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

3  
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23 **Key Words**

25 **ABSTRACT**

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

33  
34 We reviewed recent literature on ME-CSIA as an approach to improve the evaluation of sources  
35 and transformations of synthetic pesticides in agricultural soils. First, we discuss current extraction  
36 and purification techniques for pesticides in soils. Then, the analytical techniques for reliable  
37 measurements of the stable isotope composition of the pesticides are examined. Five hundred and  
38 fortyseven isotopic compositions for seventy-one active molecules and issued from different  
39 pesticide manufacturers were retrieved and listed in a database, for the following isotopes:  $^{13}\text{C}$   
40 (61%),  $^{15}\text{N}$  (14%),  $^{37}\text{Cl}$  (14%), and  $^2\text{H}$  (11%). This unique database reflects the overall isotopic  
41 variability and trackability of pesticide residues in the environment. Since the environmental fate of  
42 pesticides is governed by multiple transformation processes, including bio- and photodegradation,  
43 and other abiotic degradations, isotope fractionation values were retrieved from the peer-reviewed  
44 literature. This results in a catalogue of two hundred seventy fractionation values for thirty-three  
45 compounds to guide the interpretation of isotopic fractionation in the environment, including legacy  
46 pesticides such as atrazine, lindane, dichlorodiphenyltrichloroethane, chlordecone and  
47 organophosphorus compounds. Specific transformation processes may be identified and quantified  
48 using the Rayleigh concept for isotope fractionation during reaction. The review article summarizes  
49 existing modelling approaches and future trends to predict the reactive transport of pesticides in  
50 soils and associated hydrological systems.

51 **1. Introduction**

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

55 2012, mainly in agriculture (Fenner et al. 2013). In principle, pesticides are only registered for use if  
56 they are demonstrated not to persist too long in the environment beyond their intended period of  
57 action. However, many pesticides persist for long periods in soil, mainly in the form of non-  
58 extractable residues. On the one hand, pesticides leaching from the soil into surface and  
59 groundwater may cause severe deterioration of aquatic ecosystems (Brock et al. 2009). On the  
60 other hand, uptake of recalcitrant pesticides into crops can lead to human health risks and  
61 economic losses for farmers and distributors. Additionally, pesticide residues can impact soil living  
62 organisms with consequences on soil ecosystemic services. Over the last decades, efforts to  
63 reduce the use of pesticides have mostly failed, while global warming is expected to expand the  
64 prevalence of pests in the future and increase the societal dependence on chemical crop  
65 protection (Stokstad, 2018). The continuous and partially increasing use of synthetic pesticides in  
66 agriculture jeopardizes the ecological and chemical status of soil and aquatic ecosystems,  
67 threatening human health (Mateo-Sagasta et al. 2017). In the future, agroecology and organic  
68 farming may globally increase and clean and green chemistry in industry may give hope for change  
69 in the conception of modern pesticides. But the issue of the persistence in soils of historical and  
70 current pesticides and other pollutants still needs to be solved.

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

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

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

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

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

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

119 Nevertheless, in the last years many studies revealed that ME-CSIA can not only be applied to  
120 several pesticides such as s-triazine herbicides (Meyer et al. 2008; Reinnicke et al. 2012),  
121 phenoxyacid herbicides (Milosevic et al. 2013), substituted phenylureas such as isoproturon  
122 (Penning and Elsner 2007), glyphosate (Kujawinski et al. 2013), bentazone (Reinnicke et al. 2010),  
123 organophosphorus insecticides (Wu et al. 2014), hexachlorocyclohexanes (Badea et al. 2011),  
124 DDT (Holmstrand et al. 2004) and chlordecone (Chevallier et al. 2018) but also to transformation  
125 products amino methyl phosphonic acid (AMPA, Kujawinski et al. 2013) and 2,6-  
126 Dichlorobenzamide (Reinnicke et al. 2012). Analytical developments were made toward novel  
127 sample clean-up techniques, using for instance preparative HPLC (Schreglmann et al. 2013) or  
128 molecularly imprinted polymers (Bakkour et al. 2018), and novel injection systems including pre-  
129 concentration, like solid-phase microextraction (SPME) or large-volume injections. While all these  
130 studies brought a proof of concept for soil ME-CSIA to the scientific literature, they were  
131 sometimes restricted to artificially contaminated laboratory systems (e.g., soil microcosms or  
132 lysimeters) and higher concentrations, while field studies remain scarce (Alvarez-Zaldivar et al.  
133 2018).

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

## 147 **2. Analysis of soils**

### 148 **2.1 Extraction and clean-up for ME-CSIA of pesticide residues in soils: analytical challenges** 149 **and prospects**

150 *Challenges.* The extension of ME-CSIA of pesticide residues in soils faces three main analytical  
151 challenges limiting the evaluation of pesticide degradation in soils: (i) the need of a sufficient mass  
152 of analyte for ME-CSIA combined with low pesticide concentrations (sub-ug/g<sub>soil</sub>) following  
153 degradation, ageing or leaching, (ii) the wide range of pesticide physico-chemical characteristics  
154 (polar to apolar, non-volatile to volatile), and (iii) the co-enrichment of non-volatile soil components  
155 leading to the so-called 'matrix effect' during chromatographic separation. As for water samples,  
156 the development of appropriate sample preparation procedures, including optimized robust  
157 extraction and cleanup, is a fundamental key to maximize the analytical performance for precise  
158 and sensitive ME-CSIA without altering the isotope ratio of the target compounds (Torrento et al.  
159 2019).

160 The low concentrations of pesticides typically found in soils, the chemical properties of pesticides  
161 and the complexity of soil matrices, impose a significant pre-concentration step (from 1000 to  
162 100,000-fold to allow >10-15 ng C or Cl, >30 ng N or H on column) depending on the elemental  
163 contribution to the molar mass of the target analyte(s), as well as its solubility, polarity and  
164 volatility. In addition, the selectivity and efficiency of pesticide extraction largely varies according to  
165 the soil moisture, pH, texture and the amount of organic matter content controlling the amount of  
166 available pesticide in soils. For instance, pesticides typically sorb to soils with high clay and organic  
167 matter content and low water content (e.g. (Correia-Sa et al. 2012). Pesticide sequestration and  
168 extraction also depends on pesticide ageing, i.e., the pesticide physical and chemical distribution  
169 and diffusion over time in soil pore water, minerals and organic matter.

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

183 soil for C, H and Cl isotope analysis within the analytical precision of  $\pm 0.5\%$ ,  $\pm 5\%$  and  $\pm 0.3\%$ ,  
184 respectively. QuEChERS approaches (Anastassiades et al. 2003) involve an initial extraction with  
185 acetonitrile followed by salt addition to induce phase separation. QuEChERS with acetonitrile  
186 (Torrento et al. 2021) or modified for the use of ethylacetate (Alvarez-Zaldivar et al. 2018) or  
187 pentane:dichloromethane (3:1, v/v; Perèz et al. 2021) as extraction solvent, in pH buffered or  
188 unbuffered media, were used to extract atrazine, metolachlor, alachlor, acetochlor or metalaxyl  
189 from soil samples without causing significant isotope fractionation for carbon ( $\Delta\delta^{13}\text{C} \leq 1\%$ ) and  
190 nitrogen ( $\Delta\delta^{15}\text{N} \leq 0.5\%$ ), despite variable extraction efficiencies. However, extraction from two  
191 agricultural soils induced a systematic but reproducible isotope fractionation for Cl ( $\Delta\delta^{37}\text{Cl}$  between  
192  $+2.5$  and  $+3.5\%$ ) using a QuEChERS extraction kit with acetonitrile (Torrento et al. 2021).

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



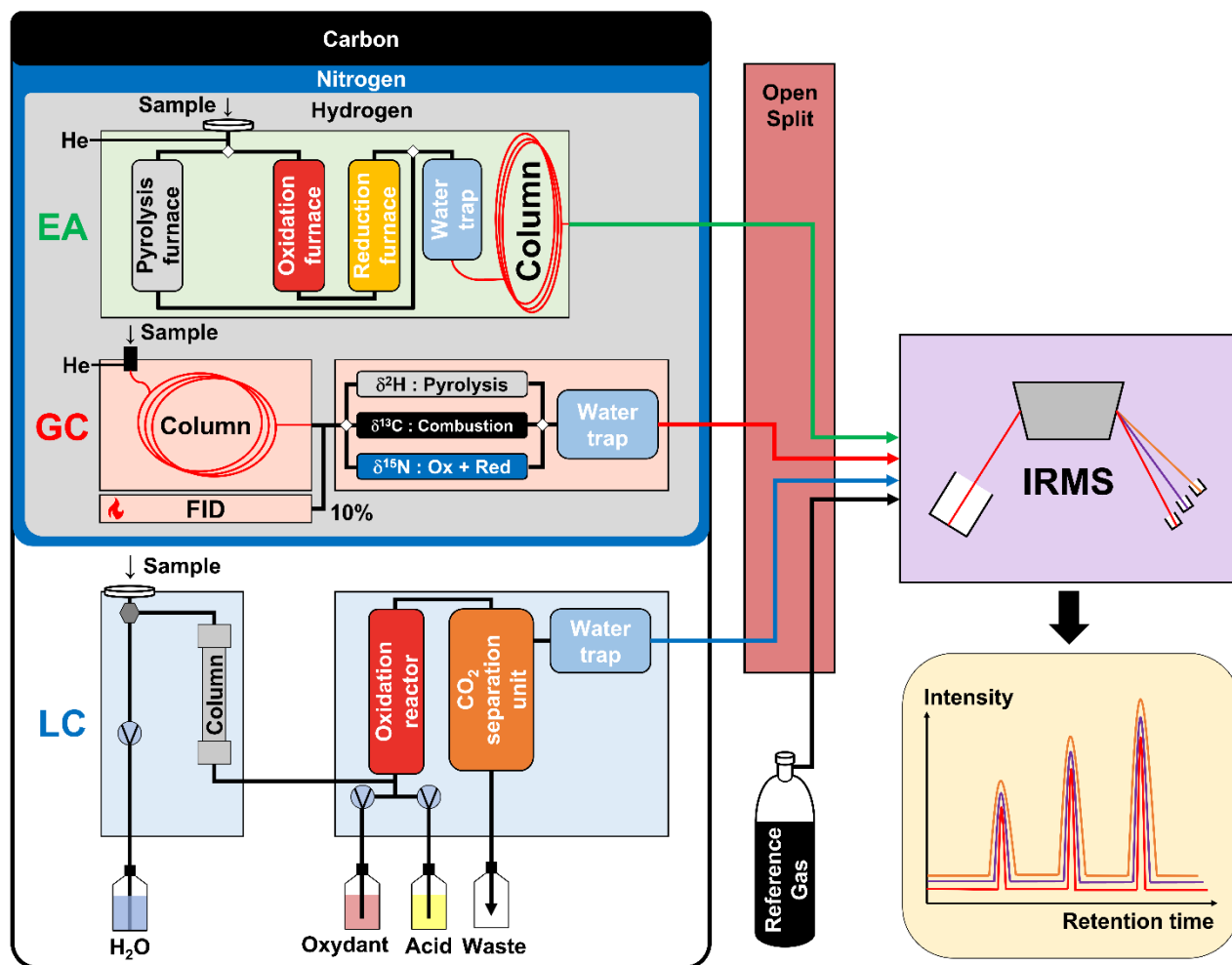
215 *Cleanup*. The pre-concentration step for pesticide CSIA and coextraction of matrix components  
216 requires cleanup strategies to purify soil extracts (e.g., Tadeo et al. 2012) and reduce co-enriched  
217 soil organic components of unknown isotopic composition. Among liquid–solid extraction  
218 techniques, the most common procedure is solid phase extraction (SPE) with cartridges or  
219 columns using sorbents, such as Florisil, silica, alumina, C18 or graphitized carbon black,  
220 depending on the selected pesticides (Buszewski et al., 2012). A current trend is to develop  
221 analytical methods where the extraction and purification are carried out simultaneously. Most soil  
222 QuEChERS approaches developed for pesticide CSIA rely on dispersive solid-phase extraction, in  
223 which a bulk amount of solid phase extraction sorbent is mixed with the extract rather than loading  
224 the extract in a column or cartridge packed with the sorbent. Primary Secondary Amine (PSA)  
225 sorbent was added to improve the cleanup of hexachlorocyclohexanes (Liu et al. 2020; Liu et al.  
226 2021) chloroacetanilide (Perez-Rodriguez et al. 2021; Torrento et al. 2021; Perez-Rodriguez et al.  
227 2021) and metalaxyl extracts (Masbou et al. 2018) with anhydrous magnesium or sodium sulfate  
228 as a cleanup and drying agent to remove soil matrix, ensure pesticide recovery and reduce GC-  
229 IRMS background. In the future, stir bar sorptive extraction (SBSE) coated with  
230 polydimethylsiloxane (PDMS) or newer materials may be used for additional clean up and  
231 enrichment following QuEChERS extraction of soil matrices for more selective pesticide extraction  
232 (Abdulra'uf and Tan 2014). For instance, molecularly imprinted polymers are synthetic polymeric  
233 materials with specific cavities designed for a template molecule that can retain the target pesticide  
234 (Farooq et al., 2018). Molecularly imprinted polymers could drastically reduce the matrix effect due  
235 to dissolved organic carbon for accurate C and N-CSIA measurements of 1*H*-benzotriazol, a  
236 typical corrosion inhibitor (Bakkour et al. 2018). In comparison with other methods, molecularly  
237 imprinted polymers offer high thermo-chemical stability and reusability and can be used for solid  
238 phase extraction as a highly selective sorbent for different types of pesticides in trace  
239 concentrations in soils (Farooq et al. 2018).

## 240 **2.2 Isotope analysis of pesticides**

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

$$244 \quad \delta = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad \text{eq. 1}$$

245 using the international  $R_{\text{standards}}$  given as e.g. in Aelion et al. (2010). The abundances of the heavy  
246 and light isotopes are generally quantified by an isotope ratio mass spectrometer (IRMS).  
247 Pesticides are often commercialized as formulations where the active molecule is mixed with  
248 additives in a solid or liquid matrix to facilitate their use. In order to trace its fate, only the  
249 abundances of isotopes from the active substance must be measured. Hence, it becomes obvious  
250 that purification and chromatographic separation of the pesticide is essential before measurement  
251 by IRMS. A large majority of isotope data on pesticides in this study (Supplementary Material  
252 Annex 1) were measured on gas chromatography coupled to an IRMS (GC-IRMS). For carbon  
253 isotope analysis of the polar herbicide glyphosate, the IRMS was coupled to liquid chromatography  
254 (LC-IRMS). And finally in an intermediate number of cases where pesticides were available as  
255 pure substances, the measurement was made on an elemental analyzer coupled to an IRMS (EA-  
256 IRMS). The latter is widely used in many labs since it permits measuring international standard  
257 materials as references and calibrate the IRMS by multiple point calibration (Schimmelmann et al.  
258 2016). The three analytical techniques are illustrated in Figure 1 and are presented in the following  
259 in more detail.



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

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

274 furnace with a pulse of oxygen. Then, the nitrous oxides are reduced to N<sub>2</sub>, and the latter is  
275 separated from CO<sub>2</sub> and other gases by the chromatographic column in the EA. The IRMS is first  
276 set to masses 28, 29, 30 to quantify the abundances of <sup>14</sup>N<sub>2</sub>, <sup>14</sup>N<sup>15</sup>N and <sup>15</sup>N<sub>2</sub> for the calculation of  
277 δ<sup>15</sup>N. The magnet of the IRMS is then switched by a peak jump to the masses 44-46 to quantify the  
278 CO<sub>2</sub> isotopologues with different masses. Correction for <sup>18</sup>O is made with the method of (Santrock  
279 et al. 1985). The mass of sample needed for accurate EA-IRMS measurement is mostly between  
280 0.2 to 0.5 milligrams of pure pesticide, which is weighted in tin or silver capsules.

281 **GC-IRMS.** This is the most widely applied method for pesticide isotope analysis, especially for real  
282 field samples, capable of measuring isotope ratios of H, C or N (Fig. 1). The main differences  
283 compared to EA-IRMS is that chromatographic separation of the compounds is made before  
284 pyrolysis or combustion, and that the injected mass of compound is much lower. The mass of  
285 carbon to reach reliable IRMS measurements of carbon stable isotope ratio is typically around 10-  
286 15 nanograms (Aelion et al. 2010). Knowing this value, the minimum injection amount into the GC  
287 can be back calculated according to dilution factors in the injector. For the hydrogen analysis,  
288 again a pyrolysis is operated at 1400°C to produce H<sub>2</sub>. For N and C analysis, a combustion furnace  
289 is used, also with reduction of NO<sub>x</sub> compounds to N<sub>2</sub>. The analysis of <sup>15</sup>N in pesticides requires,  
290 however, a separation of N<sub>2</sub> from CO<sub>2</sub>, which is done by freezing out the CO<sub>2</sub> in a cooled trap.

291 **LC-IRMS.** This technique has so far only been applied to C isotope analysis in the polar pesticide  
292 glyphosate (Fig. 1). The eluent was a 2.5 mM NaH<sub>2</sub>PO<sub>4</sub> solution adjusted to pH 1.9. The reagents  
293 to convert glyphosate to CO<sub>2</sub> were phosphoric acid and peroxodisulfate in a reactor kept at 99.9°C.  
294 Then the CO<sub>2</sub> was flushed with He to the IRMS. Details are provided in (Kujawinski et al. 2013)  
295 and Mogusu et al. (2015). This technique may also improve knowledge on the persistence of the  
296 transformation product amino methyl phosphonic acid, or other mostly polar products, following  
297 degradation processes of pesticides in soils. Apart from the three most-used techniques shown in  
298 Figure 1, other techniques were used. They were:

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

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

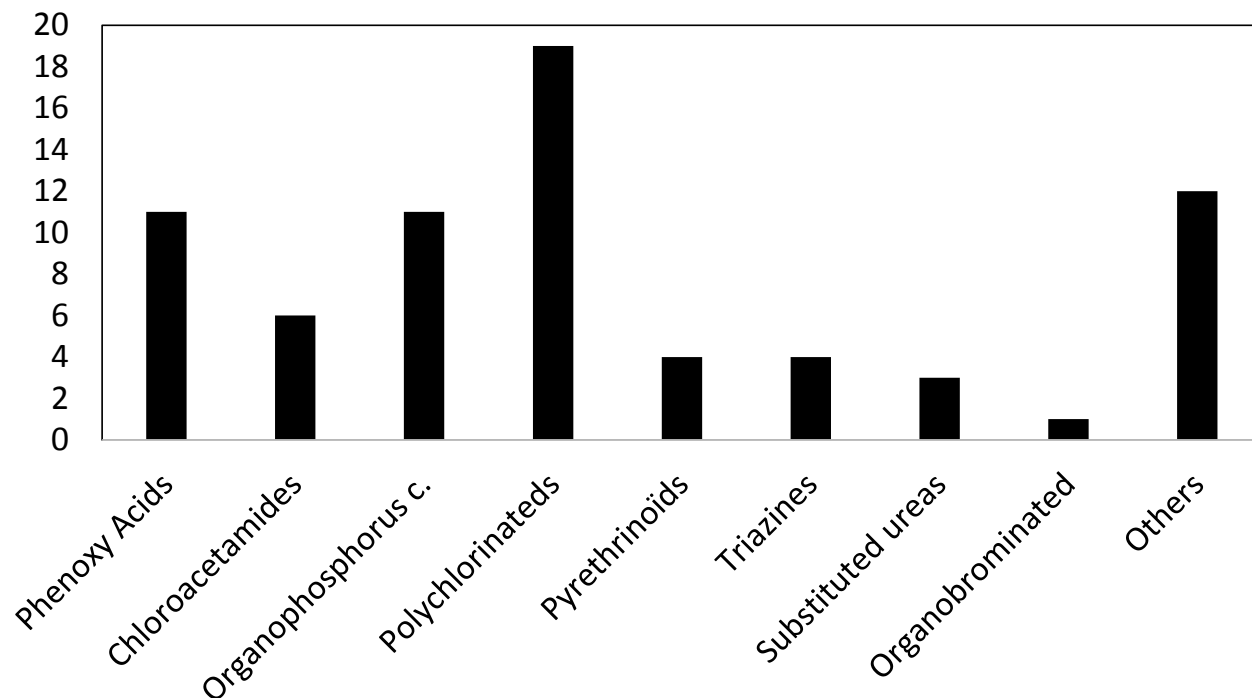
308 **GC-MC-ICPMS.** This technique consists of coupling a GC to a Multi-Collector Inductively Coupled  
309 Plasma Mass Spectrometer, first described by (Van Acker et al. 2006). For pesticides it was only  
310 used for chlorine isotope analysis. In the plasma, the masses of  $^{35}\text{Cl}$  and  $^{37}\text{Cl}$  are generated for the  
311 calculation of the isotope ratio, but care must be taken not to count also the mass of  $^{36}\text{ArH}$  when  
312 water vapor is reacting with argon gas (Horst et al. 2017). It has been applied in two studies  
313 looking at hexachlorocyclohexanes (Wu et al. 2019b), acetochlor and S-metolachlor (Lihl et al.  
314 2019). GC-MC-ICPMS measurements of pesticides may open the way to analyze the stable  
315 isotope ratios of heteroatoms, such as bromine or sulfur, in pesticide molecules.

316 **Offline MS.** Other studies converted pesticide samples by off-line methods to gaseous molecules  
317 containing chlorine or bromine and injected them to Dual-Inlet IRMS for the analysis of ratios of  
318  $^{37}\text{Cl}/^{35}\text{Cl}$  (Drenzek et al. 2002; Drenzek et al. 2004; Reddy et al. 2002) or  $^{81}\text{Br}/^{79}\text{Br}$  (Kuntze et al.  
319 2016).

### 320 **2.3 Isotopic compositions of pesticides from literature**

321 Isotopic compositions of pesticides and of key degradation products were compiled from the peer-  
322 reviewed literature (Figure 2 and Supplementary material Annex 1). The main criteria for selection  
323 of isotopic compositions of pesticides were the existence of a reliable stable isotope composition of  
324 the active molecule. Publications in which the isotope analysis by EA-IRMS targeted commercial  
325 formulations, meaning the active molecule was not pure, were not included (e.g. (Annable et al.  
326 2007). All hexachlorocyclohexane isomers were included although only the gamma isomer is a  
327 pesticide. The method of analysis is provided along with the isotopic composition. Isotopic  
328 compositions were found for 71 different compounds, in publications which appeared between  
329 2002 and 2021. A total of 547 isotopic compositions were listed, with 337 values for  $^{13}\text{C}$ , 75 values  
330 for  $^{37}\text{Cl}$ , 78 for  $^{15}\text{N}$  and 57 for  $^2\text{H}$ . One publication was found for  $^{18}\text{O}$  in glyphosate (Sandy et al.  
331 2013), and one for  $^{34}\text{S}$  in amethryn (Schürner et al 2015).

## Number of compounds for isotopic compositions



332

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

### 335 **2.4 Isotope fractionation values of pesticides**

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

341 Isotope fractionation values were found for 32 compounds (Supp. Mat., Annex 2). A total of 263  
342 isotope enrichment factors were retrieved, with 174 values for  $^{13}\text{C}$ , 19 values for  $^{37}\text{Cl}$ , 50 for  $^{15}\text{N}$ , 13  
343 for  $^2\text{H}$ , and 7 for  $^{81}\text{Br}$ , 2 for  $^{34}\text{S}$  and 1 for  $^{18}\text{O}$ . The brominated compounds all concerned reactions  
344 acting on the fumigant ethylene dibromide. For  $^{13}\text{C}$ , all enrichments were normal (i.e., negative  
345 isotope fractionation values) except two for photooxidation of atrazine where inverse effects were  
346 observed. The most pronounced normal isotope effect (the most negative enrichment factor for  
347  $^{13}\text{C}$ , -30.9 ‰) was observed for the abiotic reduction of ethylene dibromide, a small compound with

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

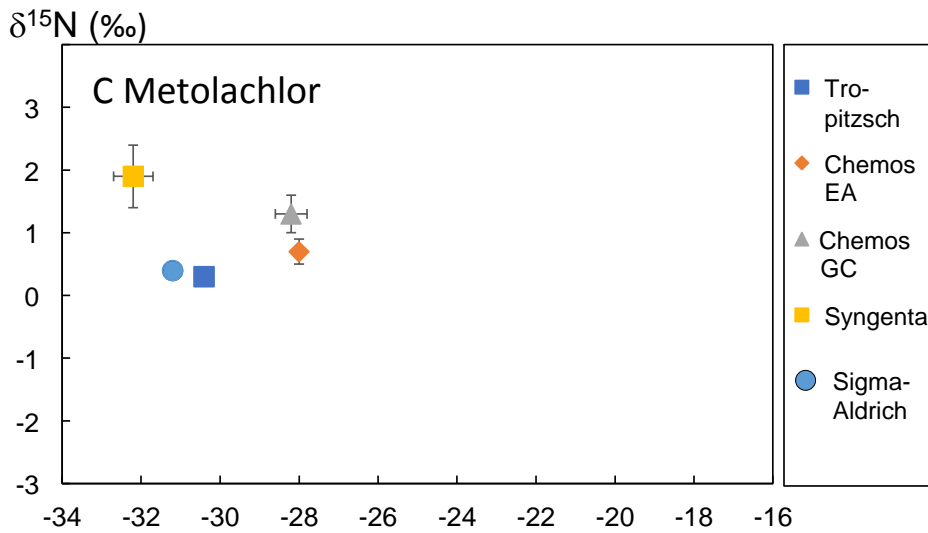
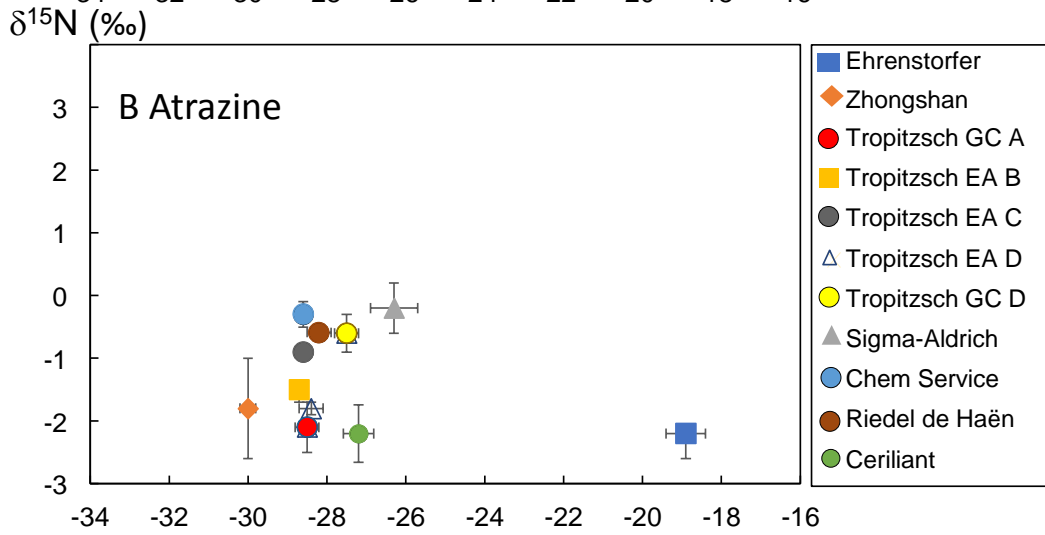
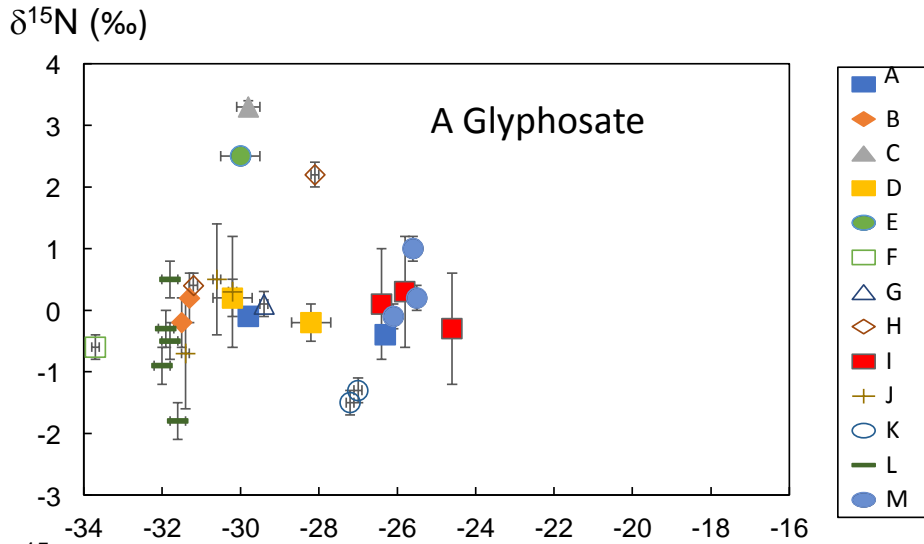
351 For  $^{15}\text{N}$ , both inverse and normal isotope effects were observed for biotic transformations. Inverse  
352 effects with positive isotope fractionation values were observed for the biotic degradation of triazine  
353 herbicides (Schürner et al. 2015; Chen et al. 2019), the aerobic degradation of bromoxynil  
354 (Knossow et al. 2020) and also for the acid hydrolysis of atrazine (Meyer et al. 2009; Masbou et al.  
355 2018).

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

### 360 **3. Applications of pesticide ME-CSIA**

#### 361 **3.1 Source identification, Forensics**

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



$\delta^{13}\text{C}$  (‰)



369 *Figure 3: Isotopic compositions of A) glyphosate from 13 manufacturers. Data from Mogusu et al.*  
370 *2015, including also data from Kujawinski et al. 2013. For list of manufacturers see Supp. Inf.*  
371 *Annex A. B) atrazine from 7 manufacturers. Data from Chen et al (2017), Masbou et al. (2018),*  
372 *Meyer et al. (2008, 2013), Ponsin et al. 2019, Torrento et al. 2019+2021. Schreglmann et al. 2013.*  
373 *C) metolachlor from 5 manufacturers. Data from Alvarez-Zaldivar et al. (2018), Masbou et al.*  
374 *(2018) and Torrento et al (2021).*

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

382 Another famous legacy pesticide, atrazine, also features highly variable isotopic composition (Fig.  
383 3B). However, the  $\delta^{13}\text{C}$  ratios of atrazine span only from -26.3 to -30 ‰ except for the atrazine of  
384 Ehrenstorfer which is at - 19 ‰, while  $\delta^{15}\text{N}$  ratios range from -2.2 to -0.2 ‰. This highlights that the  
385 power for discrimination of distinct atrazine sources is far below that of glyphosate. However,  
386 stable chlorine isotope analysis has been developed for atrazine (Ponsin et al. 2019). Further data  
387 on the stable chlorine isotope composition may help in the future to establish a more powerful  
388 basis for forensic investigations of atrazine pollution.

389 In contrast to glyphosate and atrazine, S-metolachlor analyzed from five manufacturers shows  
390 relatively little isotopic variation for carbon and nitrogen (Figure 3C), suggesting that the chlorine  
391 isotope analysis must be included for forensic studies relying on S-metolachlor (Torrento et al.  
392 2021). If isotopic signatures of S-metolachlor in commercial formulations do not vary significantly,  
393 transformation processes involving bond breaking may be followed up in the environment,  
394 independent of the used commercial formulation. This may allow catchment-scale evaluation of  
395 metolachlor transformation.

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

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

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

### 416 **3.2 Process identification by ME-CSIA**

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

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

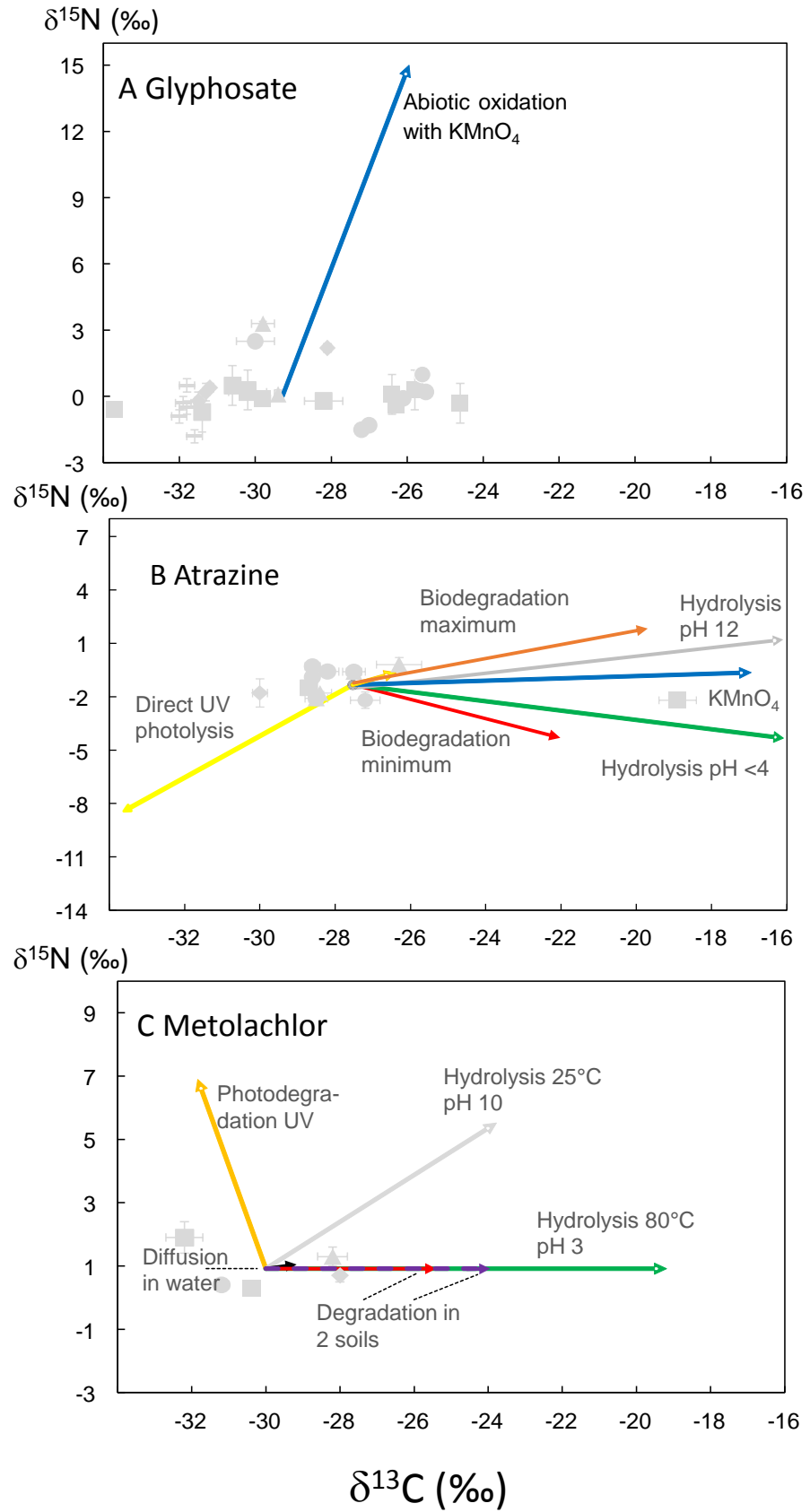
431 multi-elemental plots, like in section 3.1, for glyphosate, atrazine and metolachlor, for both carbon  
432 and nitrogen isotopes. Changes in the isotopic ratios for each process were calculated using the  
433 approximate Rayleigh equation (eq. 2), assuming a removal of 90% of the initial mass

434 
$$\Delta\delta^{13}C = \epsilon_C \ln (f)$$
 eq. 2

435 Where f is the remaining mass fraction in soil (0.1 in our calculations).  $\Delta$  is the isotope shift with  
436 respect to the initial isotope composition. For the three compounds, we calculated an average  
437 isotope composition for all manufacturers, and drew calculated isotope shifts with arrows away  
438 from this average composition (Figure 4). It is worth noting that the slope of these arrows in Figure  
439 4 corresponds to Lambda, which is generally used in the literature on ME-CSIA as (eq. 3, here for  
440 nitrogen and carbon isotopes):

441 
$$\Lambda_{N/C} = \frac{\epsilon_N}{\epsilon_C}$$
 eq.3

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



450 **Figure 4:** Change of stable isotope ratios ( $^{15}\text{N}$  vs.  $^{13}\text{C}$ ) for selected processes acting on average  
451 glyphosate (A), atrazine (B) and metolachlor (C). Grey shaded points represent variable  
452 compositions from different manufacturers, already shown in Figure 3. Note that photolysis at  
453 natural sunlight does not create an isotope shift for atrazine (Drouin et al, 2021).  $\text{KMnO}_4$ :  
454 Potassium permanganate

455 For glyphosate, only the abiotic oxidation with  $\text{KMnO}_4$  was characterized for fractionation of both  
456 stable carbon and nitrogen isotopes (Fig. 4A redrawn from (Mogusu et al. 2015)). This oxidation  
457 leads to a strong N enrichment, with a much higher shift than the range of variability of different  
458  $\delta^{15}\text{N}$ . The nitrogen isotope analysis may confirm the abiotic oxidation in soil even if the initial  
459 composition of the glyphosate was not known. In contrast, the carbon enrichment is small for  
460 abiotic oxidation, and carbon isotope analysis would not be able to confirm this removal process in  
461 soil, especially when the initial  $\delta^{13}\text{C}$  of the glyphosate was not known.

462 The C and N isotope enrichment of atrazine was studied for various processes (Torrento et al.  
463 2021; Drouin et al. 2021; Meyer et al. 2014; Meyer and Elsner 2013; Hartenbach et al. 2008), and  
464 for a range of pure bacterial strains known to degrade atrazine (Schürner et al. 2015; Chen et al.  
465 2017; Chen et al. 2019, Fig. 4B). Generally large carbon isotope shifts are expected after 90%  
466 atrazine degradation, whereas nitrogen isotope shifts remain modest. However, nitrogen shifts are  
467 either positive or negative, which can help for process identification. But the slopes of the isotope  
468 shifts between various abiotic and biotic degradations of atrazine are not very distinctive, and for a  
469 correct process identification one should add also stable chlorine isotope analysis (Ponsin et al.  
470 2019).

471 For metolachlor removal processes, large C and N isotope shifts are illustrated in Fig. 4C, with  
472 data from (Drouin et al. 2021; Masbou et al. 2018; Sun et al. 2021; Torrento et al. 2021). Photolysis  
473 under UV lamp and alkaline hydrolysis result in well-distinct isotope fractionation patterns in Figure  
474 4C, while acid hydrolysis, soil degradation and diffusion in water do not differ in their slope  $\Delta$ . They  
475 differ, however, in the magnitude of carbon isotope shifts: diffusion does almost not fractionate,  
476 and soil biodegradation leads to lower carbon isotope shifts than acid hydrolysis. To tease apart  
477 those two processes, additional analyses are needed. It is also worth noting that enantiomers of  
478 chiral pesticides, such as metolachlor, have identical physico-chemical properties, and transport  
479 processes or abiotic reactions appear not to change enantiomer proportions (Bidleman et al.  
480 2013). In contrast, degradation of chiral pesticides may be mainly microbiologically mediated and  
481 enantioselective (Liu et al. 2005). Because degradation rates often differ among enantiomers and

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

### 487 **3.3 Identification and modeling of pesticide degradation in soil and across the soil-hillslope-** 488 **water continuum**

489 Isotope data derived from ME-CSIA require adapted modeling approaches to interpret and predict  
490 the persistence and the transport of pesticides in soil and water. Reactive transport models can  
491 support this deployment as they explore numerically and quantitatively the interactions of physical,  
492 chemical and biological processes in surface and subsurface systems (Li et al. 2017, Prommer et  
493 al. 2019). Historically, the first reactive transport models integrating isotopic signatures aimed at  
494 interpreting monitored natural attenuation of legacy industrial pollutants in aquifers, e.g. (Hunkeler  
495 et al. 2008; Thouement et al. 2019; Antelmi et al. 2021) or in wetlands e.g. (Alvarez-Zaldivara et al.  
496 2016). Some reactive transport models incorporating isotope fractionation also derived the  
497 physico-chemical conditions, including redox conditions, dissolved oxygen, temperature, or pH, to  
498 predict pollutant degradation and associated isotopic fractionation across the aquifer domain  
499 (D’Affonseca et al. 2011).

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

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

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

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

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

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

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

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



*Transport* - Integrate Mischaracterization of preferential flows in soil reactive transport models for better prediction of pesticides transit time?

i) *in situ* measurements with geophysical techniques and tracer experiments for soil profiles, ii) geostatistical methods, pedotransfer functions or meta-models to parametrize preferential flow in soil reactive transport models

---

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

559 Finally, a major interest of introducing CSIA data in soil reactive transport models is to prove,  
560 characterize and quantify pesticide transformation. This, however, depends on the ability to  
561 interpret the transport of water and pesticides by preferential and matrix flows (Jarvis et al. 2012;  
562 Köhne et al. 2009a+b; Beven and Germann 2013; Gassmann et al. 2015; Weiler 2017). Including  
563 preferential flow framework in soil reactive transport models may help to improve prediction of  
564 transit times in soil. Here, CSIA may further help tease apart fast flows, mobilizing “fresh” pools of  
565 pesticides with isotopic signatures mirroring that of the applied products, from the “aged” pools of  
566 pesticides in soil, reflecting degradation in the soil matrix. *In situ* measurements of macropores,  
567 e.g., earthworm channels, soil pipes and larger soil cracks, in the soil profile may help identifying  
568 preferential flow and providing parameter values for soil reactive transport models using  
569 geophysical techniques and tracer experiments (Weiler 2017). At the hillslope and catchment  
570 scales, geostatistical methods, pedotransfer functions or meta-models, i.e., simplified statistical  
571 models built with 1D soil reactive transport models such as MACRO (Lindahl et al. 2008), may  
572 provide spatially distributed parameters. This, in turn, allows predicting preferential and soil matrix  
573 flows in Horizontal 2D soil reactive transport models with associated pesticide loads (Jarvis et al.  
574 2012).

#### 575 4. Conclusions and perspectives

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

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

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

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

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

610 Furthermore, the presence of chlorine atoms in a pesticide can cause problems during the  
611 pyrolysis of the molecule before mass spectrometry of the produced H<sub>2</sub>. Only very few studies of  
612 isotope analysis of other elements in pesticides, such as <sup>81</sup>Br (1 study, Kuntze et al. 2016), <sup>18</sup>O (1  
613 study, Sandy et al. 2013), or <sup>34</sup>S (1 study, Schürner et al. 2015) were found in literature. Future  
614 research may foster the development of stable isotope analysis for these elements to strengthen  
615 the ME-CSIA approach and enhance the distinction between transformation pathways in both  
616 laboratory and field settings.

617 More fundamentally, the mechanistic understanding of isotope fractionation and related processes  
618 should still be improved. Fractionation by diffusion has been studied in soil air (e.g. Bouchard et al.  
619 2008), water (Jeannotat and Hunkeler, 2012; Sun et al. 2021) or clay (Wanner and Hunkeler,  
620 2015). More knowledge should be gained on isotope fractionation of pesticides during diffusion in  
621 narrow soil pores, since this may be affected by Knudsen diffusion, where molecules are subject to  
622 collisions with pore walls, in addition to collisions with other gas or water molecules. Also, the  
623 fundamental understanding of isotope fractionation by sorption/desorption during multi-step  
624 partitioning and volatilization should be improved. For instance, a recent study by Zamane et al  
625 (2020) showed quite large isotope effects during volatilization of toluene and propan-2-ol from  
626 sandy soil.

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

### 632 **Supplementary Material:**

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

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640

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642

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653

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