# Investigation of pesticides and their transformation products in the Júcar River Hydrographical Basin (Spain) by wide-scope high-resolution mass spectrometry screening

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**Abstract**

The Water Framework Directive 2000/60/EC implemented by the European Union established as the main objectives to achieve a "good ecological and chemical status" of the surface water and a "good quantitative and chemical status" of groundwater bodies. One of the major pressures affecting water bodies comes from the use of pesticides and their potential presence in the water ecosystems. For this purpose, the reliable determination of pesticides and their transformation products (TPs) in natural waters (both surface and groundwater) is required. The high number of compounds potentially reaching the aquatic environment makes extraordinary difficult, if not impossible, to investigate all these compounds even using the most powerful analytical techniques. Among these, liquid chromatography coupled to high-resolution mass spectrometry is emphasized due to its strong potential for detection and identification of many organic contaminants thanks to the accurate-mass full spectrum acquisition data. This work focuses on wide-scope screening of many pesticides and their TPs in surface water and groundwater samples, collected between March and May 2017, in the Júcar River Hydrographical Basin, Spain. For this purpose, a home-made database containing more than 500 pesticides and TPs was employed. Analyses performed by liquid chromatography coupled to quadrupole-time of flight mass spectrometry (LC-QTOF MS) allowed the identification of up to 27 pesticides and 6 TPs. The most detected compounds in groundwater were the herbicides atrazine, simazine, terbuthylazine, and their TPs (atrazine-desethyl, terbumeton-desethyl and terbuthylazine-desethyl). Regarding surface water, the fungicides carbendazim, thiabendazole and imazalil, the herbicide terbutryn and the TP terbumeton-desethyl were also detected. These results illustrate the wide use of these compounds (in the present or in the recent past) in the

area under study and the vulnerability of the water bodies, and are in accordance with previous findings in other water bodies of the different Spanish Hydrographic systems.

## 1. Introduction

Pesticides are compounds used worldwide, in different sectors, but especially in the agricultural field. These substances can be degraded by living organisms (generating metabolites) and in the atmosphere, soil and/or water (transformation products, TPs). In all these cases, TPs (including also metabolites) can reach concentration levels higher than the parent substances and be even more toxic (Richardson and Ternes, 2014). As a result of their massive use around the world, pesticides and TPs move through the different environmental compartments, leading to a consequent potential risk to living organisms (including humans) or/and environment.

In the last years, it has been observed a general trend towards the use of more effective pesticides with lower environmental impact/persistence (Dumas et al., 2017; Sannino et al., 2004) and a shift from "traditional cultivation techniques" to "modern agricultural practices"(Pascual Aguilar et al., 2017). Although most pesticides cataloged as very persistent (Sousa et al., 2018) have been banned, and many compounds have been withdrawn after implementation of Directive 91/414/EEC (The Council of the European Communities, 1991), it is still possible to find products forbidden some years ago in groundwater (GW) and in soil. This is the case, among others, of atrazine (triazine herbicide) and its TPs, which 10 years after its prohibition, remain detected in GW (Pitarch et al., 2016; Talja et al., 2008; Tappe et al., 2002). So, despite the decrease in pesticides use, a comprehensive monitoring, including compounds currently used as well as those banned, is needed in order to evaluate their presence in surface water (SW) and GW (Hernández et al., 2008). In addition, the pesticides used in agriculture are constantly changing, according to the increasingly restrictive regulations and the advances in the control of pests and weeds in the chemical industries.

In order to reach an improved water status, the European Parliament established the Water Framework Directive in 2000, including as the main objective to achieve a "good ecological and chemical status" for all European Union water bodies by 2015 (Directive 2000/60/EC; European Community, 2000). This Directive stipulates that GW bodies must "register and invert any significant and sustained upward trend in the concentration of any contaminant". The Article 17 of the Directive 2006/118/EC (European Parliament and Council of European Union, 2006), announces several strategies for the prevention and control of GW pollution. This Directive shows the criteria for considering a GW body to have a "good chemical status" and to reverse the trend of increasing contaminant concentrations. Regarding SW bodies, the Spanish ordinance (Ministerio de Agricultura Alimentación y Medio Ambiente, 2015) establishes the threshold values for concentrations of pollutants (Menchen et al., 2017).

To this aim, it is necessary to go in depth on the knowledge of occurrence and concentration levels of priority substances (some of them pesticides). The first studies of the River Basin Management Plans in Europe (EU) showed that more than half of Europe´s SW bodies are in worse conditions than the "good ecological status" (Werner et al., 2012); therefore, monitoring water sources is a priority issue and needs to be periodically performed to guarantee the appropriate quality.

In general, the water bodies of the Iberian Peninsula do not present a better state than the rest of European ones ("Eurostat, Pesticide sales statistics.," 2014). Von der Ohe et al. (2011) analyzed waters collected in four European rivers (including the Llobregat Spanish River), concluding that pesticides were among the substances more frequently detected and presented high or very high risk. Spain is among the European countries with highest use of pesticides as a consequence of the intensive agricultural activity (Parris, 2011). The quality standards established in Spain for SW contaminants vary

depending on each substance (Royal Decree 817/2015, Annex IV and V) (Ministerio de Agricultura Alimentación y Medio Ambiente, 2015). In GW, the maximum allowable concentration threshold of active substance (pesticides, TPs and metabolites) is 0.1 μg/L for each individual substance, and 0.5 μg/L for the sum of all detected pesticides, TPs and metabolites (Royal Decree 1514/2009) (*Ministerio de Medio Ambiente, y Medio Rural y Marino, RD 1514/2009, de 2 de octubre, por el que se regula la protección de las aguas subterráneas contra la contaminación y el deterioro*, 2009). Currently, the River Basin (RB) Authorities are the organizations responsible for ruling on non-compliance based on current legislation.

The Júcar River Basin (JRB) District shows a very fragile balance between available resources (3,400 $hm^3$/year) and demands for water (3,200 $hm^3$/year), with more than 80% destined to irrigation. Almost 30% of the territory of the JRB District is occupied by agricultural land (either rainfed or irrigated), being agriculture the main source of diffuse pollution by an excess of fertilizers and/or pesticides. It is also relevant the high use of groundwater, which is on the order of half of total water withdrawals. Although the first studies on the presence of pesticides in JRB District were performed several years ago (Hernández et al., 2008; Marín et al., 2006), recent data have shown the continued presence of pesticides in environmental waters (Belenguer et al., 2014; Ccanccapa et al., 2016a; Menchen et al., 2017; Pascual Aguilar et al., 2017; Rousis et al., 2017) illustrating the impact of the agricultural activities, mainly, on water resources. Until now, most of those studies have focused on a limited number of pesticides, based on data reported by target analysis. This is the most common situation, where a small number of analytes is included in the target list of the analytical method. Only in a few cases, TPs have been included in the analysis (e.g. Rousis et al., 2017; von der Ohe et al., 2011).

For a comprehensive monitoring, where a large number of compounds is investigated, the application of powerful techniques, such as high-resolution mass spectrometry (HRMS), is required. The potential of ultra-high performance liquid chromatography (UHPLC) coupled to HRMS for screening of pesticides in environmental waters has already been demonstrated (Hernández et al., 2015a, 2015b; Pitarch et al., 2016; Soulier et al., 2016; Wille et al., 2011). The most commonly used analysers, Orbitrap™ (Ruff et al., 2015) and time-of-flight (TOF) (Masiá et al., 2014), provide high mass accuracy and high resolution in full acquisition mode (Cotton et al., 2016). These characteristics make these instruments very useful for the detection and identification of a huge number of pesticides as well as their TPs in a single analysis, even without reference standards (tentative identification) (Ibáñez et al., 2017).

The main objective of this work was to investigate the occurrence of a large number of compounds in GW and SW from the JRB District, based on wide-scope screening by LC-QTOF MS. An important number of TPs have also been included, in order to fill the gap of the little information available on the presence of these compounds in waters. In total, more than 500 compounds have been investigated using a generic sample treatment based on solid phase extraction (SPE) with polymeric cartridges, followed by LC- QTOF MS analysis.

## 2. Material and methods

### 2.1. Chemicals

Atrazine, atrazine-desethyl (DEA), atrazine-desisopropyl (DIA), bromacil, carbendazim, chlorpyrifos, diuron, imazalil, linuron, metolachlor, prometryn, simazine,

terbumeton, terbumeton-desethyl (TED), terbuthylazine, terbuthylazine-desethyl (TD), terbutryn, thiabendazole, 3,4-dichloroaniline (3,4- DCA), 3,5,6-trichloro-2-pyridinol (TCP) were purchased from Sigma-Aldrich (Madrid, Spain), with a purity higher than 97%. The list of pesticides and TPs available for this study, as well as some physicochemical properties, are listed in Supplementary Material (**Table SM1**).

HPLC-grade methanol (MeOH), acetone (pesticide residue analysis quality) and formic acid (98–100%, LC-MS grade), were supplied by Scharlau S.L (Barcelona, Spain). HPLC-grade water (resistivity of 18 MΩ cm) was obtained by purifying demineralised water (Millipore Ltd., Bedford, MA, USA).

Standard stock solutions (500 mg $L^{-1}$) of each compound (except carbendazim) were prepared by dissolving 25 mg (±0.01 mg) of each solid compound in 50 mL of acetone considering the purity of each solid standard. Standard stock solution of carbendazim was prepared in MeOH. Individual stock solutions and working standard solutions were stored at -20 and 4℃ in the dark, respectively.

## 2.2. General site description

The JRB Authority is a Spanish public institution, responsible, among other functions, of monitoring water resources, quality and quantity monitoring network, in the JRB District. The JRB District is an inter-regional river basin located within Spanish territory, at the Eastern part of the Iberian Peninsula, in south Europe, bordering with the Mediterranean Sea (**Figure 1**).

The area occupied by the JRB District with respect to the Spanish territory is 8.5%, this is, 42.851 $km^2$ ("Confederación Hidrográfica del Júcar, O.A," 2018). The two main

rivers located in this area are the Júcar and the Turia River, with a length of 509 and 250 km, respectively. In the East sector, it can be found the largest shallow coastal lagoon (23.94 km$^2$), named Albufera, within the Valencia Natural Park (223 km$^2$), which has a great environmental value. According to the definition of GW bodies carried out by the Water Framework Directive (WFD), the JRB District is made up of 90 GW bodies (Menchen et al., 2017). For more information about geology and hydrogeology see Supplementary Material.

Regarding land use in the JRB District, it is worth mentioning that 50.5% corresponds to forest and semi-natural areas, 35.5% are rainfed areas, 10.5% corresponds to irrigated agricultural areas, 2.8% to urban and industrial areas and finally 0.7% are SW (including wetlands). The permanent population located in the JRB District in the year 2009 were 5,162,163 inhabitants according to the Spanish National Institute of Statistics ("Instituto Nacional de Estadística," 2009). In addition, it is estimated that the seasonal population (summer) increases approximately in 500,000 inhabitants ("Confederación Hidrográfica del Júcar, O.A," 2018).

The eastern sector is formed by a succession of coastal plains in which an intense agricultural activity is developed, mainly of citrus cultivation, which represents an important sector of the export economy of the Valencian Community. The 342,000 hectares of citrus are irrigated mainly by drip systems, with an annual demand of around 2,050 hm$^3$. In the internal zones, the predominant crops are corn and cereals, with a global demand of around 1,500 hm$^3$/year. The use of pesticides is widespread in these crops (Belenguer et al., 2014; Cotton et al., 2016) .

The study area is under typical Mediterranean weather, with marked seasonality. The summer is warm and dry (July and August) and winters are relatively wet and mild.

Mean annual precipitation is around 500 mm, and varies depending on the topography (inlands and coast). In addition, torrential rains are concentrated in few days in the year, usually in months of autumn and spring. It is not uncommon to observe months with no rainfall.

**2.3 Monitoring network and sampling**

The monitoring network was designed based on previous detection of pesticides in SW and GW from this area ("Confederación Hidrográfica del Júcar, O.A." 2018). The selected sampling points belong to the JRB Authority quality-monitoring network, and all presented relatively high concentrations of pesticides in previous campaigns, including some non-compliances according to the current regulation (Ministerio de Agricultura Alimentación y Medio Ambiente, 2015; *Ministerio de Medio Ambiente, y Medio Rural y Marino, RD 1514/2009, de 2 de octubre, por el que se regula la protección de las aguas subterráneas contra la contaminación y el deterioro*, 2009). The monitoring network consisted of 19 samples: 8 corresponded to 8 SW bodies and the remaining 11 to 5 GW bodies (**Figure 1**, **Table SM2**). GW samples were collected from March-April 2017, whereas SW samples were taken in May 2017.

Polyethylene bottles (1 L volume) used for collection of samples were previously rinsed with the same water. For collection of GW samples, the wells were pumped until the pH and conductivity (EC) parameters were stabilized. Immediately after sampling, the sample bottles were placed into isothermal bags with ice to keep them cold, and shipped to the laboratory to arrive within 24 hours.

## 2.4. Qualitative validation

The analytical methodology applied was validated to evaluate its capability to detect and identify 20 compounds selected as a model, on the basis of their frequent detection in previous campaigns ("Confederación Hidrográfica del Júcar, O.A," 2018). The samples were spiked at two concentration levels (0.01 and 0.1 µg/L), and three sample volumes (100, 250 and 500 mL) were tested (n=3 each one) in the SPE procedure applied. The validation was qualitative, i.e. the objective was to evaluate whether the compounds were correctly detected and identified by LC-QTOF MS. A river sample was used as "blank" for preparation of spiked samples.

## 2.5. Sample treatment

Samples were thawed at room temperature before being processed. SPE cartridges (Oasis® HLB, 200 mg sorbent/6 mL, Waters Corp., Milford, MA, USA) were conditioned by washing with MeOH (2 x 2.5 mL) followed by rinsing with ultra-pure Milli-Q water (2 x 2.5 mL). Water samples (500 mL) were pumped through the cartridge and dried under vacuum for approximately 20 min. The analytes were eluted by gravity with MeOH (2 x 2.5 mL) and collected in glass tubes. The eluate was evaporated to dryness at 40 °C under a gentle stream of nitrogen and reconstituted in 0.5 mL MeOH:water (10:90, v/v). Analysis was performed by injecting 20 µL of the final extract in the UHPLC-HRMS system. The criteria used for confirmation of the identity are indicated in the following section. **Figure 2** shows the overall scheme of both sample treatment and data processing.

## 2.6. Instrumentation

UHPLC-HRMS analysis was performed using a Waters ACQUITY UPLC ultra-high performance liquid chromatography (UHPLC) system (Waters, Milford, MA, USA) interfaced to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (XEVO G2 QTOF, Waters Micromass, Manchester, UK), using an orthogonal Z-spray electrospray ionization (ESI) interface operating in both positive and negative ionisation modes.

The UHPLC separation was performed using an CORTECS C18 column (2.1 i.d. × 100 mm length, 2.7 µm particle size, Waters), at a flow rate of 300 µL/min. The mobile phases were (A) $H_2O$ with 0.01% HCOOH and (B) MeOH with 0.01% HCOOH. The mobile phase gradient was: 10% B at 0 min, 90% B at 14 min linearly increased, 90% B at 16 min, and finally 10% B at 18 min in order to return to initial conditions. The injection volume was 20 µL.

MS data were acquired over a *m/z* range of 50-1000. Nitrogen was used as drying and nebulizing gas. The gas flow was set at 1000 L/h. TOF-MS resolution was approximately 18000 at full width half maximum (FWHM) at *m/z* 556. Capillary voltages of 0.7 kV and 3.0 kV were used in positive and negative ionisation modes, respectively. A cone voltage of 20 V was selected for both ionisation modes. Collision gas was argon 99.995% (Praxair, Valencia, Spain). The desolvation temperature was 600 °C and the source temperature 130 °C.

For $MS^E$ experiments, two acquisition functions with different collision energies were used: the low energy (LE), selecting a collision energy of 4 eV in order to obtain information about the protonated molecule and adducts (if present), and the high energy (HE) function, with a collision energy ramp ranging from 15 to 40 eV, in order to obtain a greater range of fragment ions. The LE and HE functions settings were for both a scan

time of 0.3 s. Data were automatically processed by ChromaLynx XS software (MassLynx v 4.1, Waters).

## 2.7. Criteria for detection/identification used in the UHPLC-HRMS screening

Pesticides and TPs included in a large home-made database were investigated in the samples for a qualitative screening (i.e. detection and identification) based on mass accuracy, isotopic pattern and retention time deviation and the presence of fragment ions (European Commission, 2017). **Tables SM3-SM6** show the empirical formula of all pesticides included in the database and the exact mass corresponding to the (de)protonated molecule. For those compounds included in the reference standard mix, retention time as well as the 2-3 most important fragment ions are also showed. The extracted ion chromatograms (EIC) corresponding to (de)protonated molecules and fragment ions were automatically obtained using ChromaLynx XS. The following detection settings were applied: ±5 ppm for mass accuracy and ±0.1 min for retention time deviation. The classification of the different analytes as detected, identified/confirmed or tentatively identified depended on the fulfillment of the criteria indicated in **Table 1,** as well as on the availability of the reference standards. A compound was considered as "detected" when the most abundant ion (typically the (de)protonated molecule) was observed at the appropriate retention time (tolerance of ±0.1 min respect to a reference standard), measured at accurate mass (mass error lower than 5 ppm). For reliable "identification", the presence of the (de)protonated molecule (or any adduct, if more favorable) and, at least, one fragment ion was required, both with mass errors below 5 ppm at the expected Rt in comparison with the reference standard (±0.1 min).

When only the elemental composition was included in the database (i.e., the reference standard was not available in the laboratory), a suspect screening was carried out (Ibáñez et al., 2017). "Tentative identification" occurred when an expected ion was found with mass error below 5 ppm, together with its characteristic isotopic pattern (if exists), and one or more fragment ions were in agreement with data reported and compatible with the chemical structure of the candidate (mass error < 5 ppm). Although not applied in the present work, the use of retention time (Rt) prediction models can be useful for suspect compounds whose reference standards are not available, because it allows to discard false positives focusing the identification in those peaks that fall into the predicted Rt window (Bade et al., 2015b). Rt prediction based on artificial neural networks (ANN) will be applied in future screening (Bade et al., 2015a). For those compounds tentatively identified, the acquisition of the reference standard would be required for confirmation of their identity.

## 3. Results and discussion

### 3.1. Qualitative validation

**Table SM7** shows data obtained after testing different sample volumes (100, 250 and 500 mL) at the two concentration levels (0.01 and 0.1 µg/L) in a water sample subjected (in triplicate) to the overall SPE-LC-QTOF MS procedure.

As it can be seen, the best results were obtained when 500 mL water was loaded, as all compounds (except for chlorpyrifos) were detected and confirmed in all the 3 replicates, even at the lowest concentration level assayed (0.01 µg/L).

Although the screening was validated for only 20 compounds -selected because of their frequent occurrence in the aquatic environment of the area under study-, the accurate-mass full-spectrum acquired in the QTOF MS analysis allowed us to widen the screening to a large list of more than 500 pesticides and TPs. Under these circumstances, the positives detected in the wide-scope screening should be taken as unequivocal when the reference standard was available and allowed its full confirmation, even in the case that the compound was not subjected to validation. However, potential false negatives might occur for compounds not previously validated, especially when their physico chemical characteristics (mainly polarity) differed notably from the model compounds.

### 3.2. Results obtained in the wide-scope screening

**Table 2** shows the results obtained in the screening of GW samples. Up to 17 compounds were confirmed with reference standards: 12 pesticides (atrazine, simazine, terbumeton, terbuthylazine, bromacil, carbendazim, imazalil, thiabendazole, bentazone, imidacloprid, metalaxyl, and terbacil) and 5 TPs (DEA, DIA, TED, terbuthylazine-2-hydroxy (T2H), and TD). One more compound (metolachlor oxanilic acid (MOA)) was just tentatively identified, as the reference standard was not available at our lab.

The herbicides atrazine and simazine were found in all GW samples analysed (**Table 2**). Terbuthylazine was present in 10 out of the 11 samples (90%), and terbumeton and bromacil in 7 (63%). Regarding TPs, DEA, TD and TED were found in all GWs samples. Other TPs were DIA (72%), T2H (54%) and MOA (36%). Some other herbicides, such as diuron, bentazone and terbacil, and the insecticide neonicotinoid imidacloprid were found at least once. The fungicides thiabendazol, metalaxyl and

carbendazim were present in 3 and 4 samples, respectively, whereas the post-harvest fungicide imazalil was positive in just one water sample.

Regarding SW, the results are shown in **Table 3**. The number of compounds found in the samples increased up to 24, all confirmed with reference standards (11 of them were in fact included in the qualitative validation). Another two compounds (nicosulfuron and MOA), where tentatively identified as reference standards were not available.

Similarly to GW, the majority of compounds in SW were herbicides (13 compounds): simazine, terbumeton, terbuthylazine, terbutryn and the TPs TED, TD and T2H (triazines); nicosulfuron, linuron, metolachlor, isoproturon, and MCPA; and the TP metolachlor oxanilic acid. An important number of fungicides were also detected, specifically benzimidazoles (carbendazim and thiabendazole), triazoles (propiconazole, tetraconazole and tebuconazole), carbamates (propamocarb), strobilurins (azoxystrobin), acylalanines (metalaxyl), and imidazols (imazalil). The presence of three insecticides (acetamiprid, imidacloprid and diazinon) and the plant growth regulator paclobutrazol was also confirmed (**Table 3).**

**Figure 3** shows a summary of the results obtained, grouped by families. As observed, triazine herbicides were the most frequently detected, representing 34% of the findings in GW and 19% in SW, followed by benzimidazoles (5% and 20%, respectively). The other families were below 11%. The TPs represented 46% of the positives in GW and 22% in SW. The occurrence of TPs in GW and SW was rather similar in terms of detection frequency for TED, MOA and T2H. However, TD was detected in all GW samples but was present in only 25% of the SW analysed. DEA and DIA were found in 100% and 73% of the GW samples, but not in SW.

A rough semi-quantitative estimation was performed by comparing the area of the spiked samples (0.1 µg L$^{-1}$) used for validation of the screening with the responses of samples. Based on these data, two compounds seemed to be present at a concentration higher than 0.1 µg L$^{-1}$ in GW: the herbicide bromacil (3 out samples) and the main TP of terbumeton, TED (4 samples). In SW, the two compounds exceeding the 0.1 µg L$^{-1}$ level were the fungicides imazalil (2 samples) and thiabendazole (1 sample). Although these data should be confirmed by a validated quantitative method, it seems clear that these compounds may become a problem from a regulatory point of view if appropriate measurements are not taken.

As an illustrative example of the potential of QTOF MS, **Figure 4** shows the tentative identification of the herbicide nicosulfuron in a SW sample (reference standard non-available). The LE spectrum of the chromatographic peak at 7.51 min in ESI positive mode, showed an abundant signal at m/z 411.1087 (**Figure 4b, bottom**). It might correspond to the protonated molecule of nicosulfuron ($C_{15}H_{19}N_6O_6S^+$, expressed as protonated molecule), with a mass error of 0 ppm in relation with its theoretical exact mass. The peak corresponding to the sodium adduct was also observed at *m/z* 433.0908. The LE spectrum also showed 3 fragment ions at *m/z* 366.0510 ($C_{13}H_{12}N_5O_6S^+$, corresponding to the neutral loss of dimethylamine), *m/z* 213.0337 ($C_8H_9N_2O_3S^+$) and 182.0568 ($C_7H_8N_3O_3^+$), all with mass errors below 2 ppm. In addition, the HE spectrum (**Figure 4b, top**) showed another fragment ion at *m/z* 106.0300 ($C_6H_4NO^+$). The structure of all fragment ions was justified based on their measured accurate masses, and all were compatible with the structure of the candidate. Moreover, the 4 fragment ions were in accordance with the scientific literature ("Massbank," 2006). Although all these data strongly supported the tentative identification of the compound as

nicosulfuron, the injection of the corresponding reference standard should be required for a definitive unequivocal confirmation.

In a similar way, **Figure 5** shows the tentative identification of the metolachlor oxanilic acid in a GW sample. The LE spectrum of the chromatographic peak at 9.18 min (**Figure 5b, bottom**), showed an abundant signal at $m/z$ 280.1545, that might correspond to the protonated molecule ($C_{15}H_{22}NO_4^+$, -1.4 ppm), and at $m/z$ 302.1370, corresponding to its sodium adduct. Two fragment ions were also observed in the LE spectrum, at $m/z$ 248.1287 ($C_{14}H_{18}NO_3^+$, from a methanol neutral loss) and $m/z$ 220.1343 ($C_{13}H_{18}NO_2^+$), both with mass errors below 1 ppm. The HE spectrum (**Figure 5b, top**) showed an additional fragment ion at $m/z$ 160.1127 ($C_{11}H_{14}N$). The fragment ions were in accordance with the reported information ("Massbank," 2006); however, a plausible structure could not be proposed for fragment at $m/z$ 220. So, it would be necessary to acquire the reference standard to finally confirm or discard the identity of the compound.

### 3.2.1 Comparison of the results obtained with the legislation

In the present study, 33 compounds were detected (**Tables 2** and **3**). Among them, terbuthylazine, metolachlor, simazine and atrazine have been regularly monitored by a quantitative method since 1999 in GW (see **Table SM8**). Among these, only terbuthylazine is allowed to be used at present. In the case of metolachlor, its enantiomer S-metolachlor was allowed until July 2018 (*Commission Implementing Regulation (EU) 2017/841*, 2017). Regarding SW, diazinon has also been regularly controlled since 1999, and metolachlor, simazine, terbuthylazine, and isoproturon since 2002.

Although our data can only be considered as semi-quantitative, bromacil and TED seemed to be present in several GW samples at concentrations around or even above 0.1 µg L$^{-1}$. This herbicide was widely used for many years until its prohibition in 2003 (*Commission Regulation (EC) No 2076/2002*, 2002; Resolution of 30 June 2003 of the General Directorate of Agriculture, Ministerio de Agricultura Pesca y Alimentación, 2003). Historical data reported by JRB District showed two cases of 0.1 µg L$^{-1}$ exceedance for bromacil along the period 2010-2015, but a progressive reduction of the concentration has been observed along this period of time. The last quantitative data reported for bromacil in GW in the regular monitoring performed by JRB District corresponded to the 2017 campaign, where this herbicide was the only compound exceeding 0.1 µgL$^{-1}$ (27% of the GW samples analyzed). However, it was not found in SW. In case of TED, it is analysed in control samples since 2013. Historically, it has had defaults in 3 GW bodies. In the current sampling, the reference value of 0.1 µg L$^{-1}$ seems to have been exceeded in nearly 40% of the samples.

Although the cases of non-compliance with the regulation are scarce in GW, they always correspond to these two compounds, with the peculiarity that bromacil was withdrawn from the market more than 10 years ago. The fact that this herbicide is still regularly found in GW is a subject of concern, although its concentration seems to be in progressive decrease in the last few years.

In previous campaigns ("Confederación Hidrográfica del Júcar, O.A," 2018) performed in SW (since 2004 for imazalil, and since 2013 for thiabendazole), imazalil and thiabendazole were found above 0.1 µg L$^{-1}$ in several samples. Both are authorized post-harvest fungicides widely used at present. They are frequently found in SW samples

from this area, but there is not a maximum allowable concentration established (RD 817/2015, (Ministerio de Agricultura Alimentación y Medio Ambiente, 2015)).

For the other analytes detected, which were submitted to validation in this study, a high frequency of detection was in general observed for most of the triazines in GW and for benzimidazoles in SW (**Tables 2** and **3**). The levels did not seem to exceed the maximum concentrations set up by legislation (GW and SW), even though many of these compounds have been detected historically in different geographical areas where an intensive agricultural activity takes place.

Several compounds detected in the screening applied in this work had not been included until now in the regular monitoring performed by JRB Authority. These compounds were acetamiprid, azoxystrobin, diazinon, isoproturon, MCPA, metalaxyl, nicosulfuron, paclobutrazol, propamocarb, propiconazole, tebuconazole, tetraconazole and the TPs MOA and T2H in SW, and bentazone, carbendazim, imidacloprid, metalaxyl, terbacil, and the TPs MOA and T2H in GW. Among them, the use of terbumeton, terbacil and carbendazim was unauthorized in 2003, 2007 and 2011, respectively. This illustrates the relevance of performing large scope screening to widen the list of compounds monitored, with the aim of detecting compounds not included in the regular target quantitative analysis.

### 3.2.2 Pesticides detected in other geographical areas

The presence of pesticides in each RB District has been periodically monitored by the RB Authorities. Previous results in SW and GW revealed the presence of a notable number of pesticides in the studied areas, illustrating the impact of these compounds in

our country, where agriculture plays an important role. **Table 4** shows the compounds, among those found in the present work, also found in other geographical areas. Although a comparison of results between areas is complicated because of the different analytical methodologies applied and different compounds included in the methods, it can be observed that many of the pesticides found in other Spanish RB Districts were also present in the water bodies of the JRB District, particularly in SW. However, some pesticides/TPs frequently detected in other areas were not found in SWs of the JRB District, such as chlorfenvinphos, dichlofenthion, prochloraz, pyriproxyfen, phenoxon sulfoxide (Belenguer et al., 2014; Ccanccapa et al., 2016a).

In relation to GW, it is remarkable that triazine herbicides and some of their TPs are the predominant compounds, a fact that can be explained by their wide use in agricultural activities (Köck-Schulmeyer et al., 2014) and illustrates that most GW in Spain are vulnerable to this type of compounds. Therefore, the strict control of triazines and TPs is highly recommended. Another compound of concern is bromacil, an herbicide widely used in citrus crops until it was banned 15 years ago (Menchen et al., 2017). Its high water solubility (815 mg/L, at 20 °C) and its potential leaching index (GUS = 3.44) may explain the pollution of GW by this compound ("IUPAC, Pesticide properties database," 2007). This is also because it is relatively persistent and poorly adsorbed onto the soil (Zhu and Li, 2002).

### 3.2.3 Hydrogeological issues

In general, more pesticides were detected in SW than GW samples (**Tables 2 and 3**). This fact seems to be related with the dominant mechanisms of propagation of pollutants. The arrival of pesticides to SW bodies can be produced by surface runoff in

periods of intense precipitation (SW2 sample) or by dragging abandoned waste (Sancho et al., 2004). Frequently, discharges to natural watercourses of effluents from water treatment plants, which treat wastewater from agro-food industries, are the focus of contamination in the studied area. This seems to be the case of SW3, SW4 and SW6 samples, as imazalil and thiabendazole are frequently used in the post-harvest treatment of citrus fruits. In fact, imazalil was detected in 67% of the SW samples and Thiabendazole in 100% (**Table 3**).

In contrast, fewer pesticides, usually at lower concentration levels, were detected in GW. In this case, the origin was associated to the direct use in the crop fields. The transport mechanism from the surface to the groundwater is the percolation through the unsaturated zone, favored by rainfall or, in some cases, by intensive irrigation (flooding), which is still used in some areas.

The two main factors that govern the movement of pesticides are the thickness and nature of the unsaturated zone. Moreover, the reactivity of each compound is responsible for its leaching potential, which depends on its solubility and half-life which, in turn, is a function of its degradability and its tendency to adsorption (Morell and Candela, 1998).

In this work, however, the most significant factor seems to be the nature of the unsaturated zone. Thus, GW31, GW32 and GW51 samples are located in karstic aquifers, in which the transit of pesticides, in general, is faster and the attenuation reactions are of less intensity than in detrital aquifers. These kind of aquifers have high intrinsic vulnerability and GW is reached more easily by pesticides (Rodríguez et al., 2018).

Conversely, in detrital aquifers the attenuation processes are more intense and the leaching of pollutants is lower (Brusseau, 1995). Thus, in GW12, GW41, GW42 and GW44 samples, which correspond to detrital aquifers, the number of pesticides found was lower.

Around 30% of the GW samples with more than 10 detected compounds (**Tables SM2 and 2**) were associated with detrital materials, whereas the remaining 70% with karst materials.

## 4. Conclusions

In this work, a wide-scope screening of pesticides has been applied to surface water and groundwater samples from the Júcar River Basin District, an important geographical area in Spain where agriculture plays a relevant role with predominance of citric crops. Groundwater quality is a key issue, as irrigation by GW is about half of total water abstractions, and is the main source of drinking water supply in the area under study. Therefore, it is essential to protect GW from pesticide pollution. After LC-HRMS screening of water samples from this area, a total of 27 pesticides and 6 TPs were identified. The most frequent compounds in GW were triazine herbicides and several of their TPs, whereas in the SW were mainly fungicides, such as carbendazim, thiabendazole and imazalil, as well as the herbicide triazine and terbutryn, and the TP terbumeton-desethyl.

Several of the compounds identified in the present screening had not been included in monitoring campaigns periodically performed in the area (e.g. metalaxyl, propiconazole, tebuconazole, carbendazim, MOA, T2H), where target quantitative

methods were applied for a limited number of compounds. This fact highlights that the commonly applied quantitative target methods (although are sensitive, reliable and accurate) are restricted to the target list of compounds with the consequence that several compounds present in the samples may be ignored. Our data are useful to update the information gathered by River Basin Authorities. This is especially relevant for those compounds that are found in water bodies despite their use is not allowed according to the current legislation.

This work illustrates the need of widening the scope of the methods making use of HRMS in order to identify the most problematic compounds, including transformation products. This allows focusing subsequent quantitative analysis on those compounds actually present in the water environment. The notable number of pesticides and TPs found in water demonstrates the vulnerability of this area and the impact of the use of pesticides. The information obtained in this study should be supported by concentration data obtained in future monitoring campaigns periodically applied to waters collected in the JRB District applying quantitative methods with an updated list of target compounds.

The combination of the next three elements will allow a comprehensive overview of the pesticides impact in SW and GW in a river basin: 1) searching data of the pesticides use in the study area, 2) qualitative and quantitative assessment of pesticides occurrence in waters, and 3) development of a conceptual model, and also a mathematical one if possible, to establish the relationship between pesticides application and their presence in SW and GW.

**Acknowledgments**

**Figure captions**

**Figure 1.** Location, topography and main cities of Júcar River Basin (JRB) and monitoring network location.

**Figure 2.** Overall scheme of the screening procedure applied (sample treatment + HRMS analysis and data processing)

**Figure 3**. Percentages of the different families of pesticides identified in surface water (top) and groundwater samples (bottom).
FUNG: fungicide; HERB: herbicide; INS: insecticide; OP: organophosphorus; TPs: transformation products.

**Figure 4.** Detection and tentative identification of nicosulfuron in a SW sample. (a) nw-XICs at 0.01 Da mass window for [M+H]$^+$ in LE function and main fragments in LE/HE functions. (b) Low energy (bottom) and high energy (top) TOF mass spectra obtained for the chromatographic peak at 7.51 min.

**Figure 5.** Detection and tentative identification of metolachlor oxanilic acid in a GW sample. (a) nw-XICs at 0.01 Da mass window for [M+H]$^+$ in LE function and main fragments in LE/HE functions. (b) Low energy (bottom) and high energy (top) TOF mass spectra obtained for the chromatographic peak at 9.18 min.

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**TABLES**

**Table 1**. Criteria used in the UHPLC-HRMS screening for detection/identification of compounds

| | Reference standard | | |
| --- | --- | --- | --- |
| | Available | | Not available |
| | Detected | Confirmed/Identified | Tentatively identified |
| Accurate mass protonated molecule [M+H]$^+$ | <5 ppm | <5 ppm | <5 ppm |
| Accurate mass fragment ion | | <5 ppm | <5 ppm[a] |
| Deviation ([M+H]$^+$) respect to the reference standard | ±0.1 min | ±0.1 min | − |

ppm: mass accuracy expressed as a relative value in parts per million

[a]at least one fragment ion should be justified on the basis of the structure or on existing literature

**Table 2**. Results obtained in GW samples by UHPLC-QTOF MS screening. Samples collected between March and April 2017

| | Compounds | GW11 | GW12 | GW2 | GW31 | GW32 | GW41 | GW42 | GW43 | GW44 | GW51 | GW52 | Detection frequency (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subjected to validation | Atrazine | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | 100 |
| | Atrazine-desethyl (DEA) | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | 100 |
| | Atrazine-desisopropyl (DIA) | √ | | √ | | √ | √ | √ | √ | | √ | √ | 72 |
| | Bromacil | √ | √ | √ | | √ | | | √ | | √ | √ | 63 |
| | Carbendazim | √ | | | | √ | | | √ | | | | 27 |
| | Diuron | | | | | √ | | | | | √ | | 18 |
| | Imazalil | | | | | √ | | | | | | | 9 |
| | Simazine | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | 100 |
| | Terbumeton | | | | √ | √ | | √ | √ | √ | √ | √ | 63 |
| | Terbumeton-desethyl (TED) | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | 100 |
| | Terbuthylazine | √ | √ | | √ | √ | √ | √ | √ | √ | √ | √ | 90 |
| | Terbuthylazine-desethyl (TD) | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | 100 |
| | Thiabendazole | | √ | √ | | | | | √ | | | | 27 |
| No subjected to validation | Terbuthylazine-2-hydroxy(T2H) | | | | √ | √ | | √ | √ | | √ | √ | 54 |
| | Bentazone | | | | | √ | | | | | | | 9 |
| | Imidacloprid | | | | √ | | | | | | | | 9 |
| | Metalaxyl | | | | √ | √ | | | √ | | √ | | 26 |
| | Metolachlor oxanilic acid (MOA) | | | | t | t | | | t | | t | | 36 |
| | Terbacil | √ | | | | | | | | | √ | | 18 |

√: confirmed ((de)protonated molecule and at least one fragment ion were observed at the expected retention time).

t: tentative identification ((de)protonated molecule was observed and at least one ion fragment was justified).

**Table 3.** Results obtained in SW samples by UHPLC-QTOF MS screening. SW samples collected in May 2017

| | Compounds | SW1 | SW2 | SW3 | SW4 | SW5 | SW6 | SW7 | SW8 | Detection frequency (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Subjected to validation** | Carbendazim | √ | √ | √ | √ | √ | √ | √ | √ | 100 |
| | Imazalil | √ | √ | | √ | | √ | √ | | 62 |
| | Linuron | | | | | | | | √ | 12 |
| | Metolachlor | | | | | | | | √ | 12 |
| | Simazine | | √ | | | | | √ | √ | 37 |
| | Terbumeton | | √ | | | | | √ | √ | 37 |
| | Terbumeton-desethyl (TED) | √ | √ | √ | √ | √ | √ | √ | | 87 |
| | Terbuthylazine | √ | √ | | | | | √ | √ | 50 |
| | Terbuthylazine-desethyl (TD) | | √ | | | | | √ | | 25 |
| | Terbutryn | √ | | √ | | √ | √ | | √ | 62 |
| | Thiabendazole | √ | √ | √ | √ | √ | √ | √ | √ | 100 |
| **No subjected to validation** | Acetamiprid | | | | | √ | √ | | | 25 |
| | Azoxystrobin | | | | | | | | √ | 12 |
| | Diazinon | | √ | | | | √ | | | 25 |
| | Imidacloprid | | | | | | | | √ | 12 |
| | Isoproturon | | | | | | | | √ | 12 |
| | MCPA | | | | √ | | | | | 12 |
| | Metalaxyl | | √ | | √ | | √ | | √ | 50 |
| | Metolachlor oxanilic acid (MOA) | | t | | t | | | | t | 37 |
| | Nicosulfuron | t | | | | | | | | 12 |
| | Paclobutrazol | | | | | | √ | | | 12 |
| | Propamocarb | √ | | | | | | | √ | 25 |
| | Propiconazole | √ | | | | | √ | √ | √ | 50 |
| | Tebuconazole | √ | | | √ | | | | √ | 37 |
| | Tetraconazole | | | | | | | | √ | 12 |
| | Terbuthylazine-2-hydroxy (T2H) | | √ | | √ | | √ | √ | √ | 62 |

√: confirmed ((de)protonated molecule and at least one fragment ion were observed at the expected retention time).

t: tentative identification ((de)protonated molecule was observed and at least one ion fragment was justified).

**Table 4**. Pesticides detected in different geographical areas

| River Hydrographical Basin | Water type | Pesticides detected[a] | Reference |
|---|---|---|---|
| Duero | SW | metalaxyl, metolachlor, simazine, terbuthylazine | (Hildebrandt et al., 2008) |
| | GW | atrazine, DEA, DIA, metalaxyl, simazine, terbuthylazine | |
| Ebro | SW | carbendazim, diazinon, imazalil, imidacloprid, T2H, thiabendazole | (Ccanccapa et al., 2016b) |
| Guadalquivir | SW | simazine, terbuthylazine | (Hermosin et al., 2013) |
| | GW | atrazine, DEA, DIA, diuron, simazine, terbuthylazine | |
| Guadiana | SW | isoproturon, MCPA, terbuthylazine | (Palma et al., 2014) |
| Miño-Sil | SW | terbutylazine | (Dagnac et al., 2012) |
| Segura | SW | diazinon, simazine, TD, terbumeton, terbuthylazine, terbutryn | (Moreno-González et al., 2013) |
| Tajo | SW | diazinon, imazalil, metolachlor, terbutryn, terbutylazine | (Gómez et al., 2012) |
| Catalonia (Internal Basin) | GW | atrazine, bentazone, DEA, DIA, diuron, imazalil, simazine, terbuthylazine | (Cabeza et al., 2012) (Köck-Schulmeyer et al., 2014) |
| | SW | acetamiprid, imidacloprid, isoproturon, MCPA, simazine | (Kuster et al., 2008) (Rubirola et al., 2017) |
| Júcar | SW | carbendazim, diazinon, imidacloprid, imazalil, isoproturon, metalaxyl, metolachlor, propiconazole, simazine, TED, T2H, terbuthylazine, terbutryn, thiabendazole | (Ccanccapa et al., 2016a) (Rousis et al., 2017) (Masiá et al., 2013) (Benvenuto et al., 2010) (Kuzmanović et al., 2015) |
| | GW | atrazine, bentazone, bromacil, carbendazim, DIA, DEA, diuron, imazalil, imidacloprid, simazine, TED, TD, T2H, terbacil, terbumeton, terbuthylazine | (Pitarch et al., 2016) (Hernández et al., 2008) (Menchen et al., 2017) (Portolés et al., 2014) |

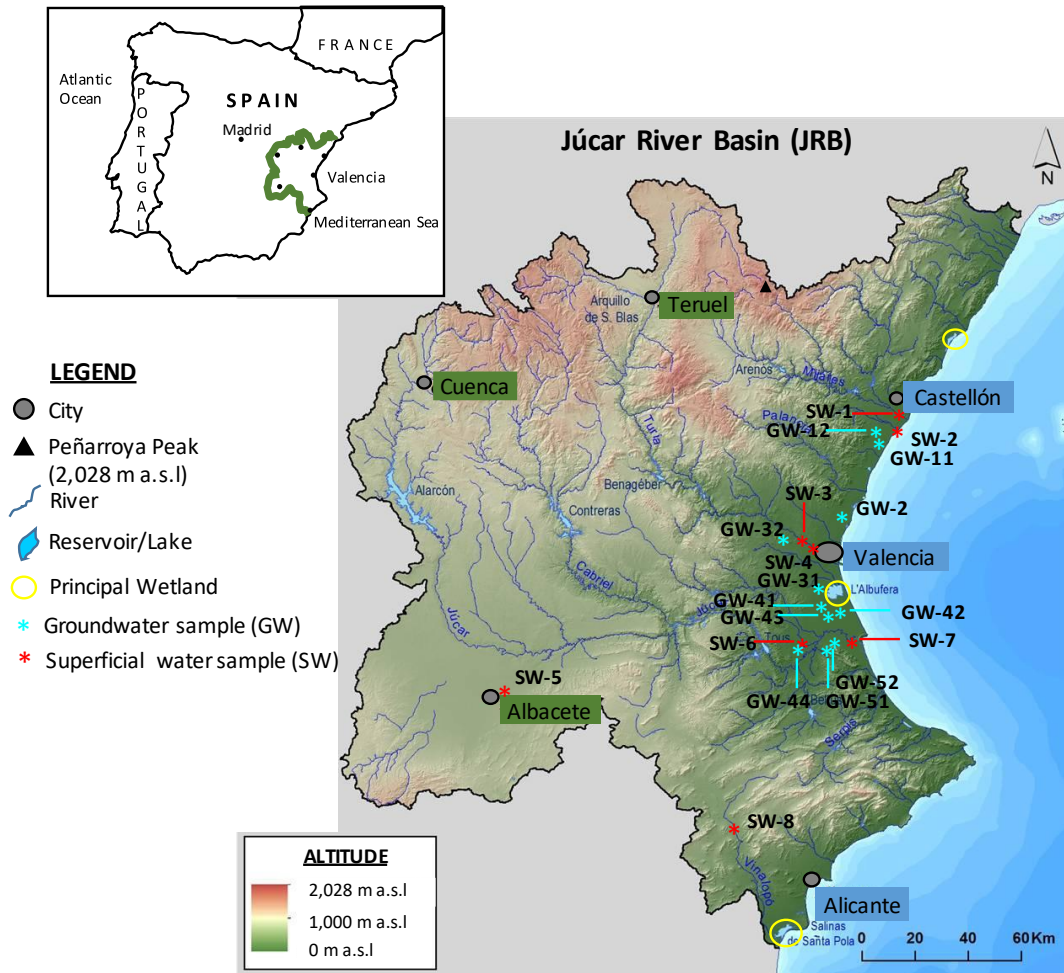a: only those found in this study have been included
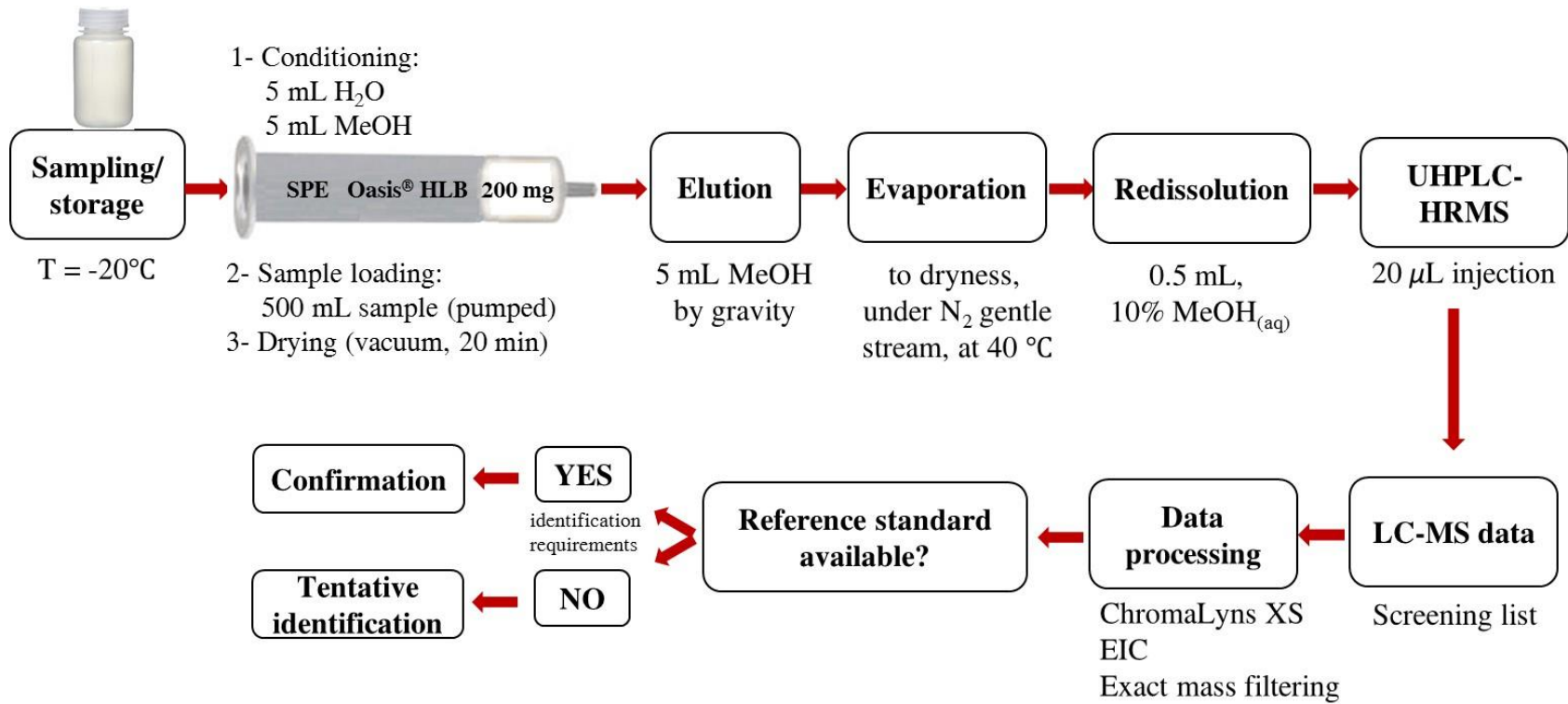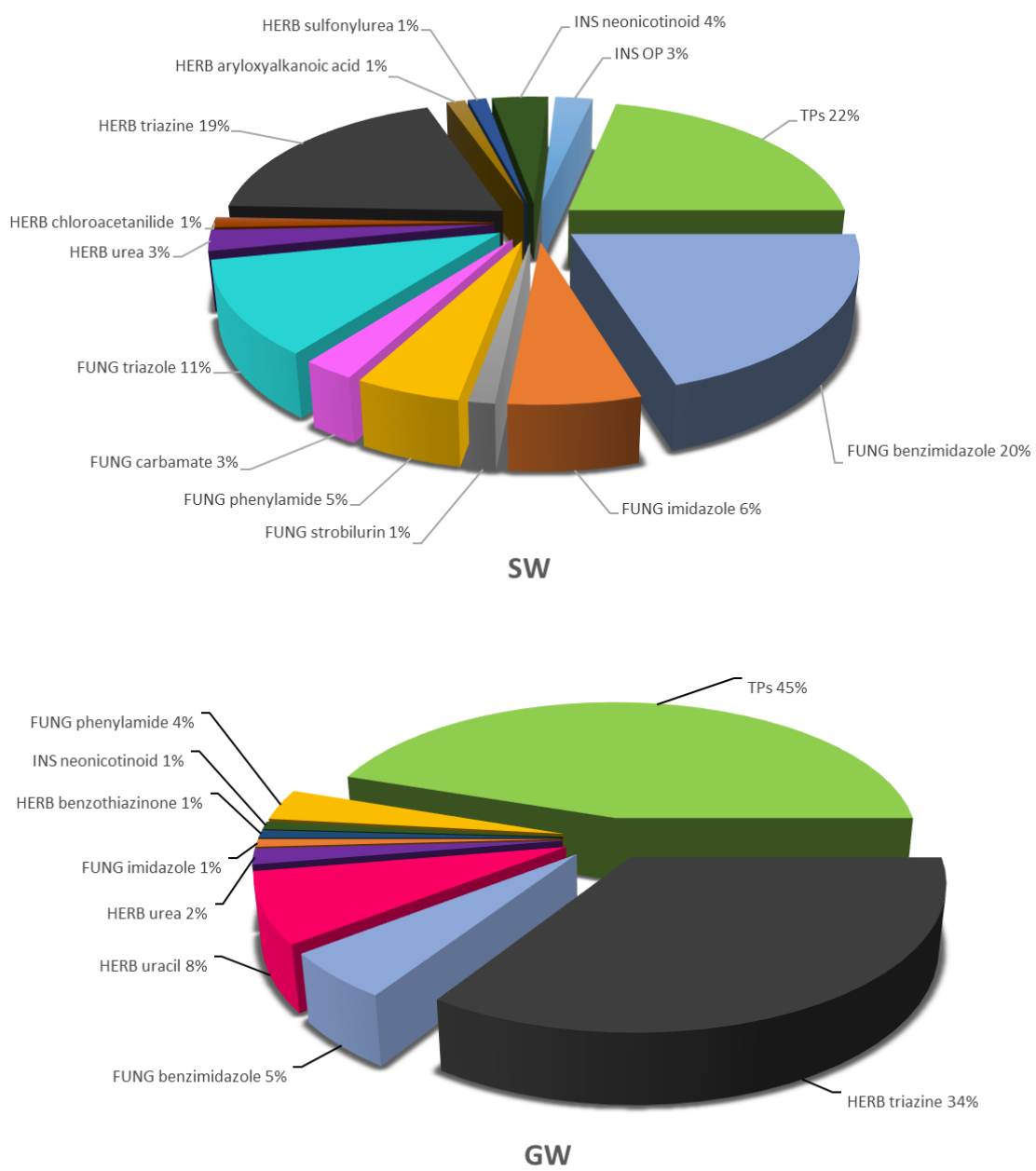
**FIGURES**



Figure 1

Figure 2

SW

HERB sulfonylurea 1%
HERB aryloxyalkanoic acid 1%
HERB triazine 19%
HERB chloroacetanilide 1%
HERB urea 3%
FUNG triazole 11%
FUNG carbamate 3%
FUNG phenylamide 5%
FUNG strobilurin 1%
FUNG imidazole 6%
FUNG benzimidazole 20%
TPs 22%
INS neonicotinoid 4%
INS OP 3%



GW

FUNG phenylamide 4%
INS neonicotinoid 1%
HERB benzothiazinone 1%
FUNG imidazole 1%
HERB urea 2%
HERB uracil 8%
FUNG benzimidazole 5%
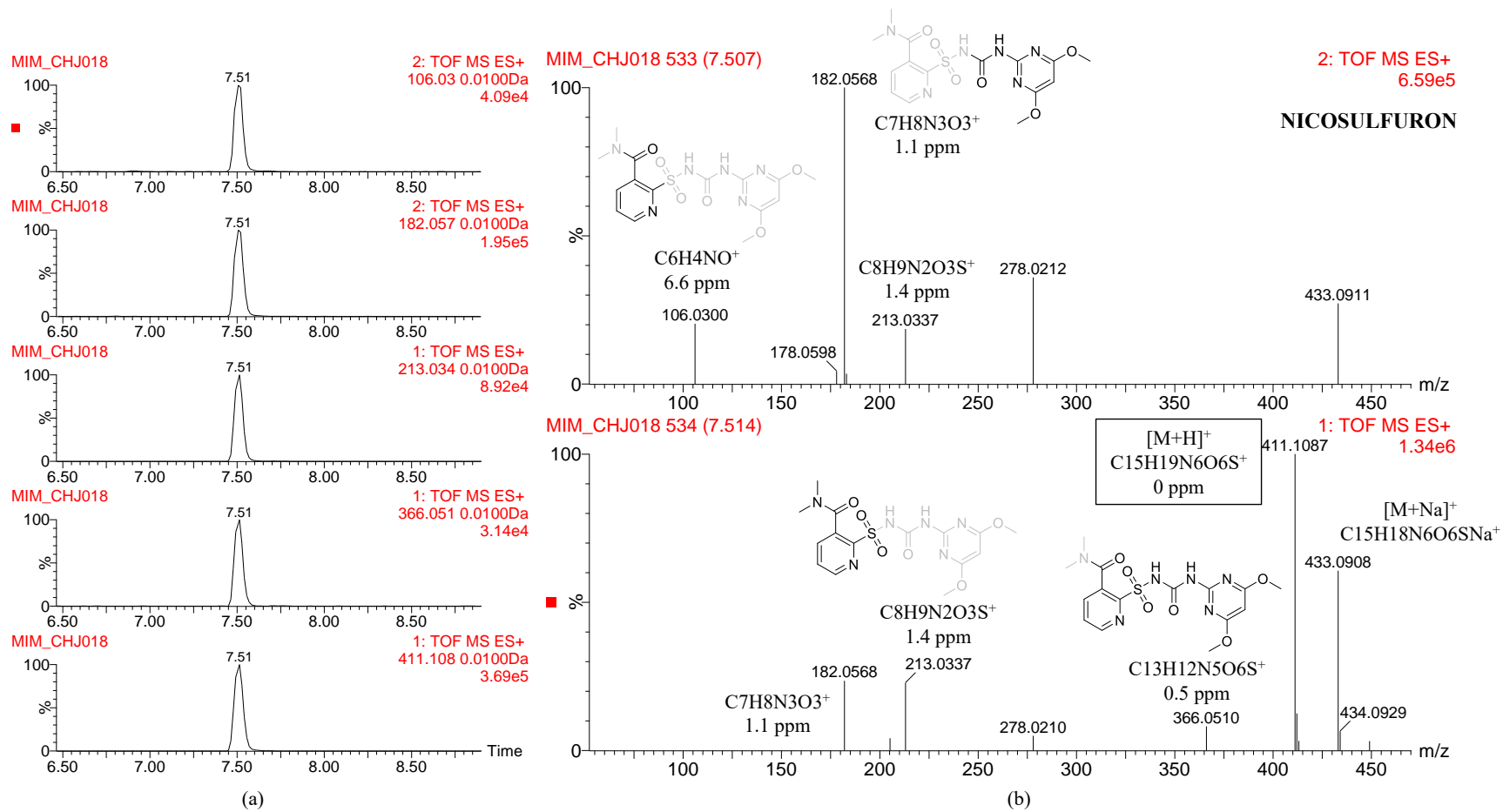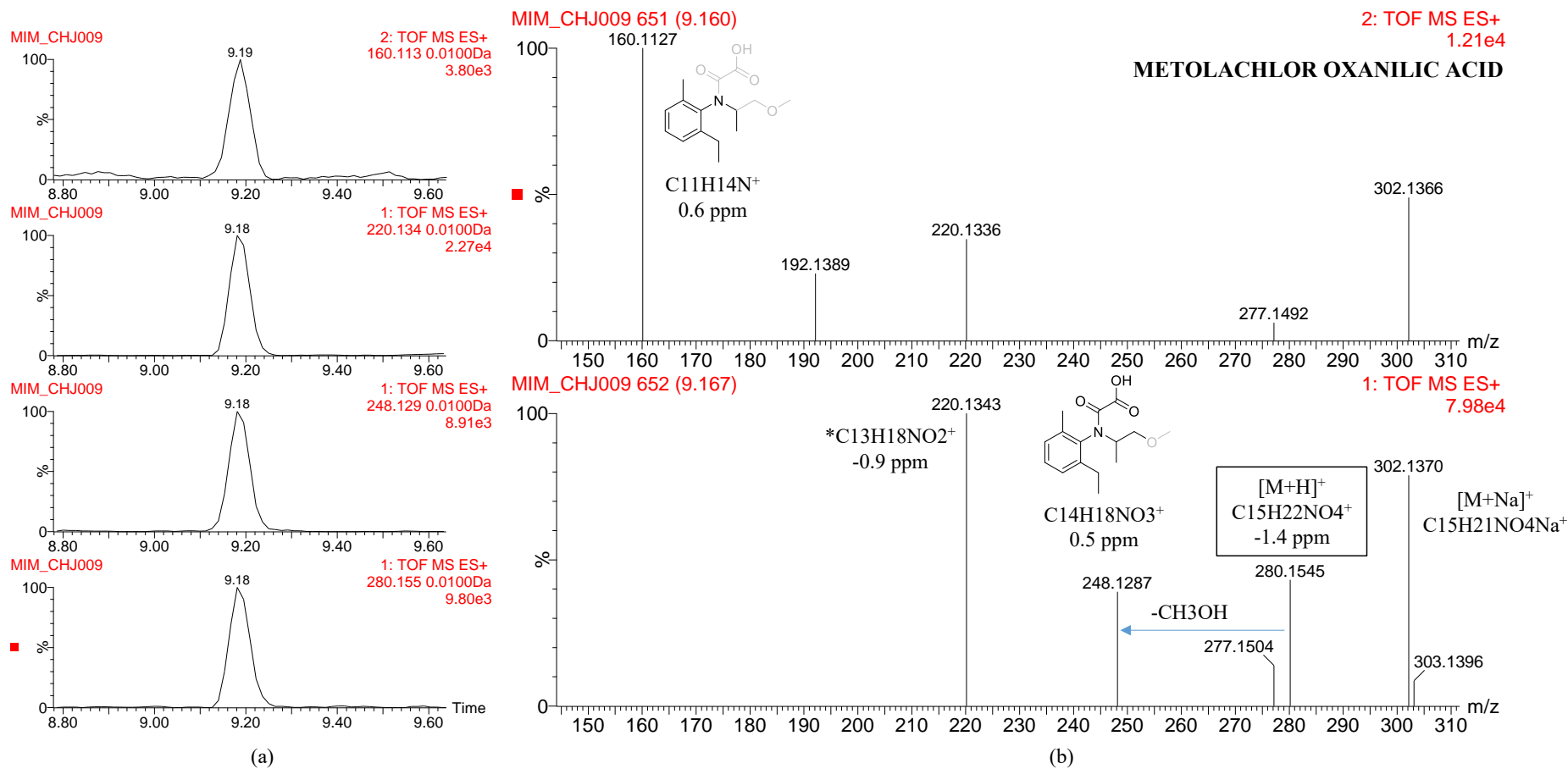HERB triazine 34%
TPs 45%

Figure 3.

Figure 4.

* A plausible structure could not be proposed for this fragment ion

Figure 5.