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1     **Identification and characterization of tebuconazole transformation products in soil by**  
2                     **combining suspect screening and molecular typology**

3

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19

20    **Keywords:** time-of-flight mass spectrometry, pesticide, triazole, clustering, environmental risk  
21    assessment.

22

23 **ABSTRACT**

24 Once released into the environment, pesticides generate transformation products (TPs) which  
25 may be of (eco-)toxicological importance. Past studies have demonstrated the difficulty to  
26 predict pesticide TP occurrence and their environmental risk by monitoring-driven approaches  
27 mostly used in current regulatory frameworks targeting only known toxicologically relevant  
28 TPs. We present a novel combined approach which identifies and categorizes known and  
29 unknown pesticide TPs in soil by combining suspect screening time-of-flight mass  
30 spectrometry with *in silico* molecular typology. This approach applies an empirical and  
31 theoretical pesticide TP library for compound identification by both non-target and target  
32 time-of-flight (tandem) mass spectrometry and structural elucidation through a molecular  
33 structure correlation program. *In silico* molecular typology was then used to group the  
34 detected TPs according to common molecular descriptors and to indirectly elucidate their  
35 environmental properties by analogy to known pesticide compounds having similar molecular  
36 descriptors. This approach was evaluated via the identification of TPs of the triazole fungicide  
37 tebuconazole occurring in a field dissipation study. Overall, 22 empirical and 12 yet unknown  
38 TPs were detected and categorized into three groups with defined environmental properties.  
39 This approach combining suspect screening time-of-flight mass spectrometry with molecular  
40 typology could be extended to other organic pollutants and used to rationalize the choice of  
41 TPs to be intensively studied towards a more comprehensive environmental risk assessment  
42 scheme.

43

44 **CAPSULE ABSTRACT**

45 Combination of time-of-flight mass spectrometry and molecular typology was shown to be of  
46 fundamental interest to identify and categorize tebuconazole transformation products in soil.

47

## 48 INTRODUCTION

49 Pesticides, used in agriculture to ensure crop yield and quality, constitute a large group of  
50 xenobiotic compounds in the environment.<sup>1,2</sup> They and their transformation products (TPs)  
51 have globally been identified as major contaminants of natural water resources. The United  
52 States Environmental Protection Agency (U.S. EPA) estimates a global release of 2.4 billion  
53 kilograms pesticides per year supporting important economic sectors of agriculture but also  
54 causing major environmental pollution.<sup>3</sup> Another aspect which makes pesticides a chemical  
55 group of particular interest is their rapidly changing and evolving market due to the  
56 continuous introduction of new molecules. In order to reach the market, new pesticide  
57 molecules have to undergo an environmental risk assessment (ERA) procedure based on a  
58 large set of data submitted by the pesticide companies and evaluated by the official authorities  
59 (e.g. the Plant Protection Products and their Residues panel<sup>4</sup> of the European Food Safety  
60 Authority for Europe (EFSA)). This procedure is periodically repeated in order to re-evaluate  
61 the already registered pesticide molecules. Once released into the agro-ecosystem, pesticides  
62 and their TPs can diffuse away from their target point, contaminate surrounding water  
63 resources<sup>5</sup> and impact non-target organisms supporting key ecosystemic services.<sup>6</sup> The  
64 properties of TPs such as bioavailability, mobility and ecotoxicity can be substantially  
65 different from the parent compound<sup>7,8</sup> and although their biological efficacy is usually lower,<sup>9</sup>  
66 they could be highly (eco-)toxicologically relevant.<sup>10</sup> In Europe, *a posteriori* monitoring of  
67 the contamination of water and air resources by pesticides is imposed by corresponding  
68 directives.<sup>11,12</sup> In contrast, no monitoring of soil contamination by pesticides is required at EU  
69 level. This is the result of the lack of a corresponding directive, although the need for a  
70 regulation was proposed to the European Commission.<sup>13</sup> Thus, if an unacceptable risk for the  
71 environment becomes evident several years after the introduction of a pesticide to the market,  
72 restrictions in its use are imposed and if monitoring studies reveal that no alleviation of the

73 risk was achieved, the pesticide is taken off the market. Past experience has shown that the  
74 knowledge of the existence of (eco-)toxicologically relevant TPs typically emerges only about  
75 20 to 30 years after the introduction of a given pesticide to the market.<sup>10</sup> Currently used  
76 monitoring-driven approaches for pinpointing environmentally and (eco-)toxicologically  
77 relevant pesticide TPs often concentrate on water samples and do not prevent the  
78 contamination of natural resources and impose mitigation actions only after contamination is  
79 reported. Novel approaches able to identify potential TPs and *in silico* assessment of their  
80 toxicity and environmental contamination could be part of the solution of this issue.

81 The gap of knowledge about the existence or properties of pesticide TPs derived by abiotic  
82 and biotic processes in the environment may be the reason why relevant TPs have been  
83 overlooked for so long. Conventional analytical methods focus on the monitoring of the so-  
84 defined ‘(eco-)toxicologically relevant’ pesticide TPs or TPs ‘relevant for groundwater  
85 contamination’.<sup>4,14</sup> Consequently, most studies focus on a limited number of molecules which  
86 have been identified as targets by monitoring-driven approaches and are calibrated against  
87 analytical standards. Therefore, they often provide only a partial view of the complex  
88 transformation patterns of pesticides in the environment. Thus, there is a need to develop an  
89 innovative approach by combining powerful analytical methods detecting also unknown TPs  
90 with categorization methods based on the chemical features of the detected TPs.

91 The analytical techniques suitable to detect unknown and suspected molecules in the absence  
92 of analytical standards are high resolution mass spectrometry (MS), namely quadrupole time-  
93 of-flight MS (QTOF-MS) and Fourier transform MS (FT-MS), as well as nuclear magnetic  
94 resonance (NMR) spectroscopy.<sup>15</sup> These techniques are often combined in forensics<sup>16</sup> or  
95 medicine,<sup>17</sup> where QTOF-MS is used for screening for new unknown molecules and then is  
96 supplemented by NMR spectra for the structural elucidation of a compound. However, NMR  
97 spectroscopy is an effective method for the confirmation of molecular structures but is rather

98 less appropriate for the analysis of TPs in environmental samples due to its rather poor  
99 sensitivity, which generally is three orders of magnitude lower than MS.<sup>15</sup>

100 Categorization of unknown and suspected molecules can be achieved by *in silico* molecular  
101 typology that enables the property-related clustering of potential TPs.<sup>18</sup>

102 In this study, we present a new approach for the *a posteriori* analysis of agricultural soil  
103 samples treated with a pesticide by combining high-resolution QTOF-MS with *in silico*  
104 molecular typology. Our approach enables profiling and molecular structure elucidation of  
105 unknown and suspected pesticide TPs in agricultural soil by the use of a compound library  
106 containing empirical (already known) and theoretical (yet unknown) TPs. The proposed  
107 approach consists of three principal steps: (i) construction of an in-house pesticide-specific TP  
108 library based on literature survey and establishment of suspect TPs, (ii) QTOF-MS analyses  
109 of soil extracts by non-target MS to screen TPs against this library, followed by target tandem  
110 MS using an ion inclusion list to propose their structure via accurate mass daughter ions and a  
111 molecular structure correlation (MSC) program and (iii) categorization of detected TPs based  
112 on *in silico* analysis using molecular typology.

113 To demonstrate the potential of this novel approach, the dissipation of the triazole fungicide  
114 tebuconazole (TCZ) was investigated in a field dissipation study. TCZ was chosen as a model  
115 compound because, despite being on the market for quite some time (27 years),<sup>19</sup> there are  
116 still significant knowledge gaps regarding its environmental fate, its transformation in soil and  
117 the ecotoxicological impact of potential TPs.<sup>20</sup> The potential formation of various triazole TPs  
118 known to be recalcitrant to biodegradation and to possibly interact with the hormone  
119 regulation of non-target organisms by inhibition of the cytochrome P450-dependent  
120 conversion of lanosterol to ergosterol is particularly relevant.<sup>21,22</sup> The applied approach is of  
121 interest for improving future environmental fate studies to become more comprehensive, thus  
122 strengthening ERA.

## 123 MATERIAL AND METHODS

### 124 Library setup of TCZ TPs

125 An in-house TCZ TP library was constructed based on a thorough literature review including  
126 research papers (cited in the results section entitled ‘Empirical TCZ TPs’) and data sets of the  
127 pesticide company on the transformation pattern of TCZ in various matrices. Search was done  
128 by means of ‘Web of Science’, ‘Google’ and others using search terms like ‘tebuconazole’,  
129 ‘metabolite’, ‘transformation product’, ‘degradation’ and others. The developed library was  
130 composed of 47 empirical (already detected in at least one study) and 29 theoretical TPs. The  
131 latter were created based on expert knowledge taking into account common reaction processes  
132 in organic chemistry and biochemistry likely leading to probable molecules. The TCZ TP  
133 library contains the name, molecular formula, molecular structure and mass of each molecule  
134 (Supporting Information, table S1). Initial pretests of chemical analysis were carried out on a  
135 subset of soil samples randomly chosen from the field study to search for TCZ TPs. Retention  
136 times of TPs proposed via MSC were then added to the library as an additional parameter for  
137 identification purposes in MS screening.

### 138 TCZ field dissipation study

139 The field dissipation study was conducted on a North Italian field (45°04’80’’N, 09°45’60’’E)  
140 in autumn/winter 2013/14. The field soil was characterized as loamy sand (4.2 % clay, 13.5 %  
141 silt, 82.3 % sand) with a pH of 7.3 and 4.5 % of organic matter. It was not treated with  
142 pesticides for more than five years and cropped with winter wheat. A commercial formulation  
143 of TCZ (Folicur SE, Bayer Garden) was applied on field plots of 60 m<sup>2</sup> each at 2.5 kg TCZ/ha  
144 (5x the recommended dose).<sup>23</sup> Soil samples were taken at 0, 3, 7, 21, 35, 56, 70, 105 and 125  
145 days after pesticide application according to the ISO standard.<sup>24</sup> For each plot and each  
146 sampling date, a composite soil sample was prepared from 10 sub-samples collected with an  
147 iron cylinder of 5 cm diameter, taking a soil depth of 5 cm. Soil samples were manually

148 homogenized, sieved (5 mm) and kept frozen at -20°C until further processing. The field  
149 dissipation study was part of a larger study investigating field dissipation of three model  
150 pesticides including TCZ at various application rates (four plots are used in this study: two  
151 non-treated control plots and two TCZ 5x dose plots). The field dissipation of TCZ was  
152 monitored in soil extracts via high performance liquid chromatography coupled to photodiode  
153 array detector (HPLC-PDA) and followed a biphasic pattern with a half-life (DT<sub>50</sub>) of 2.5  
154 days, as calculated with a Hockey-stick kinetic model.<sup>25</sup> After 125 days, 14.2 % of applied  
155 TCZ were remaining in the investigated soil layer.

#### 156 **TCZ and TP extraction from soil**

157 TCZ and its related TPs were extracted with acetone from 40 g of soil as explained in the  
158 Supporting Information (SI-2). The final obtained residues were re-dissolved in 1 mL of  
159 70 v% methanol and 30 v% ultrapure water containing 0.01 % phosphoric acid and used for  
160 chemical analysis. The extraction efficiency for TCZ was 86.36 % ± 0.45 %.

#### 161 **UHPLC-ESI-QTOF MS analysis**

162 In order to investigate TCZ TPs, samples were first analyzed by non-target UHPLC-ESI-  
163 QTOF mass spectrometry. TCZ TPs were primarily identified by screening of the MS-only  
164 raw data against the TP library, using accurate mass, isotope spacing and isotope ratio for  
165 identification. A difference in accurate mass of 5 ppm, together with an isotopic pattern  
166 overall score higher than 85 % were used as cutoff values. Compounds that passed both  
167 thresholds, that had a plausible peak chromatogram feature and that were not detected in the  
168 controls (non-treated soil samples), were chosen for further analysis by target UHPLC-ESI-  
169 QTOF tandem MS. For this purpose, the three most abundant precursor ions (generated  
170 during MS-only) and the retention time (RT) of each MS-only detected TP were exported to  
171 be included into a target list for tandem MS. Method details are explained in the Supporting  
172 Information (SI-3).



## 173 **Molecular structure correlation**

174 Elucidation of the molecular structure of compounds detected by tandem MS was obtained  
175 through a molecular structure correlation (MSC) program (Agilent MassHunter Molecular  
176 Structure Correlator B.05.00). The software correlated accurate mass daughter ions for a  
177 compound of interest with the molecular structures available in the in-house custom library by  
178 using a ‘systematic bond-breaking’ approach as described by Hill and Mortishire-Smith.<sup>26</sup>  
179 Briefly, an overall correlation score was generated by taking into account the individual  
180 scores from each daughter ion, the mass accuracy of these fragments, and the overall  
181 percentage of ion intensities being plausibly explained with substructures.

## 182 **Molecular typology for categorization of detected TCZ TPs**

183 TCZ and its detected TPs were categorized using the molecular typology approach ‘TyPol’  
184 (Typology of Pollutants) proposed by Servien et al. (2014) allowing clustering of chemicals  
185 based on the use of molecular descriptors and of environmental parameters.<sup>18</sup> Environmental  
186 parameters of TPs of TCZ being unknown, clustering was only done using a set of 40  
187 molecular descriptors. After clustering, TyPol was used to estimate the environmental  
188 parameters (vapor pressure, dissipation half-life, sorption coefficient and bioconcentration  
189 factor) of TCZ TP clusters by analogy to reference compounds for which environmental  
190 parameters are known. Details about the method are given in the Supporting Information (SI-  
191 4).

## 192 **RESULTS AND DISCUSSION**

### 193 **Empirical TCZ TPs**

194 Literature review revealed that studies reporting TCZ dissipation were often ignoring  
195 potential TP occurrence and were mainly following targeted analytical approaches.<sup>27,28</sup> It is  
196 noteworthy that newly discovered TPs detected by non-target approaches were observed  
197 during degradation of TCZ by bacterial or fungal cultures,<sup>29</sup> as photodegradation products,<sup>30</sup>

198 in field soil studies<sup>31,3</sup>, in soil microcosm studies<sup>33,34</sup> and in fruits.<sup>35</sup> Additional information  
199 was found in regulatory documents mainly citing unpublished or confidential studies  
200 reporting TPs in soil and crops partially based on MS and NMR spectroscopy.<sup>36,37,38</sup>  
201 Altogether, literature review led to the construction of a library containing 47 empirical and  
202 29 theoretical TCZ TPs (Supporting Information, table S1) which was used for TCZ TP  
203 suspect screening in soil samples from the field dissipation study described before.

#### 204 **Detection of TCZ TPs**

205 The QTOF-MS analyses of soil extracts gained from the field dissipation study led to the  
206 detection of the parent compound TCZ, as well as of 22 empirical and 12 yet unknown TPs,  
207 which were contained in the library. Among the 34 detected features, some of them showed  
208 the same exact mass and retention times. 12 TPs passed all steps of identification from non-  
209 target MS-only identification to target tandem MS and structure correlation. Three TPs passed  
210 only the first two steps (selected by tandem MS but not further identified through structural  
211 correlation) and 19 TPs were only detected by MS identification with plausible chromatogram  
212 features, but were not further selected by tandem MS due to either method restrictions or used  
213 selection criteria or molecular characteristics (e.g. low mass, high volatility,...). Table 1  
214 summarizes the data of all detected TCZ TPs, reporting compound name, molecular structure,  
215 mass, reference if empirical, and retention time of each TP. Moreover, the time points are  
216 provided (expressed in days after the application of the TCZ treatment) at which the TCZ TPs  
217 were detected and information regarding the confidence of identification. TPs are coded  
218 according to their level of identification confidence (a and orange hooklet: detected by MS-  
219 only; a,b and yellow hooklet: detected by MS and selected by tandem MS; a,b,c and green  
220 hooklet: detected by MS, selected by tandem MS and identified by MSC). According to  
221 Schymanski et al. (2014) level 'a' (MS-only detection) could be assigned to 'level 4 -  
222 identification confidence by unequivocal molecular formula'; level 'a,b' (selection by tandem

223 MS) to 'level 3 - tentative candidate - identification confidence by structure, substituent and  
224 class'; level 'a,b,c' (structure proposition by MSC) to 'level 2,b - probable structure -  
225 identification confidence by diagnostic evidence'.<sup>39</sup> The molecular formulas of compounds  
226 that have only been detected by non-target MS but were not further selected by target tandem  
227 MS are rather unlikely to be false positives, as they (i) were not detected in the controls, (ii)  
228 have plausible chromatogram features and isotopic patterns and (iii) do not differ in RT  
229 between replicates and different time points. However, their molecular structure rests tentative  
230 as the compound was not selected by tandem MS and as the molecular structure elucidation  
231 by MSC is only possible based on the detected product ions produced during tandem MS.  
232 More likely, reasons for the impossibility of selection by target tandem MS could be either  
233 peak saturation at high concentrations (as it has been seen for the parent molecule TCZ when  
234 injected at high volumes), or a high sensitivity of compounds present at low concentrations, or  
235 too low masses of the product ions or a high volatility of the precursor. Indeed, electron spray  
236 ionization (ESI) conditions and the chosen collision energies have to be efficient enough to  
237 produce precursor and product ions while avoiding fragmenting the precursor into too small  
238 product ions, as the minimum m/z value was chosen to be 60 due to noise exclusion.  
239 Regarding low m/z values, it must be mentioned that the low molecular weight of putative  
240 TPs not detected by the non-target MS-only screening such as the well-known triazole  
241 fungicide TP 1-H-1,2,4-triazole (TP 63)<sup>35-38</sup> may be considered as false negatives as they may  
242 not be efficiently ionized by ESI due to their volatility at the high temperature of the used ion  
243 source. Nevertheless, it should be noted that a compound, which was detected by MS-only  
244 and selected by target tandem MS but whose molecular structure could not be proposed by  
245 MSC, might be correct in its molecular formula but might have a molecular structure not  
246 present in the TP library. Additionally, the poor observed correlation for the low molecular  
247 weight TPs might be affected by the formation of product ions, which were below the m/z

248 threshold and thus were missing for MSC. Strictly, all the detected molecules by our approach  
249 still remain inferred until confirmed by retention time and fragmentation pattern of pure  
250 standards, which are not available on the market for most of them.

251 Despite of this weakness, the benefits of suspect screening coupled to MSC for the elucidation  
252 of molecular structures represent a powerful alternative when standards are not available.  
253 These benefits can be illustrated by the example of TPs 13 and 15, which are constitutional  
254 isomers. As they have the same molecular formula and mass, they could not be discriminated  
255 by MS-only and tandem MS (Supporting Information, figure S1) even using MS instruments  
256 with higher resolution power. MSC was able to correlate the product ions to the respective  
257 precursor for elucidation of their molecular structure (Supporting Information, figure S2) and  
258 hence had the capacity to discriminate between these TPs without need of any additional  
259 separation step or the use of standards. Furthermore, it must be pointed out that an even  
260 higher number of TPs can be taken into account through suspect screening than in targeted  
261 approaches, thus providing more comprehensive information on the transformation of one or  
262 more pesticides to a series of TPs in the environment.

263 The pesticide dissipation study carried out at field scale was found to be suitable for the  
264 purposes of our study. The applied TCZ concentration was in the range of concentrations  
265 commonly applied in agriculture<sup>23</sup> and nine time points within 125 days after treatment  
266 enabled the comprehensive exploration of TP formation and decay patterns although detailed  
267 data on the temporal patterns were not established due to the absence of analytical standards.

268 As TCZ TPs were extracted from the top 5 cm layer of soil, it has to be noted that only those  
269 TPs present at this soil layer could be studied. TP properties can strongly deviate from those  
270 of the parent molecule<sup>40</sup> and might be vulnerable to leaching, runoff or volatilization although  
271 the parent molecule is considered as relatively immobile or non-volatile.<sup>36,41</sup> Thus, TPs which  
272 potentially dissipated from the top soil layer, which were not extractable from soil via the

273 applied extraction method or which were not listed in the TP library were disregarded in this  
274 study.

275 Moreover, TCZ and several of its TPs have two enantiomers or do even possess more than  
276 one chiral center. This may be important concerning stereoselective toxicity and dissipation<sup>42</sup>  
277 but it was not considered in this study. For the completion of our analytical approach and for  
278 the provision of comprehensive information on actual environmental fate, molecular typology  
279 can be applied to estimate potential properties of the detected TPs in a theoretical manner.  
280 This technique can be used to estimate properties of TPs of any anthropological substance in  
281 nature.

### 282 **Categorization of TCZ TPs**

283 TCZ and its 34 detected TPs were categorized by TyPol into 3 different clusters (table 1,  
284 figure 1) according to their molecular descriptors including molecular weight, number of  
285 atoms, dipole moment, polarizability or total energy (Supporting Information, table S2). TCZ  
286 and 23 of its TPs were categorized into cluster 1 exhibiting the highest simple and valence  
287 molecular connectivity indices (MCIs), energy of the highest occupied molecular orbital  
288 ( $E_{\text{HOMO}}$ ), molecular weight (MW), surface (S), polarizability ( $\alpha$ ), number of atoms and the  
289 lowest total energy ( $E_{\text{tot}}$ ). Cluster 2 was composed of 7 TPs that were characterized by the  
290 highest dipole moment ( $\mu$ ), energy of the lowest unoccupied molecular orbital ( $E_{\text{LUMO}}$ ) and  
291 the lowest number of chlorine atoms. The final 4 TPs were grouped in cluster 3. Those  
292 molecules were characterized by the lowest MCIs,  $E_{\text{HOMO}}$ , MW, S and  $\alpha$  but by the highest  
293  $E_{\text{tot}}$ .

294 The different molecular properties of the TPs within each cluster also adumbrate deviating  
295 environmental behaviors, once more emphasizing the importance of the comprehensive  
296 knowledge of the existence of each occurring TP. This means that TPs which were classified  
297 into the same cluster as their parent molecule TCZ (including newly detected and yet

298 unknown TPs) may have similar behavior in the environment compared to TCZ. TPs which  
299 were clustered away from TCZ may have different environmental fate and ecotoxicological  
300 impact compared to TCZ. This can be especially problematic if their formation is ignored and  
301 thus only TCZ and known TPs are quantified and monitored in the environment. To evaluate  
302 these hypotheses, TyPol was applied for a second time in order to categorize 116 various  
303 well-known pesticides and 19 TPs available in the TyPol database by analysis of both  
304 molecular descriptors and their known environmental parameters (half-life in soil ( $DT_{50}$ ),  
305 sorption coefficient ( $K_{OC}$ ), vapor pressure ( $P_{vap}$ ) and bioconcentration factor (BCF)). The  
306 compounds formed 6 clusters and median values of the molecular descriptors for each cluster  
307 were obtained (an example is given for  $K_{OC}$  in the Supporting Information, tables S3 and S4,  
308 and figure S3). In order to obtain a preliminary idea of the potential environmental behavior  
309 of the detected TCZ TPs, their molecular descriptors were compared to the median values of  
310 the 6 clusters formed by clustering the 116 pesticides and 19 TPs. When the molecular  
311 descriptors of the well-known compounds of one cluster were highly similar to those of a  
312 TCZ TP cluster, it was assumed that environmental parameters could be in a similar range.  
313 TPs that were in the same cluster as their parent compound TCZ (cluster 1) were found to  
314 overlap with a cluster of pesticides characterized by i) low  $P_{vap}$  (median  $P_{vap} = 0.026$  mPa),  
315 indicating a low volatility from soil and plants and thus low risk for short or long range  
316 transfer through the atmosphere,<sup>43</sup> ii) low mobility in soil (median  $K_{OC} = 825$  L/kg)  
317 suggesting a low risk for groundwater contamination,<sup>44</sup> iii) low persistence in soil  
318 (median  $DT_{50} = 37$  days) and iv) low potential for bioaccumulation (median BCF = 78)  
319 (Supporting Information, table S2). In the same manner, TCZ TPs forming cluster 2 had  
320 molecular descriptor values similar to pesticides and TPs exhibiting i) a higher soil mobility  
321 than TCZ (median  $K_{OC} = 217$  L/kg), thus posing a moderate potential risk for groundwater  
322 contamination,<sup>44</sup> ii) a higher potential for volatilization losses (median  $P_{vap} = 0.098$  mPa) than

323 TCZ, iii) lower persistence in soil (median  $DT_{50} = 20$  days) and iv) a lower potential for  
324 bioaccumulation (median BCF = 44). Finally, TCZ TPs forming cluster 3 were the most  
325 mobile molecules among the identified TPs (median  $K_{OC}$  of 123 L/kg) suggesting a  
326 potentially higher risk for groundwater contamination. Furthermore, the TPs of cluster 3  
327 posed the highest risk for volatilization losses (median  $P_{vap} = 1.600$  mPa) suggesting a  
328 significant risk for long or short range atmospheric transfer. Their persistence in soil was  
329 assumed to be similar to that of cluster 2 ( $DT_{50} = 20$  days) and lower than that of TCZ (cluster  
330 1) and their potential for bioaccumulation was estimated to be low (BCF = 1) in comparison  
331 to cluster 1 (BCF = 78) and cluster 2 (BCF = 44). These results are in agreement with  
332 previous observations showing that when  $E_{HOMO}$ , MCIs, and MW decrease, sorption of  
333 organic compounds tend to decrease, and when  $\alpha$ ,  $\mu$  and MW increase,  $P_{vap}$  tend to decrease.<sup>45</sup>  
334 Overall, the *in silico* approach using TyPol suggests that 23 of the 34 TCZ TPs targeted in this  
335 study are expected to have a similar environmental behaviour as the parent compound. On the  
336 other hand, the remaining 11 TPs may be more mobile and volatile than TCZ suggesting a  
337 higher risk for groundwater and air contamination. However, their lower persistence and  
338 lower bioaccumulation potential than TCZ may counterbalance this risk. This combined  
339 method allows a first assessment of the fate of theoretical TPs in the environment identifying  
340 those of potential concern that need to be intensively studied in a refined risk assessment  
341 approach. If we consider TCZ as a case study, certain TPs from each cluster could be selected  
342 based on their molecular features (e.g. selecting between some constitutional isomers, keto-  
343 enol tautomers or others) and further considered for a refined ERA.

344 To our knowledge, our approach is the first which combines (i) comprehensive screening of  
345 environmental samples (particularly soils) for suspected pesticide TPs based on a library of  
346 empirical and theoretical TPs, (ii) detection of TPs without the need of analytical standards,  
347 (iii) proposition of their molecular structures without the need of NMR spectroscopy and then

348 (iv) categorization of the detected TPs according to their physicochemical properties aiming  
349 to assess their environmental behavior. The approach described in this study could be applied  
350 for refining pesticide risk assessment and thus enable regulators and agrochemical industries  
351 to identify potential pesticide TPs showing relevant properties for further environmental and  
352 ecotoxicological assessment. Once the selected TPs are synthesized, absolute confirmation  
353 and quantification can be enabled and thus TP fate, environmental concentration and toxicity  
354 to ecosystems and human health can be assessed providing a refined and comprehensive risk  
355 assessment of a given pesticide.

## 356 **ASSOCIATED CONTENT**

357 Supporting information is available regarding the TCZ TP library, TP extraction from soil, the  
358 analytical method, chromatogram and MSC examples, the molecular typology method,  
359 detailed TyPol results for TCZ and its TPs and for the 116 pesticides and 19 TPs from the  
360 TyPol database. This material is available free of charge via the Internet.

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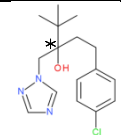
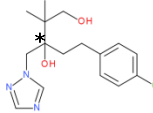
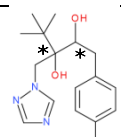
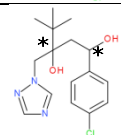
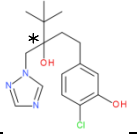
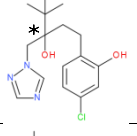
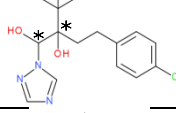
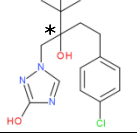
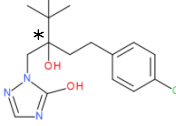




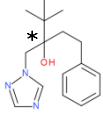
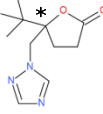
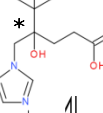
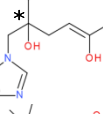
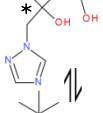
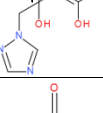
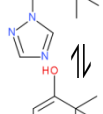
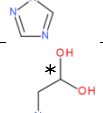
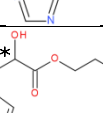
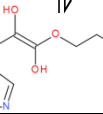
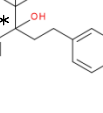
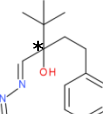
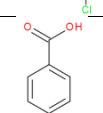

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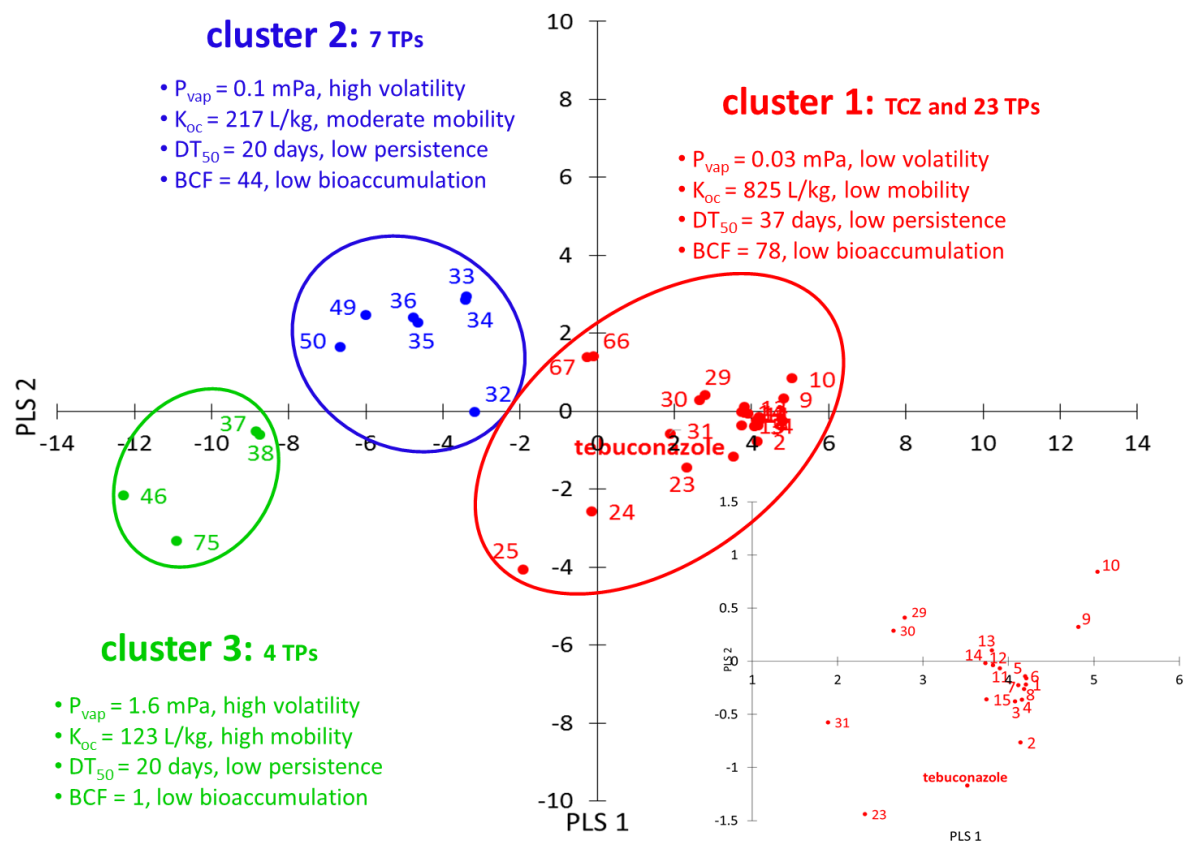
558 Table 1: TCZ TPs detected in this study by suspect screening analysis. Reference to the  
 559 degree of confidence on identification (a. detected by MS (orange hooklet); b. selected by  
 560 target tandem MS (yellow hooklet); c. proposed molecular structure by MSC (green hooklet)),  
 561 name, structure, mass and reference if empirical of each TP are provided. The time points at  
 562 which a TP was detected and the cluster into which it was categorized are given. Blue boxes  
 563 group TPs having the same mass.

compound name	molecular structure	mass [g/mol]	reference	detected in this study	RT [min]	details	TyPol cluster
TCZ, 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol		307.15	38	✓ a,b,c	9.38	detected at 0,3,7,21,35, 56,70,105 and 125 d	1
TP 1, 5-(4-chlorophenyl)-2,2-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentane-1,3-diol		323.14	29	✓ a	9.24	detected at 21,35 and 70 d	1
TP 2, 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentane-2,3-diol		323.14	37	✓ a	9.24	detected at 21,35 and 70 d	1
TP 3, 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentane-1,3-diol		323.14	theoretical TP	✓ a	9.24	detected at 21,35 and 70 d	1
TP 4, 2-chloro-5-[3-hydroxy-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentyl]phenol		323.14	theoretical TP	✓ a	9.24	detected at 21,35 and 70 d	1
TP 5, 5-chloro-2-[3-hydroxy-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentyl]phenol		323.14	37	✓ a	9.24	detected at 21,35 and 70 d	1
TP 6, 2-[2-(4-chlorophenyl)ethyl]-3,3-dimethyl-1-(1,2,4-triazol-1-yl)butane-1,2-diol		323.14	theoretical TP	✓ a	9.24	detected at 21,35 and 70 d	1
TP 7, 1-[2-[2-(4-chlorophenyl)ethyl]-2-hydroxy-3,3-dimethyl-butyl]-1,2,4-triazol-3-ol		323.14	theoretical TP	✓ a	9.24	detected at 21,35 and 70 d	1
TP 8, 2-[2-[2-(4-chlorophenyl)ethyl]-2-hydroxy-3,3-dimethyl-butyl]-1,2,4-triazol-3-ol		323.14	theoretical TP	✓ a	9.24	detected at 21,35 and 70 d	1

TP 9, 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentane-1,3,5-triol		339.14	37	✓ a	9.06	detected at 3,7,21,35, 56,70,105 and 125 d	1
TP 10, 1-[2-[2-(4-chlorophenyl)ethyl]-2-hydroxy-3,3-dimethyl-butyl]-1,2,4-triazole-3,5-diol		339.14	theoretical TP	✓ a	9.06	detected at 3,7,21,35, 56,70,105 and 125 d	1
TP 11, 1-(4-chlorophenyl)-3-hydroxy-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-1-one		321.12	30-33	✓ a,b,c	9.41	detected at 3,7,21,35, 56,70,105 and 125 d	1
TP 12, (E)-1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pent-1-ene-1,3-diol		321.12	37	✓ a,b,c	9.41	detected at 3,7,21,35, 56,70,105 and 125 d	1
TP 13, 1-(4-chlorophenyl)-3-hydroxy-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-2-one		321.12	theoretical TP	✓ a,b,c	9.41	detected at 3,7,21,35, 56,70,105 and 125 d	1
TP 14, (Z)-1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pent-1-ene-2,3-diol		321.12	theoretical TP	✓ a,b,c	9.41	detected at 3,7,21,35, 56,70,105 and 125 d	1
TP 15, 2-[2-(4-chlorophenyl)ethyl]-2-hydroxy-3,3-dimethyl-1-(1,2,4-triazol-1-yl)butan-1-one		321.12	theoretical TP	✓ a,b,c	9.41	detected at 3,7,21,35, 56,70,105 and 125 d	1
TP 23, 1-(4-chlorophenyl)-4-methyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol		293.13	37	✓ a,b,c	10.15	detected at 3,7,21,35, 56,70,105 and 125 d	1
TP 24, 4-(4-chlorophenyl)-2-methyl-1-(1,2,4-triazol-1-yl)butan-2-ol		265.10	37	✓ a	6.54	detected at 105 and 125 d	1
TP 25, 4-(4-chlorophenyl)-1-(1,2,4-triazol-1-yl)butan-2-ol		251.08	37	✓ a	4.24	detected at 3,7,21,35, 56,70,105 and 125 d	1
TP 29, 4-[3-hydroxy-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentyl]phenol		289.18	theoretical TP	✓ a,b	8.20	detected at 105 and 125 d	1
TP 30, 2,2-dimethyl-5-phenyl-3-(1,2,4-triazol-1-ylmethyl)pentane-1,3-diol		289.18	theoretical TP	✓ a,b	8.20	detected at 105 and 125 d	1

TP 31, 4,4-dimethyl-1-phenyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol		273.18	theoretical TP	✓ a,b,c	9.65	detected at 3,7,21,35,56,70,105 and 125 d	1
TP 32, 5-tert-butyl-5-(1,2,4-triazol-1-ylmethyl)tetrahydrofuran-2-one		223.13	29,34	✓ a,b,c	5.13	detected at 35,56,70,105 and 125 d	2
TP 33, 4-hydroxy-5,5-dimethyl-4-(1,2,4-triazol-1-ylmethyl)hexanoic acid		241.14	33	✓ a	8.38	detected at 3,7,21,35 and 56 d	2
TP 34, 5,5-dimethyl-4-(1,2,4-triazol-1-ylmethyl)hex-1-ene-1,1,4-triol		241.14	33	✓ a	8.38	detected at 3,7,21,35 and 56 d	2
TP 35, 3-hydroxy-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentanoic acid		227.13	32	✓ a	4.72	detected at 3,7,21,35 and 56 d	2
TP 36, 4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pent-1-ene-1,1,3-triol		227.13	32	✓ a	4.72	detected at 3,7,21,35 and 56 d	2
TP 37, 3,3-dimethyl-1-(1,2,4-triazol-1-yl)butan-2-one		167.11	33	✓ a,b,c	9.28	detected at 35,56,70,105 and 125 d	3
TP 38, (E)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)but-1-en-2-ol		167.11	33	✓ a,b,c	9.28	detected at 35,56,70,105 and 125 d	3
TP 46, 2-(1,2,4-triazol-1-yl)ethane-1,1-diol		129.05	35,36	✓ a,b	0.68	detected at 56,70,105 and 125 d	3
TP 49, butyl 2-hydroxy-3-(1,2,4-triazol-1-yl)propanoate		213.11	38	✓ a	7.27	detected at 3,7,21,35 and 56 d	2
TP 50, (Z)-1-butoxy-3-(1,2,4-triazol-1-yl)prop-1-ene-1,2-diol		213.11	38	✓ a	7.27	detected at 3,7,21,35 and 56 d	2
TP 66, (NE)-N-[2-[2-(4-chlorophenyl)ethyl]-2-hydroxy-3,3-dimethylbutylidene]formamidine		280.13	29	✓ a,b,c	8.14	detected at 7,21,35,56,70 and 105 d	1
TP 67, 1-(4-chlorophenyl)-4,4-dimethyl-3-[(E)-(methylenehydrazono)methyl]pentan-3-ol		280.13	29	✓ a,b,c	8.14	detected at 7,21,35,56,70 and 105 d	1
TP 75, 4-chlorobenzoic acid		156.00	37	✓ a	9.05	detected at 70 and 105 d	3

\* Chiral center



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566 Figure 1: Categorization of TCZ and its 34 detected TPs into 3 clusters according to their  
 567 molecular descriptors on the two main components of the PLS regression (PLS 1 and PLS 2)  
 568 and their estimated environmental parameters. Formed clusters are defined by a different  
 569 color code. Each TP has its respective number. The inserted graph serves as enlargement of a  
 570 part of cluster 1.