R}{Rstandard} - 1\right) * 1000 \qquad \text{(eq. 6.1)}$$

$\Delta\delta$ Notation: Change of δ with respect to its initial value.

The change of δ from its initial value is expressed $\Delta\delta$ and is measured in per thousand.

Fraction of non-reacted compound

The fraction of a compound that has not yet been transformed, also called remaining fraction f. It is obtained either from the residual mass (remaining mass/initial mass), or from the remaining concentration (if the volume is constant, we have $f=C/C_{initial}$).

Transformation

A transformation is defined here being either a phase change (e.g., a liquid-vapor equilibrium or a sorption process) or a chemical reaction of a compound, including biochemical and microbiological reactions.

Isotope effect

An isotope effect is the result of isotopic fractionation. It can be kinetic or at equilibrium.

Normal isotope effect

A normal isotope effect is observed when the transformation of the light isotope is favored in relation to the transformation of the heavy isotope. This corresponds to a fractionation factor α **< 1** and negative enrichment factors ε in the recent environmental literature.

Inverse isotope effect

An inverse isotope effect is observed when the transformation of the heavy isotope is preferred to the transformation of the light isotope. This corresponds to fractionation factors with α **>1** and positive enrichment factors ε.

Slope Lambda Λ of a multi-elemental isotope analysis

The representation of data in a dual plot that traces the δ of an element 1 versus the δ of an element 2 often (but not always) gives a straight line. The slope of this straight line is called Λ and it is approximately equal to $\Lambda \approx \varepsilon_1/\varepsilon_2$**.** A non-linear correlation may result if the enrichment factors are of very different magnitudes, for example in a graph δ^2H versus δ^{13}C (Dorer et al. 2014), (Höhener and Atteia 2014).

To quantify Λ correctly from measured data in delta notation, one must plot $1000\ ln\left(\frac{1000+\delta}{1000+\delta_0}\right)$ of one element versus the same expression of the second element (Höhener and Imfeld 2021).

Rayleigh equation

The Rayleigh equation links the change in the isotope ratio to the progress of a transformation. There are different ways of writing Rayleigh equations: either by expressing the change in the R ratio versus the fraction of non-reacted compound f, or the change of δ to f. The most used forms of Rayleigh equations are given in Table IV.1. They apply to obtain fractionation factors and enrichment factors from measured data

when a substrate is transformed into a product. A Rayleigh equation that applies to intermediate products in degradation chains is given elsewhere (Höhener and Atteia 2014).

Table IV.1: Rayleigh equations in different forms

Category	Equation	Validity for	Reference
1: Equations for substrate: Non-linear forms			
Eq. 2a	$\frac{R}{R_0} \approx f^{(\alpha-1)}$	Approximation for the case of rare heavy isotopes: valid for ^{2}H, ^{13}C, ^{18}O, …	(Hunkeler et al. 2008)
Eq. 2b	$\frac{R}{R_0} = \frac{(1+R_0)}{(1+R)} f^{(\alpha-1)}$	Exact equation, valid for cases where the heavy isotope is not rare, e.g experiments with deuterated compounds	(Hunkeler et al. 2002)
Linear forms for substrate			
Eq. 2c	$ln\frac{R}{R_0} \approx (\alpha-1)\, ln\, f$	Equation 2a in linear form. Conditions like for 1a. To obtain α from measured R and f.	
Eq. 2d	$ln\frac{R}{R_0} = (\alpha-1) ln\frac{f(1+R_0)}{(1+R\ )}$	Eq. 2b in linear form.	
Eq. 2e	$1000\, ln\left(\frac{1000+\delta}{1000+\delta_0}\right) = \varepsilon\, ln\, f$	Conditions like for 2a. To obtains ε from measured δ and f.	(Elsner 2010)
Eq. 2f	$\Delta\delta \approx \varepsilon ln f$	Like 2e, but approximative. Valid only for changes $\Delta\delta$ of less than 20 per thousand.	(Hunkeler et al. 2008)
2: Equation for product			
Eq. 3	$\delta_p \approx \delta_{s0} - \varepsilon_s \frac{f_s ln f_s}{1-f_s}$	Used when changes in the isotope ratio of a product was measured but using the remaining fraction of the substrate f_s.	(Mariotti et al. 1981)

Annex IV: Addresses of Laboratories which offer services for CSIA

Isodetect GmbH

https://www.isodetect.de/en/isodetect/

Isodetect Leipzig Deutscher Platz 5b D-04103 Leipzig Phone: +49 – (0) 341 – 355 35 – 855	Isodetect München Richard-Wagner-Str. 15 D-80333 München Phone: +49 – (0) 89 – 8908 – 4187

Hydroisotop GmbH

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ITES Université de Strasbourg

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